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Mechanical, physicochemical and color properties of chitosan based-films as a function of *Aloe vera* gel incorporation

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ABSTRACT

The objective of the present study was to investigate the effect of *Aloe vera* gel incorporation at different proportions on chitosan-based films. Consequently, the thickness of films was affected significantly by the addition of the gel and decreased from F0 (plain chitosan film) to F50 (the film containing 50% gel). The gel incorporation did not have a considerable effect on water vapor permeability (WVP); however, a significant difference was observed for F50. Addition of the gel significantly improved the water solubility (WS), wherein the F10 (the film with 10% of gel) showed the lowest. All mechanical properties increased by introducing the gel and, after reaching the peak for F20, started to reduce. Color properties were affected by the gel addition as the higher the gel, the darker the films. Overall, the results showed that incorporating the gel into film-forming solution of chitosan up to 20% (F20) was promising.

1. Introduction

Chitosan, a natural carbohydrate copolymer [β-(1–4)-2-acetamido-D-glucose and β-(1–4)-2-amino-D-glucose units], which is yielded from deacetylation of chitin [poly-β-(1–4)-N-acetyl-2D-glucosamine] as a major component found in shellfish industry, has been highlighted as a biocompatible, non-toxic, and biodegradable cationic polysaccharide (Karlsen, 1991). This biopolymer has been focus of interest for multiple applications including photography due to its resistance to abrasion, optical characteristics, and film-forming ability; in cosmetic products such as creams, nail lacquers, lotions, and permanent waving lotions; as an improving lactose intolerances agent and as a chromatographic support due to presence of free amino and hydroxyl groups in chitosan (Wani, Hasan, & Malik, 2010); drug-loaded films by dissolving or dispersing active ingredients within these films (Fernández-Cervera et al., 2004; Ritthidej, Phaechamud, & Koizumi (2000); Shu, Zhu, & Song (2001)); chitosan membranes (Muzzarelli, Isolati, & Ferrero (1974)), and food preservation because of chitosan ability to be used as food coating materials to extend the shelf life of different food products (Moreira, Pereda, Marcovich, & Roura (2011); Sanchez-Gonzalez, Chafer, Hernandez, Chiralt, & Gonzalez-Martinez (2011)); wherein the antibacterial activity of chitosan films is under influence of several factors such as pH of chitosan solutions, intrinsic factors of chitosan (i.e., molecular weight and degree of deacetylation), chitosan–metal complex, as well as sorption and bactericides properties of chitosan films (Aider, 2010).

Chitosan has been reported as an excellent film-forming substance, wherein positively charged groups occurring in chitosan can come in interaction with groups possessing opposite charges and yield three-dimensional networks (Despond, Espuche, & Domard, 2001). However, the properties of the obtained chitosan films are mainly proportional to several variants including chitin source, chitosan characteristics (i.e., molecular weight and deacetylation degree), type and amount of solvents, plasticizers, copolymers, dispersants, compatibilizers, among others and the method used for film preparation (Chillo et al., 2008; Fernández-Cervera et al., 2004; Muzzarelli, Tanfani, Emanuelli, & Mariotti (1983); Park, Marsh, & Rhim, 2002; Suyatma, Copinet, Tighzert, & Coma, 2004).

Aloe vera, a cactus-like plant, is a perennial succulent belonging to the Liliaceal family which grows in hot and dry climates (Choi & Chung, 2003). The plant has triangular, fleshy leaves with serrated edges, yellow tubular flowers and fruits containing countless seeds. Each leaf has three layers as follows: (1) an inner clear gel which is composed of 99% water and the rest is glucomannans, amino acids, lipids, sterols and vitamins, (2) the middle layer of latex, a bitter yellow sap, which contains glycosides and anthraquinones and (3) the outer thick layer of 15–20 cells, known as rind, being protective

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and synthesizing proteins as well as carbohydrates (Tyler, 1993). For centuries, the yellow latex (exudate) and clear gel (mucilage), exuded from the large leaf parenchymatic cells of *Aloe vera*, has been employed for medical and pharmaceutical purposes such as anti-inflammatory effects, treatment of skin burns, protection of the skin against UV and gamma radiation damage, treatment of frostbite and psoriasis, supporting and enhancing the immune system, antiviral and antitumor activity, laxative effects, and, last but not least, wound healing (Wani et al., 2010). However, the main use of *Aloe vera* gel is mainly in the cosmetology and medication; more recently, it has found its place in the food industry as a source of functional foods in ice-cream, drinks and beverages (Moore & MacAnalley, 1995), and, due to antifungal activity of *Aloe vera* gel, as an unique edible coating (plain or in combination with other components) to extend the post-harvest storage of arctic snow (Ahmed, Singh, & Khan, 2009), apple slices (Chauhan, Raju, Singh, & Bawa, 2010), sweet cherry (Martinez-Romero et al., 2006), papaya fruits (Marpudi, Abirami, Pushkala, & Srividya (2011)) and table grapes (Serrano et al., 2006; Valverde et al., 2005).

