Preparation of biopolymer film from chitosan modified with lipid fraction

Vanderlei C. Souza, Micheli L. Monte & Luiz A. A. Pinto*

Unit Operation Laboratory, School of Chemistry and Food, Federal University of Rio Grande (FURG), PO Box 474, Zip 96201-900, Rio Grande, RS, Brazil

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Summary
Films formed from polysaccharides, as chitosan, present a high permeability in water vapour. In order to increase resistance to water vapour for chitosan-based films, different lipid fractions were incorporated into a filmogenic matrix: fish and vegetable oils, stearic and oleic acids. The chitosan showed a molecular weight of 150 kDa and a deacetylation degree of 86 ± 1%. Results showed that incorporation of different lipid fractions decreased the water vapour permeability (WVP) (1.3–1.8 g mm m\(^{-2}\) day\(^{-1}\) kPa\(^{-1}\)) as compared with pure chitosan film (3.8 g mm m\(^{-2}\) day\(^{-1}\) kPa\(^{-1}\)). A higher reduction in WVP (65%) was found with the addition of refined fish oil to the continuous matrix of the films than with the addition of refined rice oil, oleic or stearic acid (50–60%). However, pure chitosan films showed better tensile strength (TS = 33 MPa) and elongation percentage (E = 18%) than lipids fraction–chitosan films (7–19 MPa and 7–13%, respectively).

Keywords
Biopolymer films, chitosan, fatty acid, fish oil, lipids, vegetable oil.

Introduction
Owing to concerns about limited natural resources and environmental impacts caused by the use of synthetic polymers and rigid packaging, there is a great interest in developing biodegradable films that act as packing material and/or component (Arvanitoyannis, 1999; Ferreira et al., 2009). Two major promising applications of such films are the replacement of short-life plastic in food packaging and use as edible food films (Adebiyi et al., 2008). Films formed from polysaccharides have good mechanical properties; however, the fact that they are highly permeable to water vapour owing to their strong hydrophilic character limits their potential applications, because an effective control of moisture transfer is desirable for most foods (Arvanitoyannis et al., 1998; Liu et al., 2006; Fernandez-Saiz et al., 2009).

Chitosan is an amine polysaccharide type of carbohydrate with antimicrobial, nontoxic and biocompatibility properties, which when combined with its cationic character and film-forming capacity makes it one of the biopolymers with the greatest potential in obtaining food packaging, especially as edible films (Arvanitoyannis et al., 1997; No et al., 2007).

In order to improve the water barrier properties, the main lipid fractions incorporated into the base of biopolymer films are fatty acids (Srinivasa et al., 2007; Fabra et al., 2008; Vargas et al., 2009), vegetable oils (Bourtoom & Chinnan, 2009), hydrogenated oils (The et al., 2009) and waxes (Hambleton et al., 2009). For example, Bourtoom & Chinnan (2009) added lipids to chitosan–starch composite films, decreasing the water vapour permeability (WVP) when palm oil and margarine were used. The effect of adding some vegetable oils, such as oregano essential, has also been investigated (Chi et al., 2006). However, little information exists about the impact of fish oil incorporation on biopolymer films properties.

Waxes have been the most widely used lipids in polysaccharide films. Studies show that they are more effective than saturated fatty acids in improving the water vapour barrier property, and this is because of its high hydrophobicity (Fabra et al., 2008; Hambleton et al., 2009). Owing to the hydrophobic nature of the fish oil, it could be combined with polysaccharides; furthermore, use of fish oil as the dispersed phase could aggregate value to the film, mainly due to the presence of eicosapentaenoic (C20:5 EPA \(\omega-3\)) and docosahexaenoic (C22:6 DHA \(\omega-3\)) acids (Crexi et al., 2010).

The aim of this study was to increase resistance to water vapour of chitosan-based films, incorporating different lipid fractions into the filmogenic matrix: fish
oil (refined from carp viscera), vegetable oil (refined from rice), saturated fatty acid (stearic acid) and unsaturated fatty acid (oleic acid). Moreover, mechanical properties (tensile strength and elongation percentage at break point) of the films were evaluated.

