



Carboxymethyl cellulose-based films: Effect of organosolv lignin incorporation on physicochemical and antioxidant properties

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ABSTRACT

Organosolv lignin was incorporated in carboxymethyl cellulose (CMC)-based films as a reinforcing and bioactive agent. Films were produced by the casting method using compatible and environmentally friendly solvents. The incorporation of lignin in CMC-based films was evaluated in term of morphology, physicochemical, barrier, mechanical and antioxidant properties. Solubility tests showed that lignin improved the water resistance of the films for approx. 60%. This behavior was also observed for the water vapor permeability, achieving a 20% reduction. Morphological characteristics suggested low compatibility between organosolv lignin and CMC matrix, revealed by the small aggregates homogeneously distributed in the film, through scanning electron microscopy, related to the self-assembly behavior of lignin. CMC/lignin-based films also presented higher thermal stability. Fourier transform infrared spectroscopy showed that incorporation of lignin led to small changes in the film's structure. Finally, organosolv lignin proved to be a promising material for the development of active CMC-based films due to its demonstrated antioxidant activity that can be useful to pack food products that suffer from undesirable oxidation reactions.

1. Introduction

The worldwide interest in bio-based polymers has increased due to the need to reduce fossil fuels dependence. Thus, there is a tendency to substitute petrochemical-based polymers by bio-based ones that are easily bio-degraded and less likely to cause environmental pollution. Several bio-based polymers have shown to be promising for use as films and coatings in food packaging applications, such as alginate, chitosan, starch, cellulose derivatives and gelatin (Ballesteros et al., 2018).

Among the various biopolymers, carboxymethyl cellulose (CMC) is commonly used as a food additive, but also in pharmaceutical applications. It is a water-soluble polysaccharide composed of 1,4- β -D-glucan monomers, in which hydroxyl groups are partly substituted by CH₂COOH groups (Shahbazi et al., 2016). Concerning packaging, CMC has been considered as a promising biopolymer to replace non-degradable polymer in some applications (Shahbazi et al., 2016; Sonkaew et al., 2012). It may be associated with other ions that will influence its physicochemical properties. For example, sodium CMC shows a high hydrophilic capacity, absorbing more than 50% in weight

of water (Britto and Assis, 2009). In fact, CMC-based films, as almost all biodegradable films, have low water resistance and present weak water vapor barrier properties, that make them frequently non-competitive when compared with other non-biodegradable polymers (Shahbazi et al., 2016). In this context, CMC-based films have been filled with other polymers aiming to improve these properties (Ibrahim and El Salmawi, 2013; Oun and Rhim, 2017).

Lignin is the second most abundant natural renewable biopolymer (Hambardzumyan et al., 2015). It is expected that the amount of commercially available lignin increases due to the new advances in biorefineries. Currently, it has been obtained mainly by sulfite and kraft pulping. The problem is that these lignins present sulfur chemically bonded to them that is difficult to remove, precluding their use in high value-added applications (Wörmeyer et al., 2011). Alternatively, organosolv lignin is characterized by its high purity (low inorganic impurities and sulfur-free) and low molecular weight (Mw), making it attractive for applications in bio-based chemicals and materials (Michelin et al., 2018). It presents several properties, such as biodegradability, high thermal stability, antioxidant activity, UV blocking and

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antimicrobial properties, hydrophobicity, adhesive and other properties (Naseem et al., 2016). Due to its high availability, renewable and biodegradable character, lignin has been incorporated as filler in different polymeric matrices to enhance the film properties (Crouvisier-Urien et al., 2017; Gordobil et al., 2015; Núñez-Flores et al., 2012). It has been shown to improve water resistance, UV blocking properties, thermal stability, processability and mechanical strength of bio-based films. In addition, lignin plays an important role in antioxidant properties as a stabilizer because the phenolic hydroxyl groups can scavenge free radicals (Ballesteros et al., 2018).

Several works have incorporated water soluble lignin, such as lignosulphonates and alkali lignins in different biopolymers (Aadil et al., 2016a; Aadil et al., 2016b; Núñez-Flores et al., 2012, 2013; Bhat et al., 2013; Miranda et al., 2015; Shankar et al., 2015; Crouvisier-Urien et al., 2016, 2017). Aadil and co-authors (2016a,b) reported that alkali lignin reduced the water affinity of alginate and gelatin films, respectively. Similarly, incorporation of alkali lignin in starch films significantly reduced the water vapor permeability and solubility, and improved the thermo-mechanical properties (Bhat et al., 2013). Additionally, alkali lignin/agar-based films exhibited higher UV and mechanical properties and also lower water vapor permeability (Shankar et al., 2015).

On the other hand, few studies have reported the use of organosolv lignin with water soluble polymers due to its insolubility in water. To overcome this issue, these studies have used a mixture of solvents as a strategy, but the use of non-environmental friendly solvents, high price and complex multistep processes can be some of the disadvantages. For instance, Alzagameem et al. (2019) have studied the antimicrobial and antioxidant capacity of composite films based on organosolv lignin and HPMC (hydroxypropylmethylcellulose) using the casting method for the film production and solvents such as DMSO, HCl and ethanol. Guo et al. (2019) have assessed the antioxidant potential and UV-shielding performance of cellulose/technical lignins composite films (produced by the casting method), including organosolv lignin, in what avicel® PH-101 cellulose was activated with methanol and DMAc and dissolved at DMAc/LiCl solvent system. Hambardzumyan et al. (2015) produced films based on organosolv lignin and cellulose nanocrystals, using Fenton's reagent to improve the grafting process of lignin onto the surface of cellulose nanocrystals. They evaluate the antioxidant properties, UV-Vis properties and water sensitivity. It is clear from these studies that a mixture of solvents can be used to produce composite based on organosolv lignin and biopolymers, but can also be seen that the combination can be improved in terms of solvent and biopolymer. Besides, the effect of the incorporation of organosolv lignin in bio-based films is unknown for their physical properties since most of these works have reported mainly the bioactive properties of the composite films.