Thereby, the aim of the present study was to evaluate the physicochemical, mechanical and color properties of chitosan–*Aloe vera* gel films.

2. Materials and methods

2.1. Materials

Chitosan (Mv = 750 kDa, degree of deacetylation ≥ 75%) was obtained from Sigma (Aldrich Chemical Company Inc., Milwau-kee, WI, USA). *Aloe vera* gel was obtained from fresh *Aloe vera* leaves farmed in the Faculty of Agriculture (Tarbiat Modares University, Tehran, Iran) by scraping the outer epidermis.

2.2. Preparation of films

(1) Plain chitosan film (F0): chitosan 2% (w/w) was dispersed in 2% (w/w) lactic acid solution (Merck, Germany) under gentle stirring (Heidolph MR 3001K, Germany). The suspension was filtered through cheesecloth in order to eliminate the insoluble materials. The filtered solution was dispensed on the surface of Petri dishes (9 cm of diameter) and dried in an ambient temperature at 30 °C for 48 h. After film constitution, it was separated from the dish and placed in a controlled temperature chamber (IranKhodsaz IKHRH, Iran) at 25 °C with relative humidity (RH) of 50% prior to the analysis. (2) Chitosan–*Aloe vera* gel blend films: the extracted *Aloe vera* gel (at different ratios of 10, 20, 30, 40 and 50%) was added to the chitosan solution, and the blend solution was homogenized by using magnetic stirrer (Heidolph, MR 3001K, Germany) at ambient temperature (30 °C) and 300 rpm for 30 min; since the higher temperature along with longer time of exposure, in especial, may alter the beneficial properties of the gel (Grindlay & Reynolds, 1986; Ahlawat & Khatkar, 2011; Maughan, 1984). Then, to obtain the blend films, the same procedure as producing F0 was followed. The generated films were coded and given in Table 1.

Table 1
Illustration of the generated films; film's code and ratios.

Film's code	Ratio
F0	100% chitosan/*Aloe vera* gel 0%
F10	90% chitosan/*Aloe vera* gel 10%
F20	80% chitosan/*Aloe vera* gel 20%
F30	70% chitosan/*Aloe vera* gel 30%
F40	60% chitosan/*Aloe vera* gel 40%
F50	50% chitosan/*Aloe vera* gel 50%

2.3. Edible film analysis

2.3.1. Thickness

The films thickness (mm) was measured, using a hand-held digital micrometer (Mitutoyo, Japan), at five different positions in each specimen to the nearest 0.001 mm.

2.3.2. Physicochemical properties

2.3.2.1. Water vapor permeability. The water vapor permeability (WVP) of films was determined gravimetrically, using a modified ASTM E00996-00 (ASTM, 2000a) procedure. The cup had an internal diameter of 3.0 cm and an external diameter of 4.5 cm (exposed area: 7.065 cm^2), and was 3.5 cm deep. The cup was filled with 8 ml of distilled water in order to generate a 100% RH and covered by the film, and an O-ring rubber and vacuum grease helped to assure a good seal. The film and O-ring rubber were attached to the cup by four metal clips. Then, the cup was placed inside a desiccator containing silica gel in order to provide 0% RH. Changes in the weight of the cup (to the nearest 0.001 mg) were recorded under room conditions as a function of time for 12 h. The tests were conducted in triplicate. The water vapor permeability was calculated as follows:

$$\mathrm{WVP}(\mathrm{g\,m^{-2}\,h^{-1}\,Pa^{-1}\,mm}) = \frac{\Delta m \times X}{A \times t \times \Delta P} \tag{1}$$

where Δm is the mass change over time (g); X is the thickness (mm); t is the time (h); A is the film area (m^2) and ΔP is the partial vapor pressure difference of the atmosphere with silica gel and pure water (3179 Pa, at 22 °C).