Material and methods

Production of chitosan

Chitin was obtained through the steps of demineralisation, deproteinisation and deodorisation of shrimp (Farfantepenaeus brasiliensis) waste. Chitin was dried in a tray drier until commercial moisture content (< 10%). Chitosan was obtained from chitin according to the methodology of Weska et al. (2007) through alkaline deacetylation (NaOH 0.42 kg L\(^{-1}\), at 130 °C for 90 min). Chitosan was purified and dried in spouted bed according to the methodology of Halal et al. (2011).

The viscosity average molecular weight of chitosan was determined by viscosimetric method (model Schott Gerate, GMBH-D65719; Cannon-Fenske, Mainz, Germany). The intrinsic viscosity was obtained by Huggins equation and converted into molecular weight by Mark–Houwink–Sakurada equation, eqn 1, where\( k = 1.81 \times 10^{-3} \text{mL g}^{-1} \) and \( \alpha = 0.93 \).

\[
\eta = KM_v^\alpha
\]  

where \( \eta \) is intrinsic viscosity (mL g\(^{-1}\)) and \( M_v \) is viscosity average molecular weight (Da) (Weska et al., 2007).

Deacetylation degree was verified through Fourier transform infrared spectroscopy, FT-IR (Prestige 21, Kyoto, Japan) analysis. Chitosan powder was prepared with potassium bromide (WF Científica, Pelotas, Brazil) (Sakkayawong et al., 2007). Deacetylation degree was determined according to eqn 2 (Cervera et al., 2004).

\[
\%DD = 87.8[3(A_{C-O}/A_{-OH})]\]  

where \( \%DD \) is deacetylation degree (%); \( A_{C-O} \) is absorbance of \( \text{C} = \text{O} \) group; and \( A_{-OH} \) is absorbance of -OH group.

Obtainment of lipid fractions

Crude fish oil was obtained from carp (Cyprinus carpio) viscera by fishmeal process according to the methodology proposed by Crexi et al. (2009). Crude oil was subjected to chemical refinement, comprising degumming, neutralisation, washing, dehumidification, bleaching, winterisation and deodorisation steps (Crexi et al., 2010). Fatty acids (stearic and oleic acids) were provided by the WF Científica, and commercial rice oil purchased from a local market.

Physicochemical characterisation of the refined oils was carried out. Thiobarbituric acid value (TBA) was found according to Vyncke (1970), using spectrophotometric method (model Q-108 DRM; Quimis, São Paulo, Brazil), and calculated from a standard curve obtained by reacting known amounts of 1,1,3,3 tetramethoxypropane with TBA. Free fatty acid (FFA) content was found according to the methods of American Oil Chemists Society (AOCS, 1980). FFA method (Ca 5a-40) was used, based on titration of the oil with a sodium hydroxide solution (phenolphthalein as an indicator), suitably diluted with an ethyl alcohol–ethyl ether mixture.

Through the preparation of methyl esters, it was possible to determine the fatty acid profile of refined oil in accordance with Metcalf & Schimtz (1966). Fatty acid methyl esters were identified using gas chromatography (model Varian-3400 CX, Minneapolis, MN, USA).

