Structure and permeability of low-methoxyl pectin (LMP)-Sodium alginates (NaAlg) films

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Alginate and pectin form synergistic mixed gels that lead to a microstructure totally different from that of pure biopolymers. Synergistic mixed gels are of particular interest as makers of films with new improved characteristics. The objectives of the present work were, (a) to develop composite biodegradable films based on sodium alginate (NaAlg) and low-methoxyl pectin (LMP), (b) to evaluate the water vapor (WVP) and oxygen permeability, and (c) to characterize film microstructure by electron microscopy. A film-forming solution was prepared by adding 2% w/w of NaAlg, LMP or their mixtures, in a constantly stirred solution of NaCl (0,1M), during 4 h and then poured into Petri glass plates and allowed to dry at ambient, resulting in about 40µm films. WVTR was determined gravimetrically using a modified ASTM Method E 96-95, and oxygen permeability of the prepared films was measured according to ASTM D3985. Films structure was characterized using both transmission (TEM) and scanning (SEM) electron microscopy. Results indicated that water vapor permeation follows Henry's law after two weeks of assay. Water permeability reduced in mixed biopolymer films, indicating NaAlg/LMP synergism. Pure pectin films resulted a better water vapor barrier, indicating the molecular weight has not effect on permeability. On the other hand, the reduction in permeability by alginate adding was assumed to be a result of the filling of pectin net. Permeability to oxygen was irrelevant. Confirming permeability assays, SEM micrographs of 1:1 alginate/pectin films show surface with lower porosity than pure (pectin or alginate) films. Moreover, TEM studies at the higher magnification (140,000X) also show a closer and denser nature in the case of mixed networks.

1. Introduction

It is well known that agricultural and seafood commodities produce high amounts of surplus and wastes that are unprofitable sources of biopolymers, such as several polysaccharides with good film-forming capability, including alginate and pectin. It is also known that alginate and pectin form synergistic mixed gels. Since synergism can lead to a microstructure totally different from that of pure biopolymers, synergistic mixed gels are of particular interest as makers of films with new improved characteristics (Parris et al., 1995; Pavlath et al., 1999a; Pavlath et al., 1999b).

Alginate is of interest as a potential biopolymer film or coating component because of its unique colloidal properties (Moe et al., 1995). Alginate is a hydrophilic colloidal carbohydrate extracted with dilute alkali from various species of brown seaweeds (*Phaeophyceae*). In molecular terms, it is a family of unbranched binary copolymers of $(1 \rightarrow 4)$-linked β-D-mannuronic acid and α-L-guluronic acid residues of widely varying composition and sequential structure (King, 1983; Moe et al., 1995). The most useful property of alginates is their ability to react with polyvalent metal cations, specifically calcium ions, to produce strong gels or insoluble polymers (King, 1983). Such Ca-alginate gels are used in the food processing industry for producing restructured foods such as meat products, onion rings, pimento olive fillings, crabsticks, and cocktail berries (Moe et al., 1995).

On the other hand, pectin is a linear chain of α-(1-4)-linked D-galacturonic acid that forms the pectin-backbone, a homogalacturonan (Kertesz, 1951). Into this backbone, there are regions where galacturonic acid is replaced by (1-2)-linked L-rhamnose. From rhamnose, sidechains of various neutral sugars branch off. A third structural type of pectin is Rhamnogalacturonan II, which is a less frequent complex, highly branched polysaccharide. Isolated pectin has a molecular weight of typically 60 – 130,000 g/mol, varying with origin and extraction conditions. In nature, around 80% of carboxyl groups of galacturonic acid are esterified with methanol. This proportion is decreased more or less during pectin extraction. The ratio of esterified to non-esterified galacturonic acid determines the behavior of pectin in food applications. Pectins are classified as high (HM) or low (LM) methoxyl pectins, with more or less than half of all the galacturonic acid esterified, respectively.

Although the nature of the synergistic interaction between pectin and alginate in mixed gels is not fully known, it appears to be specific rather than based on incompatibility or exclusion effects (Oakenfull et al., 1990). The rheological behavior of alginate/pectin mixed gels has been largely studied (Garncarek & Garncarek, 1993; Oakenfull et al., 1990; Rao and Cooley, 1995). Unlike the rheological behavior, the microstructure of alginate/pectin mixed films has been less studied. Based on intrinsic viscosity measurements on HM pectin and alginate with a high M/G ratio, Oakenfull et al. (1990) suggested aggregation as a step preceding gel formation.

Though edible films prepared from hydrocolloids like alginate form strong films, they exhibit poor water resistance because of their hydrophilic nature (Kester and Fennema, 1986). The ability of alginate to make strong and insoluble gels with calcium ions can be utilized to improve such properties of alginate films (Pavlath et al., 1999). However, gel formation of alginate with calcium ions is so instantaneous that it prevents casting to make films in some cases. The water permeability of edible films is one of the most important properties in food or pharmaceutical applications (Sothornvit and Krochta, 2000), and most of the published studies on biopolymer films deal mainly with film formation and film properties such as mechanical and barrier properties (Kim et al., 2002).