2.3.2.2. Water solubility. The water solubility (WS) was defined as the percentage of film dry matter solved after 24 h of immersion in distilled water (Gontard, Guilbert, & Cuq, 1992). To determine the initial dry matter of the films, 1 cm × 4 cm strips were dried in an oven at 105 ± 1 °C for 24 h. Other strips were cut, weighed and immersed in 50 ml of distilled water (with periodic stirring) for 24 h at 25 °C. Then, to determine the final dry matter of the films, they were taken out and dried at 105 ± 1 °C for 24 h. All the tests were conducted in triplicate and the means were reported. The water solubility was calculated by following equation:

$$\mathrm{WS}(\%) = \frac{[\text{initial dry weigh(g)} - \text{final dry weigh(g)}]}{\text{initial dry weigh(g)}} \times 100 \tag{2}$$

2.3.3. Mechanical properties

All mechanical properties of the films were determined by a texture analyzer (Zwick B2 2.5/TH 1S, Germany) using a modified ASTM D00882-00 (ASTM, 2000b) procedure. The films were cut into 1 cm × 6 cm strips for testing. The measurements were performed using a cross-head speed of 50 mm min^{-1} with an exposed area of 1 cm × 4 cm for each film strip. Four strips were prepared from each film. The tensile stress was plotted against the elongation in order to give a stress–strain curve, and the ultimate tensile strength (TS, in MPa), elongation at break (%*E*) as well as elastic modulus (EM, in MPa) of films were reported.

2.3.4. Color properties

Color values of the films were measured with HnterLab (Colorflex 4510, USA). The Hunter Lab color scale was used for measuring color values: L^*, the lightness variable; a^*, from green to blue and b^*, from yellow to red. The total color difference (ΔE^*) and chroma (C^*) were calculated as follows:

$$\Delta E = \left(\sqrt{\Delta a^2 + \Delta b^2 + \Delta L^2}\right) \tag{3}$$

$$C = \left(\sqrt{a^2 + b^2}\right) \tag{4}$$

where $\Delta L = L^*_{standard} - L^*_{sample}$, $\Delta a = a^*_{standard} - a^*_{sample}$ and $\Delta b = b^*_{standard} - b^*_{sample}$. The standard plate (calibration plate CX0738, $L^* = 92.23$, $a^* = -1.28$, and $b^* = 1.22$) was used as a standard. Five measurements were taken on each film, one at the center and four around the perimeter.

2.4. Statistical analysis

A completely randomized experimental design was used for characterizing the films. Analysis of variance (one-way ANOVA) was used to compare mean differences of the samples. If differences existed in means, multiple comparisons were performed using Duncan's Multiple Range Test (DMRT) (confidence level, α: 0.05). SPSS 16.0.0 statistical software for windows (SPSS Inc., Chicago, USA) was used for data treatment and statistical analysis.

3. Results and discussion

3.1. Thickness

As can be seen in Table 2, the thickness of the films was significantly affected by *Aloe vera* gel incorporation ($p < 0.05$), so that, an increase in the ratio of *Aloe vera* gel versus chitosan from 0% to 50% led to a considerable reduction in thickness of films from 0.213 ± 0.003 mm for F0 (plain chitosan film) to 0.163 ± 0.005 mm for F50 (blend film containing 50% *Aloe vera* gel). The film thickness was in proportion to the film's nature and composition, as shown in the present study (Table 2). This has also been demonstrated by other researchers (Abugoch, Tapia, Villamán, Yazdani-Pedram, & Díaz-Dosque, 2010; Di Pierro et al., 2006; Sebti, Chollet, Degraeve, Noel, & Peyrol, 2007), in which a relationship has been observed between film thickness and, the content and nature of the film-forming polymer.

3.2. Physicochemical properties

3.2.1. Water vapor permeability (WVP)

Water vapor permeability is defined as the ease of moisture for penetrating and passing through the hydrophilic portion of film (Hernandez, 1994). Hence, the hydrophilic–hydrophobic ratio of the film composition plays a considerable role in WVP. In the literature, there are several available reports which demonstrate plain chitosan films as highly permeate to water vapor (Butler, Vergano, Testin, Bunn, & Wiles, 1996; Caner, Vergano, & Wiles, 1998), which limits their applications especially in highly humid environments. Therefore, a variety of attempts have been made to improve weak water vapor barrier property of chitosan films by incorporating various components such as thermally gelatinized corn starches (Xu, Kim, Hanna, & Nag, 2004), lysozyme (Park, Daeschel, & Zhao, 2004), different fatty acids (Srinivasa, Ramesh, & Tharanathan, 2007; Vargas, Albors, Chiralt, & González-Martínez, 2009; Wong, Gastineau, Gregorski, Tillin, & Pavlath, 1992) and vitamin E (Park & Zhao, 2004). The effect of *Aloe vera* gel incorporation is presented in Table 2. Addition of *Aloe vera* gel slightly reduced WVP, whereas it did not reach a significant difference until incorporating the *Aloe vera* gel at an equal portion with chitosan (F50), which obtained a considerable effect ($p < 0.05$).