Film preparation

Film-forming dispersions (FFDs) were obtained by dissolution, at room temperature, of chitosan powder (1% w/v) in an aqueous solution of glacial acetic acid (1% v/w). When required, Tween 80 at 5% (w/w) with respect to the amount of chitosan and lipid fraction (fish oil, vegetable oil, oleic acid or stearic acid) was added (chitosan/lipid ratio of 4:1). FFD were emulsified (model 1100-01; Dremel Stylus, Rancine, WI, USA) at 10 000 rpm for 4 min. Subsequently, pH (MB10/MB-10P; Marte, São Paulo, Brazil) was adjusted to 5.0 with NaOH solution (1 m) (Vargas et al., 2009). Before the incorporation of lipid fraction, this was dissolved through its heating by a hot plate (752A; Fisaton, São Paulo Brasil). The dispersions were filtered through a paper filter under vacuum. Thickness was controlled through the volume of the filmogenic solution on petri dishes. All the films were prepared with 100 mL of filmogenic solution per petri dish. The films were obtained by evaporating the solvent in an oven (model Q-314M; Quimis, São Paulo, Brazil) with 0.0100 ± 0.0005 mm resolution. The films were obtained by evaporating the solvent in an oven (model Q-314M; Quimis) with air circulation at 25 °C for about 48 h. After the storage period, the film thickness was measured using a digital calliper (model VTC; Stainless Hardened, São Paulo, Brazil) with 0.0100 ± 0.0005 mm resolution. The thickness was set as the arithmetic mean of twelve random measurements over the area of the film.

Water vapour permeability of films was determined gravimetrically at 25 °C, using the ASTM standard method E96-95 (ASTM, 1995). Samples of each film in
the form of discs (diameter = 70 mm) were fixed with paraffin cell permeation of aluminium, containing anhydrous calcium chloride. These cells were placed in desiccators at 25 °C and 75% relative humidity. By increasing the mass of anhydrous calcium chloride (measured in intervals of 24 h for 7 days), it was possible to determine the water vapour transferred through the film according to eqn 3.

\[
WVP = \frac{W_{ma}}{t} \frac{L}{A \Delta P}
\]

where \(W_{ma}\) is the weight of absorbed moisture (g); \(t\) is the time duration of the test (days); \(L\) is the average film thickness (mm); \(A\) is the area of the exposed film surface (m²); and \(\Delta P\) is the partial vapour pressure difference across the film (Pa).

Tensile strength (TS) and elongation percentage (E) at break point were measured uniaxially by stretching the specimen in one direction using a Texture Analyzer (model TA.XP; Stable Microsystems SMD, Godalming, UK) according to the ASTM D-882 standard (ASTM, 2001), with a 50 N load cell. Sample films were cut into 25-mm-wide and 100-mm-long strips. The initial grip separation and cross-head speed were set to 50 mm and 50 mm min⁻¹, respectively.

The films were analysed using FT-IR spectrophotometry (Prestige 21), in a spectral range from 4000 to 400 cm⁻¹. Film samples were examined for surface characteristics using a scanning electron microscope (SEM) (model JSM-5800 LV, Jeol, Tokyo, Japan) operated at 10 kV. Five samples were mounted on a bronze stub and sputter-coated (Sputter coater SPI-Module, Santa Clara, CA, USA) with a layer of gold prior to imaging.

Statistical analysis

One-way analysis of variance and Tukey’s multiple comparison tests were used to statistically determine significant differences (\(P \leq 0.05\)) among averages, using the software Statistic 6.0 (Statsoft, Tulsa, OK, USA).

Results and discussion

Chitosan powder characterisation

Chitosan showed viscosity average molecular weight of 150 ± 5 kDa (eqn 1). The FT-IR spectrum was analysed (figure not shown): a strong band at 1556 cm⁻¹ showed a typical chitosan amino group (-NH₂) (Ziani et al., 2008). At 1640 cm⁻¹, an axial deformation of C=O (amide band I) (Martínez-Camacho et al., 2010) was observed. Weak bands at 1020 and 1080 cm⁻¹ were relative to C-N links and at 2933 cm⁻¹ to primary amine stretching. These peaks are related to functional chitosan amino group. In addition, at 3470 cm⁻¹, hydroxyl groups linked in chitosan structure (Baskar & Kumar, 2009) were observed. Deacetylation degree obtained from FT-IR analysis was 86 ± 1%.

