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Structural properties of films and rheology of film-forming solutions based on chitosan and chitosan-starch blend enriched with murta leaf extract

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ABSTRACT

Chitosan (CH) and chitosan-corn starch (CH-CS) film-forming solutions (FFS) and films, with or without polyphenol-rich aqueous extract from murta (*Ugni molinae* Turcz) leaves (PEML) were prepared. The impact of the FFS type and PEML, considering pH values of the FFS, on dynamic and steady-shear behavior of FFS, interaction mechanisms of PEML with polymer chains and changes on infrared spectra of films were investigated. Mechanical properties, thickness and color from films were also evaluated.

Blending CH with PEML produced huge aggregates that were visible to the naked eye. Rheological parameters, *K* and *n*, were affected significantly by the FFS type and the PEML presence. Viscosity of both FFS increased with the addition of PEML, leading a gel-like structure and thixotropic behavior. Sol–gel transition occurred when PEML was added to FFS, increasing strongly their elasticity.

The addition of PEML to CH-CS blend film leads to a reduction ($p < 0.05$) of elongation at break and tensile strength, increasing its thickness and yellow color. The PEML formed electrostatic interactions with chitosan. Ester linkages and hydrogen bonds were also formed between PEML and both chitosan and starch blends.

1. Introduction

Hydrocolloids, such as chitosan and starch, are biodegradable and non-toxic biopolymers based on renewable resources, used as coatings and films in food and pharmaceutical applications. The cationic character of chitosan offers an opportunity to establish electrostatic interactions with other compounds, depending -on the pH and -on the acid type used to dissolve it (Alvarado et al., 2007). Due to these characteristics, chitosan has been widely used for the production of edible films with adequate barrier to water vapor (Aider, 2010; Alvarado et al., 2007; Rivero, García, & Pinnoti, 2010). However, considering the cost of chitosan preparation, it seems economically feasible to combine it with other film forming biopolymers (Aider, 2010). One of them, starch, is the most common carbohydrate in the human diet. Blending two different hydrocolloids can strongly change both the physical and rheological properties of the composite film-forming solutions (FFS), affecting the functionality of the resulting coatings and films. These changes occur due to the compatibility/incompatibility between both macromolecules, which depend on their molecular weight, chemical structure, pH, conformation and hydration behavior among others.

Incorporation of antioxidant and/or antimicrobial compounds into films or coating materials can provide food protection against oxidation, microorganism growth, enzymatic browning and vitamin losses (Bonilla, Atarés, Vargas, & Chiralt, 2012). In previous works, the polyphenol-rich aqueous extracts from murta leaves have been shown to induce antioxidant capacity to fish gelatin films (Gómez-Guillén, Ihl, Bifani, Silva, & Montero, 2007) and also to carboxymethylcellulose-montmorillonite nanocomposite films (Quilaqueo Gutiérrez, Echeverría, Ihl, Bifani, & Mauri, 2012). The composition of polyphenol-rich aqueous extract could modify the physical properties and chemical structure of films and coatings. The effect of those additives on the film properties will depend on their chemical structure, concentration, dispersion degree in the film and interaction with the polymers (Kester & Fennema, 1986).

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Murta or murtilla *(Ugni molinae* Turcz) is an endemic shrub in central-southern Chile, which belongs to the Myrtaceae family. Aqueous extracts from *murta* leaves showed high antioxidant activity *in vitro* (Rubilar et al., 2005) and decreased the growth of *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Staphylococcus aureus* (Shene et al., 2009). Phenol acids like gallic acid, as well as flavonoid aglycones and glycosides from quercetin, myricetin and kaempferol are among the main compounds found in those extracts (Bifani et al., 2007; Rubilar et al., 2005).

The rheological properties of biopolymer solutions can affect spreadability, thickness and uniformity of liquid coating layer and film performance. A reduction in the solution's viscosity provides a processing advantage during high shear processing operations, such as pumping, filling and spraying application; whereas high apparent viscosity at low shear rates provides a desirable mouth-feel during mastication and a better application of FFS by dipping (García et al., 2009, pp.169).

In this context, the purpose of the present work was to investigate the effect of the polyphenol-rich aqueous extract from murta leaves (PEML) on the dynamic and steady-shear rheological behavior of FFS based on chitosan and chitosan-starch blend, and on chemical structure, morphology and physical properties of the resulting films.

2. Materials and methods

2.1. Materials

Fresh murta leaves (*U. molinae* Turcz) of ecotype 27-1 were sampled near Temuco, Chile (38°35′39″ South latitude) at the Instituto de Investigaciones Agropecuarias, INIA Carillanca. Corn starch (CS), containing 27% amylose, and medium molecular weight chitosan (CH), 190–310 kDa, with a deacetylation degree (DD) of 75%, were purchased from Sigma–Aldrich Co. Glacial acetic acid (CH_3COOH, 98% purity, Merck) and glycerol ($CH_2OH–CHOH–CH_2OH$, 87% purity, Merck) were also used for obtaining FFS.