Mainly the high WVP of the chitosan film is related to its hydrophilic nature, which lets water to come in interaction with matrix and results in increasing their rate of permeation (Fajardo et al., 2010). Thereby, the observed reduction of WVP in the present study (Table 2), by increasing the *Aloe vera* gel ratio, was attributed to possible interactions between chitosan and the components contained in *Aloe vera* gel, which decreased the availability of the hydrophilic groups in chitosan, diminishing their interactions with water; therefore, the water vapor transmittance was reduced. Ultimately, the authors concluded that *Aloe vera* gel incorporation had the potential of enhancing the chitosan film barrier property to water vapor.

3.2.2. Water solubility (WS)

The obtained results from the present study indicated that the presence of *Aloe vera* gel reduced water solubility to a considerable extend (Table 2). In comparison with the plain chitosan films (F0), which were completely soluble in water ($100.00 \pm 0.00\%$), the lowest water solubility belonged to the blend film consisting of 10% *Aloe vera* gel and 90% chitosan (F10: $45.122 \pm 2.11\%$) and increasing the portion of *Aloe vera* gel was followed by an increase in the water solubility of *Aloe vera* gel–chitosan blend films.

3.3. Mechanical properties

The results of film's mechanical analysis are summarized in Table 3. All mechanical properties, tensile strength (TS, in MPa) indicating the maximum tensile stress that the film can sustain, elongation at break (*E*, in %) as the maximum change in the length of a test film before being broken, and elastic modulus (EM, in MPa) which is a measure of the stiffness of the film (Srinivasa et al., 2007), as a function of *Aloe vera* gel incorporation, showed the same behavior pattern. These mechanical characteristics started to increase slightly as a result of increasing the *Aloe vera* gel ratio, from plain chitosan films (F0: 7.89 ± 0.65 MPa, $65.35 \pm 1.81\%$ and 9.62 ± 0.63 MPa; for TS, *E* and EM, respectively) to the highest values in blend films containing 20% *Aloe vera* gel (F20: 8.49 ± 0.70 MPa, $80.89 \pm 7.72\%$ and 10.50 ± 0.42 MPa; for TS, *E* and EM, respectively), however this increase did not obtain any significant difference ($p > 0.05$), except for elongation at break (Table 3). This increase was followed by a significant reduction in all mechanical properties by introducing higher quantities of *Aloe vera* gel, as the lowest values obtained by blend films containing 50% *Aloe vera* gel (F50: 6.20 ± 1.37 MPa, $59.42 \pm 8.04\%$ and 6.66 ± 0.75 MPa; for TS, *E* and EM, respectively), which can be related to the high moisture content of incorporated *Aloe vera* gel (98.6 ± 0.1), since the moisture has been reported as an effective plasticizer (Krochta, 2002). Clearly, the blend films containing 20% *Aloe vera* gel (F20) showed the highest values for mechanical characteristics, which indicated that this amount of *Aloe vera* gel incorporation (20%) was a threshold for obtaining films with suitable toughness and flexibility.

3.4. Color properties

Color properties of packaging are a matter of prime importance with regard to general appearance and consumer acceptance. Changes of rectangular coordinates (L^*, a^*, and b^*) are given in Table 4, where can be seen that these color properties all have the same tendency and increasing the level of *Aloe vera* gel results in significant increase in L^*, a^*, and b^* of films ($p < 0.05$). In terms of chroma (C^*), the same change as rectangular coordinates (Table 4) was observed and an increase in *Aloe vera* gel ratio was followed by a raise in C^* (from F0: 3.85 ± 0.08 to F50: 20.15 ± 0.82) (Table 4). However, *Aloe vera* gel incorporation had a significant reducing effect on the total color difference (ΔE^*), that is, the higher the *Aloe vera* gel, the lower the ΔE^* (from F0: 88.57 ± 0.03 to F50: 76.08 ± 1.02) (Table 4). In general, introduction of *Aloe vera* gel at higher levels yielded blend films with darker appearance (brown), which can be attributed to the fact that the extracted *Aloe vera* gel, applied in this study, could contain anthranquinones from the rind, since they are difficult to be completely eliminated and, are air and light sensitive, hence the oxidation of phenol occurred (Yamaguchi, Mega, & Sanada, 1993).