Refined oil characterisation

Thiobarbituric acid and FFA values of carp refined oil (TBA = 5.9 ± 0.2 mg malonaldehyde kg⁻¹; \(FFA = 0.09 ± 0.01\)%) and rice oil (TBA = 0.14 ± 0.02 mg malonaldehyde kg⁻¹; \(FFA = 0.22 ± 0.01\)%) are in accordance with the standards required for the acceptability of refined oils for human consumption (TBA = 7–8 mg malonaldehyde kg⁻¹ and \(FFA = 1.8–3.5\)% (Sathivel et al., 2003; Boran et al., 2006). The refined oil thus obtained offer quality and oxidative stability and thereby can be used as edible components.

The fatty acid profiles and lipid classes of refined oils are shown in Table 1.

According to Table 1, the major fatty acids present in refined carp (Cyprinus carpio) oil are palmitic acid (C16:0), palmitoleic acid (C16:1), oleic acid (C18:1 9–9), linoleic acid (C18:2 9–6) and linolenic acid (C18:3 9–3), which constitute approximately 68% of the total fatty acids. Furthermore, the refined oil showed 69% of unsaturated and polyunsaturated fatty acids (MUFA + PUFA), of which 28% correspond to 9–3 and 9–6. These proportions are in agreement with those found by Crexi et al. (2010). The majority of fatty acids present in rice oil are palmitic acid (20.2%), stearic acid (1.2%), oleic acid (41.9%) and linoleic acid (28.6%).

<table>
<thead>
<tr>
<th>Fatty acid (%) profiles and lipid classes (% of total fatty acids) of the carp and rice refined oils</th>
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<tbody>
<tr>
<td>Fatty acids and lipid classes</td>
</tr>
<tr>
<td>C16:0</td>
</tr>
<tr>
<td>C18:0</td>
</tr>
<tr>
<td>C16:1 9–7</td>
</tr>
<tr>
<td>C18:1 9–9 cis</td>
</tr>
<tr>
<td>C18:1 9–9 trans</td>
</tr>
<tr>
<td>C18:2 9–6 trans 9,12</td>
</tr>
<tr>
<td>C18:2 9–6 cis</td>
</tr>
<tr>
<td>C18:3 9–6</td>
</tr>
<tr>
<td>C18:3 9–3</td>
</tr>
<tr>
<td>C20:5 9–3 (EPA)</td>
</tr>
<tr>
<td>C22:6 9–3 (DHA)</td>
</tr>
<tr>
<td>(\sum) ni</td>
</tr>
<tr>
<td>(\sum) SFA</td>
</tr>
<tr>
<td>(\sum) MUFA</td>
</tr>
<tr>
<td>(\sum) PUFA</td>
</tr>
<tr>
<td>9–3</td>
</tr>
<tr>
<td>9–6</td>
</tr>
</tbody>
</table>

Mean value ± standard error (in replicate). EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; \(\sum\) ni, sum of unidentified fatty acids; \(\sum\) SFA, sum of unsaturated fatty acids; \(\sum\) MUFA, sum of monounsaturated fatty acids; \(\sum\) PUFA, sum of polyunsaturated fatty acids.

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The proportion of linolenic acid (1.2%) in rice oil is lower when compared with that of fish oil (7.6%) (Table 1). The metabolic conversion of linolenic acid to EPA and DHA is not efficient, and for this reason, it is considered essential and must be supplied in the diet (Horrocks & Yeo, 1999).

**Film characterisation**

All the films showed an average thickness of 0.030 ± 0.001 mm and were characterised in terms of WVP and mechanical (tensile strength and elongation percentage at break point) properties (Table 2).

Table 2 shows significant differences ($P \leq 0.05$) between the permeability and mechanical properties of films through Tukey’s mean comparison test.

The incorporation of different lipid fractions decreased the WVP [WVP, (eqn 3)] as compared with pure chitosan film (Table 2). The results showed that the addition of refined fish oil or oleic acid resulted in a higher reduction in WVP than the addition of refined rice oil or stearic acid. For the film containing fatty acids, WVP decreased with an increase in the unsaturation degree of lipids.