The objectives of the present work were basically: (a) to develop composite biodegradable films based on sodium alginate (NaAlg) and low-methoxyl pectin (LMP); (b) to evaluate the water vapor (WVP) and oxygen permeability; and (c) to characterize film microstructure by electron microscopy.

2. Materials and Methods

Pectin was a low methoxyl citric pectin (LM 104) AS from GENU PECTIN (Denmark) Sodium alginate (NaAlg), was obtained from Fluka (Switzerland). Molecular weight (Mw) and polydispersion degree (Mw/Mn) of NaAlg were 280,000 g/mol and 1.88 respectively, with a ratio-D-mannuronic acid to α-L-guluronic (M/G)= 0,43. Pectin was standardized resulting in a LMP with M_w= 30,000 g/mol; and degree of esterification and amidation were 31% and 17% respectively. All other chemicals used were of analytical reagent grade. A film-forming solution was prepared by slowly adding 2% w/w of NaAlg, LMP or their mixtures, in a constantly stirred solution of NaCl (0,1M), during 4 h. Approximately 20g of filtered solutions were poured into 9cm Petri glass plates and allowed to dry at ambient temperature for 24 h, resulting in about 40µm film. Samples were made by triplicate. WVTR was determined gravimetrically using a modified ASTM Method E 96-95. The oxygen permeability of the prepared films was measured by a MOCON OX-TRAN 2/21 (USA) Permeameter according to ASTM D3985.Films structure was characterized using both transmission and scanning electron microscopy, with a SEM EVO 40 XVP at 5,8 kV, and a TEM JEOL 100 CXL (JEOL, Tokyo) microscope at 80 kV, respectively. Films for TEM were crio-fractured, and gold-covered with Pelco model 3 Sputter Coater 91000 (Ted Pella Inc.,NY). TEM Assayed films were fixed with OsO_4 and ruthenium red, then dehydrated and embedded with Spurr resin and finally cut with a LKB Microtome.

3. Results and Discussions

3.1 Films Permeability

Mean permeability coefficients P were determined from the slope of the permeation curve according to the equation (1):

$$P = \frac{Q}{t}\frac{X}{\Delta p A} \qquad (1)$$

where Q= mass permeated; X= film thickness; Δp: partial pressure difference (driving force); A: film area; and t= time. In the case of water vapor permeability (WVP) partial pressure difference can be written as:

$$\Delta p = \frac{p^s}{100}\left(HR_1 - HR_2\right) \qquad (2)$$

where $HR_{1,2}$ are relative humidity on both sides of the film.

Figs. 1a,b shows the water permeation rate through films, at both 35% and 75% relative humidity (RH) driving force. As figure shows, water vapor permeation follows Henry's law after two weeks of assay. Mean permeability coefficients were determined from the slope of the permeation curve. Table 1 shows water vapor permeability of alginate and pectin films. WVP of the alginate films was 2 orders of magnitude lower than that of κ-carrageenan film (another carbohydrate from red algae), of $3.3\pm0.09\times10^{-9}$ g m/m^2 s Pa (Rhim et al., 1998). Parris et al. (1995) reported the WVP of alginate films prepared using alginate and glycerin (7:3 by weight) was $0.65\pm0.01\times10^{-9}$ g m/m^2 s Pa.
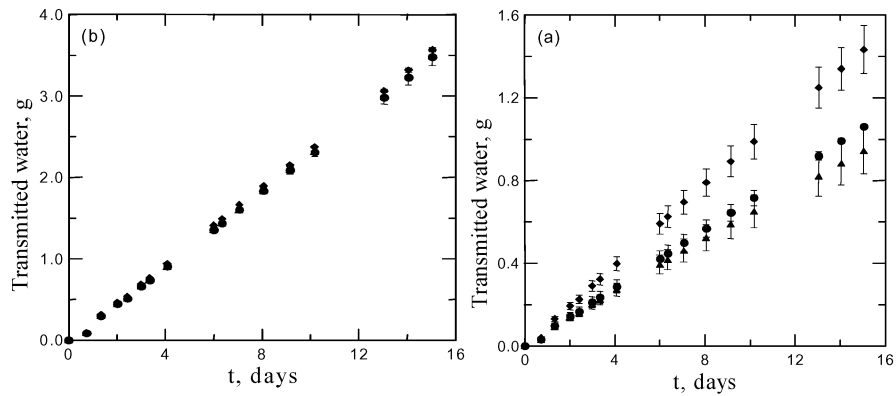
Figure 2: Water vapor permeated through films; (♦) NaAlg, (●) LMP and (▲) 1:1NaAlg/LMP blend, at 23 °C. (a) HR 35 %; (b) HR 75 %

Reportedly, water vapor barrier properties of biopolymer films decrease by adding a plasticizer like glycerin (Gontard et al., 1993) and WVP of films decrease with increasing temperature of WVP measurement (Rhim et al., 2003).