Table 2
Thickness and physicochemical properties of plain chitosan and chitosan–*Aloe vera* gel blend films.[a]

Film's code	Thickness (mm)	Water Vapor permeability (WVP) ($gm^{-2} h^{-1} Pa^{-1}$ mm)	Water solubility (WS) (%)
F0	0.213 ± 0.003^{f}	0.302 ± 0.027^{b}	100.00 ± 0.00^{d}
F10	0.203 ± 0.001^{e}	0.296 ± 0.007^{ab}	45.12 ± 2.11^{a}
F20	0.193 ± 0.002^{d}	0.291 ± 0.009^{ab}	48.14 ± 3.51^{ab}
F30	0.186 ± 0.004^{c}	0.291 ± 0.023^{ab}	52.05 ± 8.06^{abc}
F40	0.172 ± 0.001^{b}	0.287 ± 0.008^{ab}	55.91 ± 6.52^{bc}
F50	0.163 ± 0.005^{a}	0.266 ± 0.004^{a}	59.99 ± 4.40^{c}

[a] Data, followed by their standard deviations, are means of three replicates. Treatment means were separated using the Duncan test and values followed by the different letter in a same column are significantly different (α: 0.05).

Table 3
Mechanical properties of plain chitosan and chitosan–*Aloe vera* gel blend films.[a]

Film's code	Tensile strength (TS) (MPa)	Elongation at break (E) (%)	Elastic modulus (EM) (MPa)
F0	7.89 ± 0.65^{b}	65.35 ± 1.81^{a}	9.62 ± 0.63^{b}
F10	8.44 ± 0.59^{b}	68.96 ± 4.32^{ab}	10.18 ± 0.45^{b}
F20	8.49 ± 0.70^{b}	80.89 ± 7.72^{c}	10.50 ± 0.42^{b}
F30	7.15 ± 0.69^{ab}	79.62 ± 2.34^{bc}	7.52 ± 0.72^{a}
F40	6.20 ± 0.16^{a}	60.11 ± 8.97^{a}	7.48 ± 1.52^{a}
F50	6.20 ± 1.37^{a}	59.42 ± 8.04^{a}	6.66 ± 0.75^{a}

[a] Data, followed by their standard deviations, are means of three replicates. Treatment means were separated using the Duncan test and values followed by the different letter in a same column are significantly different (α: 0.05).

Table 4
Effect of *Aloe vera* gel incorporation at different ratios to chitosan solution on obtained film's color values of a^*, b^*, L^*, ΔE^* and C^*.[a]

Film's code	a^*	b^*	L^*	ΔE^*	C^*
F0	-3.82 ± 0.08^{a}	-0.52 ± 0.06^{a}	3.72 ± 0.03^{a}	88.57 ± 0.03^{f}	3.85 ± 0.08^{a}
F10	-3.18 ± 0.09^{b}	3.20 ± 0.10^{b}	5.22 ± 0.22^{b}	87.05 ± 0.22^{e}	4.51 ± 0.01^{a}
F20	-2.99 ± 0.28^{b}	8.00 ± 0.43^{c}	7.81 ± 0.08^{c}	84.71 ± 0.05^{d}	8.43 ± 0.51^{b}
F30	-2.84 ± 0.21^{b}	9.38 ± 0.27^{d}	10.27 ± 0.53^{d}	82.28 ± 0.52^{c}	9.80 ± 0.30^{c}
F40	-1.59 ± 0.55^{c}	12.98 ± 0.77^{e}	15.05 ± 0.86^{e}	78.07 ± 0.94^{b}	13.08 ± 0.70^{d}
F50	2.43 ± 0.03^{d}	20.01 ± 0.83^{f}	18.60 ± 0.85^{f}	76.08 ± 1.02^{a}	20.15 ± 0.82^{e}

[a] Data, followed by their standard deviations, are means of three replicates. Treatment means were separated using the Duncan test and values followed by the different letter in a same column are significantly different (α: 0.05).

4. Conclusion

The results of this study suggested that the incorporation of *Aloe vera* gel into film-forming chitosan solution can have a considerable influence on the properties of the obtained chitosan–*Aloe vera* gel blend films. The physicochemical properties of the plain chitosan film were improved to the high extend by incorporating *Aloe vera* gel, the lower water solubility and permeability to water vapor. The mechanical analyses of films indicated that the introduction of *Aloe vera* gel up to 20% slightly increased the tensile strength, elongation at break and elastic modulus values of films, and the maximum quantities were obtained. Lastly, the color of films became darker (from light yellow for F0 to brown for F50) as the level of *Aloe vera* gel proportion increased. In conclusion, incorporation of *Aloe vera* gel at 20% level was found to be a critical quantity, since along with presenting an appropriate water vapor barrier and low solubility in water for obtained blend films (F20), the films with the best mechanical properties were formed, in comparison with other films.

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