2.2. Obtaining and characterization of PEML

Murta leaf samples were air-dried for 48 h to about 7% moisture content at 35 °C. Dried leaves were milled and the leaf powder (10 g) was macerated with distilled water (ratio = 1:10) at 170 rpm for 90 min at 25 °C. The mixture was filtered (Whatman N° 1 paper) and sterilized through a membrane PES of 0.22 μm to obtain the polyphenol-rich aqueous extract from murta leaves (PEML). The PEML showed 1.4% solids and 0.98 g/ml density. Antioxidant capacity, expressed as the PEML concentration required to scavenge 50% of $ABTS^{\cdot+}$ (2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonate) free radical, was 0.038 mg gallic acid equivalent (GAE)/mL, and the total phenol content was 40.67 mg GAE/g d.m. murta leaves.

2.3. Preparation of film-forming solutions (FFS)

The concentration of components in each FFS for hydrocolloid blend formulation is shown in Table 1. *Starch-based FFS*: Aqueous solution from CS (0.5% w/w) was prepared by heating beyond their gelatinization temperature (70 ± 5 °C) for 20 min under gentle magnetic stirring, and cooled until 25 °C at a rate of 2–3 °C/min. *Chitosan-based FFS:* Best chitosan films were prepared from 2% chitosan in 1% acetic acid, as described before (Siripatrawan & Harte, 2010). Chitosan was dispersed in acetic acid (1% v/v) to prepare solutions of 1.5 and 2% w/w. This dispersion was mechanically stirred at 500 rpm for 2 h (Ultra-Turrax T25, Janke & Kunkel, Germany), and filtered through cheese-cloth. The incorporation of PEML was 40% of the dissolution solvent of CH solution.

Table 1
Composition of film-forming solutions, with or without polyphenol-rich aqueous extract from murta leaves (PEML).

Hydrocolloid				Glycerol	PEML	
FFS	[% w/w]			[g/g H_T]	[mL/g H_T]	
$H_1–H_2$	H_1	H_2	H_T[a]		Without	With[b]
CH	2.00	–	2.00	0.25	0	20
CH-CS	1.50	0.50	2.00	0.25	0	20

[a] H_T: Total concentration of hydrocolloids in solution.
[b] Total phenol content from PEML expressed as gallic acid equivalent (GAE): 81.33 mg GAE/g H_T.

Considering the results obtained before (Bifani et al., 2007) in relation to the reduction in the oxygen permeability of films obtained, after incorporation of 40 mL of aqueous extract of murta leaves in 100 mL of film-forming solution of carboxymethylcellulose. *Chitosan-starch blend FFS:* The concentration of total hydrocolloids in solution (H_T) was maintained at a constant 2% w/w value. CH-CS blend FFS was prepared by mixing the CH and CS solutions shown above, with or without added PEML (20 mL/g H_T, 81.33 mg GAE/g H_T). The addition of 25% w/w glycerol was reproduced from reported formulations of films based on chitosan and chitosan-starch films (Mathew, Brahmakumar, & Abraham, 2006; Mayachiew & Devahastin, 2010) and a gentle stirring of the solution at controlled conditions for 30 min at 45 °C was performed. Vacuum was applied to remove air bubble formation in blend FFS. The pH value of blend FFS was determined at 23 °C. Samples for rheological analysis were stored at 5 °C for 3 days, until use.

2.4. Rheological measurements: Fundamentals and methods

Dynamic viscoelasticity and steady state flow measurements were carried out in a controlled-stress rheometer (Bohlin CVO Instruments, Inc. Grandbury, NJ) with a cone-plate geometry (cone angle 4°, diameter = 40 mm, gap = 150 μm). Before analysis, the sample was placed into the rheometer, which was equilibrated at 25 °C (Gómez-Guillén et al., 2007).

2.4.1. Steady shear measurements

Flow curves and thixotropic properties were obtained by registering the shear rate when shear stress was increased from 0 to 250 Pa and decreased from 250 to 0 Pa, at 25 °C. Experimental data were fitted to Ostwald–de Waele model or rheological Power law model according to Equation (1).

$$\tau = K\dot{\gamma}^n \quad (1)$$

where τ is shear stress (Pa), K is consistency index (Pa s), n is flow index (–) and $\dot{\gamma}$ is shear rate (s^{-1}).

The changes in the solution's apparent viscosity or steady-shear viscosity (η, Pa s), were investigated according to Equation (2). Three repetitions were performed for each sample.

$$\eta_{(\dot{\gamma})} = K\dot{\gamma}^{n-1} \quad (2)$$

2.4.2. Dynamic measurements of viscoelastic properties

Three dynamic studies were performed: (a) An oscillatory stress sweep test from 0.03 to 400 Pa, at a constant frequency of 0.1 Hz and 25 °C was made to set the upper limit of the linear viscoelastic region (LVR). (b) Frequency sweep over a range of 0.01–50 Hz at 25 °C was performed at an oscillatory stress within LVR for each solution. Viscoelastic parameters, storage or elastic modulus (G', Pa), loss or viscous modulus (G'', Pa), complex modulus (G^*, Pa), complex viscosity (η^*, Pa s) and tangent of the phase angle (tan