Table 1. Water vapor permeability of NaAlg, LMP and 1:1 blend films, at 23 °C.

ΔHR^* [%]	Film	$P \cdot 10^{11}$ [g s^{-1} m^{-1} Pa^{-1}]	Error [%]
35	NaAlg	5,56 ± 1,24	22,3
	1:1 Blend	3,12 ± 0,31	9,9
	LMP	4,85 ± 0,09	1,8
75	NaAlg	6,62 ± 1,67	25,2
	1:1 Blend	5,53 ± 0,22	4,0
	LMP	5,81 ± 0,90	15,5

*$\Delta HR\%$ (Relative humidity difference)

Water permeability reduced in mixed biopolymer films, indicating NaAlg/LMP synergism. Pure pectin films resulted a better water vapor barrier than pure alginate films, indicating the molecular weight has not effect on permeability. On the other hand, the reduction in permeability by alginate adding was assumed to be a result of the filling of pectin net. Permeability to oxygen was practically insignificant, bellows the effective resolution of the used permeameter.

3.2 Films Microstructure

Microstructure of alginate, pectin, and alginate/pectin (1:1) mixed films, studied both by SEM and TEM, is shown in Fig. 3 revealing the general nature of the network configuration. It was observed that that pectin influences the network density of pure alginate films. While SEM micrographs (Fig. 3 a, b and c) show the effect of biopolymer blend on film surface, the network characteristics are better revealed by TEM studies at the highest magnification (Fig. 3 d, e, and f). Confirming permeability assays, SEM micrographs of 50:50 alginate/pectin films show surface with lower porosity than pure (pectin or alginate) films.
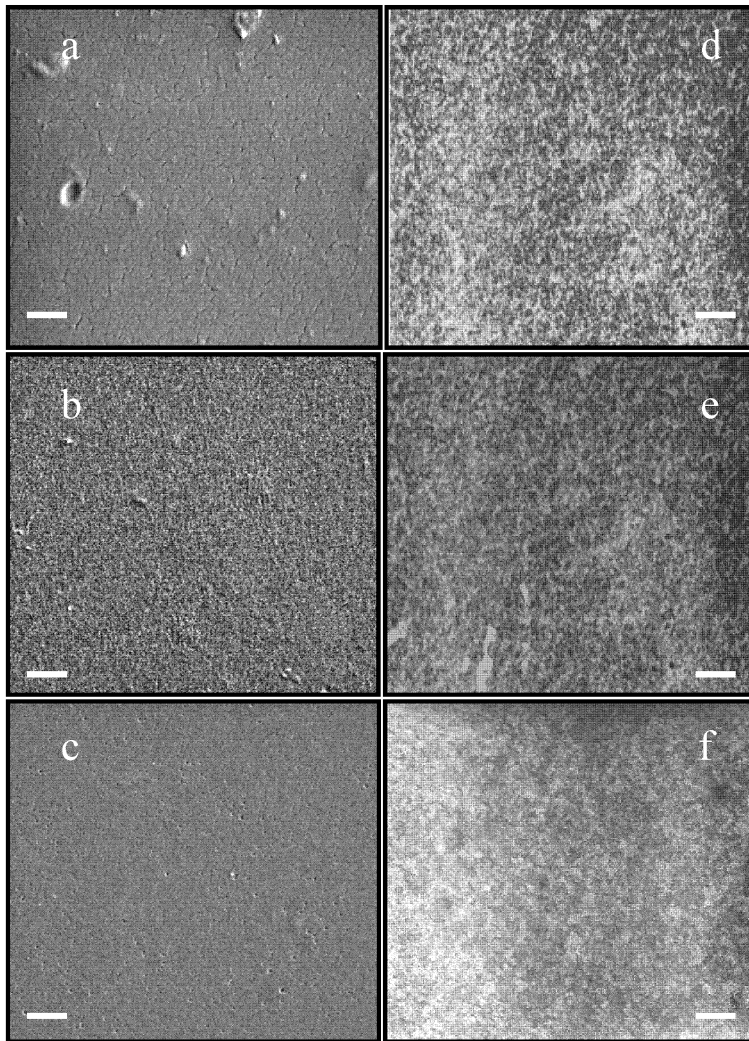
Figure 3: SEM, [11000X- bar: 2.00μm] (a) NaAlg; (b) 1:1 NaAlg:LMP; (c) LMP and TEM [80,000X; bar: O.12μm](d) LMP; (e) 1:1 NaAlg:LMP (f); NaAlg films micrographs.

Moreover, TEM at the higher magnification (140,000X) also show a closer and denser nature in the case of mixed networks. As the studied films are water soluble, the authors are working at the present on less soluble mixed films formulations, also with better mechanical properties.

4. Conclusions

Alginate:LM pectin mixed films show oxygen permeability comparable with other synthetic and biopolymer films. A synergistic interaction between pectin and alginate in

mixed gels, reducing permeability, was observed. Future studies will continue to focus on the improvement of water resistance and mechanical properties

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