The low transfer of water vapour between the polymer chains of the composite (chitosan–refined oil) films may be due to low interaction of highly polar molecules such as hydroxyl fatty alcohols and carboxyl groups of fatty acids present in the oil (Table 1). In this sense, the obtained results indicate that the predominance of long-chain lipid molecules, especially EPA and DHA, may have been responsible for the higher resistance to water vapour diffusion of the chitosan–fish oil composite films, where EPA + DHA contents in fish and rice oils were 5.3% and 0.1%, respectively (Table 1). Hence, molecules of the polar portion may have had little influence on the matrix of long-chain hydrocarbons, and the water migrated preferentially through the continuous matrix (hydrophilic) and the dispersed lipid phase, increasing the tortuosity in the polymer chains and decreasing the diffusion of water vapour (Shellhammer & Rhim, 2005).

Oleic acid has a certain degree of mobility because of its double bond, which might result in reducing WVP properties (Fabra et al., 2008; Monedero et al., 2009). Despite the high amount of oleic acid present in refined rice oil (Table 1), the films containing pure oleic acid (CH-OA) had lower values of WVP than those that contained rice oil (CH-RRO). The results of CH-RRO films could be due to the development of particular interactions of oleic acid with chitosan matrices, which did not increase the hydrophobicity of the matrix when the refined rice oil was incorporated.

Elongation percentage ($E$) at break point and tensile strength ($TS$) values of pure chitosan (CH) film (Table 2) are in the range found by the literature, around 40 MPa (Srinivasa et al., 2007) and 20% (Vargas et al., 2009) for $E$ and $TS$, respectively. The addition of the different lipid fractions decreased $TS$ and $E$ values, and similar results were found by Srinivasa et al. (2007) and Vargas et al. (2009), who added saturated fatty acids (palmitic and stearic acids) and unsaturated fatty acids (oleic acid) to a chitosan matrix, respectively, and by Bourtoom & Chinnan (2009) through the incorporation of oleic acid, palm oil or margarine to a chitosan–starch matrix. The lower tensile strength of composite films can be attributed to a reduction in proximity and interaction between the polymers chains, caused by the surfactant addition. According to Bourtoom & Chinnan (2009), the lower elongation is the consequence of a reduction in interaction between the lipid molecules (nonpolar) and carbohydrates (polar).

Films obtained with refined rice oil showed higher elongation compared with those obtained with fatty acids or refined fish oil. The high amount of oleic acid in the oils (Table 1), especially the refined rice oil, caused a plasticising effect of the films (Monedero et al., 2009). If plasticisation does occur, the tensile strength should decrease and at the same time the elongation increases. However, it was not the case when the elongation value of the chitosan-only film is compared with that of the composites. It may be a case of phase separation caused by incompatibility. This can be deduced from imaging techniques such as scanning electron microscopy, where surface microstructure was observed for films. According to the classification established by Krochta & De Mulder-Johnston (1997) for biopolymer films, the mechanical attributes obtained by the composite chitosan and lipid films developed in this study can be considered as poor, compared with synthetic films.

FT-IR spectroscopy was used to examine the interactions between chitosan and refined fish oil. The infrared

<table>
<thead>
<tr>
<th>Biopolymer films*</th>
<th>WVP (g mm m$^{-2}$ day$^{-1}$ kPa$^{-1}$)</th>
<th>Tensile strength (MPa)</th>
<th>Elongation at break point (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH</td>
<td>3.80 ± 0.03*</td>
<td>33.0 ± 0.4a</td>
<td>18.0 ± 0.6a</td>
</tr>
<tr>
<td>CH-SA</td>
<td>1.80 ± 0.01b</td>
<td>19.0 ± 0.4b</td>
<td>7.0 ± 0.4b</td>
</tr>
<tr>
<td>CH-OA</td>
<td>1.41 ± 0.03c</td>
<td>16.0 ± 0.5c</td>
<td>12.0 ± 0.7c</td>
</tr>
<tr>
<td>CH-RRO</td>
<td>1.53 ± 0.02d</td>
<td>14 ± 1d</td>
<td>13.0 ± 0.1d</td>
</tr>
<tr>
<td>CH-RFO</td>
<td>1.32 ± 0.06*)</td>
<td>7.1 ± 0.5*</td>
<td>8.5 ± 0.1*</td>
</tr>
</tbody>
</table>

Mean values ± standard error (in replicate). Different superscripts letters in the same column indicate significant differences ($P \leq 0.05$) between the properties of the biopolymer films.

*CH, pure chitosan; CH-SA, chitosan–stearic acid; CH-OA, chitosan–oleic acid; CH-RRO, chitosan–refined rice oil; CH-RFO, chitosan–refined fish oil films; WVP, water vapour permeability.

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Figure 1 Infrared spectra of film: (a) pure chitosan film (CH); (b) chitosan–refined fish oil composite film (CH-FRO).

Figure 2 Scanning electron microscope micrographs of films. (a) CH, pure chitosan; (b) CH-OA, chitosan–oleic acid; (c) CH-RFO, chitosan–refined fish oil; (d) CH-RRO, chitosan–refined rice oil; (e) CH-SA, chitosan–stearic acid film.
spectra of pure chitosan and chitosan–refined fish oil composite film are presented in Fig. 1.

The IR spectra of pure chitosan film showed peaks at 3292 cm⁻¹ of OH stretching, which overlaps the NH stretching in the same region. The peaks at 2877, 2972 and 2981 cm⁻¹ are related to the symmetric and asymmetric axial deformation of CH (CH₃ and CH₂). The band at 1560 cm⁻¹ was the NH bending (amide II). A small peak near 1415 cm⁻¹ was because of C=O stretching (amide I), and a peak at 1707 cm⁻¹ suggested the presence of a carbonyl group in the film (Xu et al., 2005). In the spectrum of chitosan–refined fish oil composite film, the amino peak of chitosan has not moved with the addition of oil. Unlike the work of Xu et al. (2005) that prepared composite films, this result indicated that interactions were not present between the hydroxyl groups of fatty acids present in oil and the amino groups of chitosan. Peaks at 1535–1251 cm⁻¹ refer to the predominance of unsaturation present in the refined oil. The peaks at 2924 and 2852 cm⁻¹ correspond to CH₃ and CH, respectively.

Scanning electron microscope micrographs of CH film (Fig. 2a) show increased surface irregularity in the film with the incorporation of lipids. However, CH-OA (Fig. 2b) and refined fish oil (CH-RFO) (Fig. 2c) appeared to be well incorporated and embedded in the chitosan matrix, resulting in a relatively smooth and continuous surface, whereas the films with CH-RRO (Fig. 2d) and stearic acid (CH-SA) (Fig. 2e) had a highly irregular surface, suggesting a phase separation because of incompatibility. The differences in surface structure may have contributed to some extent to the differences in properties (mechanical and WVP) of the two films. Aggregation was probably more difficult for formulation with refined rice oil or stearic acid because of the phase separation or higher surface tension of liquid droplets. This separation could explain the higher WVP (as compared with pure chitosan and refined fish oil films) of the films because of preferential paths for water vapour diffusion and the low TS (as compared with pure chitosan film) for structure discontinuities (Table 2).

Conclusions

Film-forming dispersions were obtained by dissolution at room temperature (chitosan/lipid ratio of 4:1), and it was emulsified at 10 000 rpm for 4 min with pH adjusted to 5.0. Results showed that the incorporation of different lipid fractions (fish oil, vegetable oil, saturated fatty acid and unsaturated fatty acid) decreased the WVP (from 50% to 65%) as compared with pure chitosan film. Addition of refined fish oil to the continuous matrix of the films resulted in a higher reduction in WVP (WVP) than the addition of refined rice oil, oleic acid or stearic acid. However, the films with different lipid fractions showed lower mechanical properties, tensile strength and elongation percentage at break point (7–19 MPa and 7–13%, respectively), than the pure chitosan film (33 MPa and 18%). These results suggest that the resulting films mainly of chitosan–fish oil may be classified as packaging material for foods.

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References