To address this issue, organosolv lignin was incorporated in the CMC polymer, since the CMC can be solubilized in water-miscible solvents, such as ethanol (up to 50%), avoiding issues related to the polymers compatibility in the mixture, solvent toxicity and complexity of the processes. To the best of the authors' knowledge, never organosolv lignin was used in combination with CMC for the production of films. In this context, the aim of this work was to incorporate organosolv lignin (an industrial by-product) in CMC-based films aiming their reinforcement (better mechanical properties), as well as improved barrier properties and lower solubility, including increased functionality (antioxidant capacity) using an 50% ethanol solution. For that the developed CMC-based films without and with the incorporation of organosolv lignin were evaluated in terms of films' morphology, physicochemical, barrier and antioxidant properties.

2. Materials and methods

2.1. Materials

Lignin from corncob with 92% purity was obtained after sequential liquid hot water (200 °C, 30 min) and ethanol organosolv (140 °C, 40

min, 60% EtOH) pretreatments according to Michelin et al. (2018). Sodium carboxymethylcellulose – CMC (Blanose, 7M65) was obtained from Ashland Inc (Düsseldorf, Germany); Glycerol (99.95%) from Fisher Chemical; Ethanol (99.8%) from Sigma-Aldrich. All other reagents were of analytical grade.

2.2. Preparation of films

CMC aqueous solution was prepared by dissolving 2% (w/w) CMC in an ethanolic solution (50%, v/v), under stirring at 200 rpm, overnight, at room temperature (22 ± 2 °C). A concentration of 0.5% (v/v) of glycerol was added in the solution. Lignin solution was obtained by dispersing 0.25%, 0.5% and 1% (w/v) ethanol organosolv lignin (EOL) in an ethanolic solution (50%, v/v), under stirring at 200 rpm, overnight, at room temperature. Finally, the film-forming solutions were obtained by mixing these two solutions (CMC and lignin, 1:1, v/v) at 200 rpm, during 1 h at 80 °C to obtain a water/ethanol ratio of 50:50 (v/v). Although CMC is a water-soluble polymer, it can be solubilized in water-miscible solvents, such as ethanol (up to 50%) allowing to overcome compatibility-related issues with polymers soluble in organic solvents, such as organosolv lignin. The final concentration of EOL in the film was 12.5%, 25% and 50% $w_{\text{lignin}}/w_{\text{CMC}}$. Neat CMC film (without lignin) was also prepared as control.

To produce the films, 28 mL of this film-forming solution was cast onto a Petri dish (9.5 cm diameter) and dried initially at room temperature for 24 h and after that at 35 °C for plus 24 h. Films were peeled off from the Petri dish and stored inside a desiccator at room temperature and 54% relative humidity (RH) at least 24 h before analysis. This RH was achieved by using a saturated salt solution of $\text{Mg}(\text{NO}_3)_2$ in a desiccator.

2.3. Color and opacity of the films

A Minolta colorimeter (CR 400; Minolta, Japan) was employed to the color measurements of the films, as reported by Martins et al. (2012). For that, it was used a white color plate as a standard for calibration and as a background. L^* , a^* , b^* values of the film were determined through reflectance measurements. The Hunterlab method was used to determine the film opacity, through the relationship of the film opacity on a black standard and a white standard. Three replicates were carried out with ten measurements each.

2.4. Scanning electron microscopy (SEM)

The surface morphology of the films was examined by SEM (Quanta FEG 650, FEI, USA). Films were affixed on aluminium stubs using carbon adhesive tape and sputter-coated with gold. An acceleration voltage of 5 kV at different magnifications was applied to obtain the images.

2.5. Fourier-transform infrared (FTIR) spectroscopy

Chemical groups and bonding arrangement of constituents of the films were determined by FTIR spectroscopy. Bruker FT-IR VERTEX 80/80v (Boston, USA) in Attenuated Total Reflectance mode (ATR) with a platinum crystal was used to obtain the FTIR spectra. The measurements were recorded from 4000 to 400 cm^{-1} wavenumber range, at a resolution of 4 cm^{-1} and 16 scans. The analysis was performed in duplicate.

2.6. Moisture content and water solubility

Moisture content measurement of the films was performed by gravimetric analysis, placing approximately 20 mg of film at 105 °C until constant weight. After that, the moisture content (expressed as percentage) was determined through the weight loss of the films.

For water solubility determination, 2 cm diameter of dried films (moisture-free) were weighed and subsequently immersed, under

constant agitation (150 rpm), into 50 mL of distilled water for 24 h at room temperature. After that, the undissolved films were withdrawn and dried at 105 °C until constant weight. The water solubility of the films (expressed as percentage) was calculated based on the difference in weight between the dry matter of non-solubilized films after 24 h of dissolution and the films before immersion. Both analyses were performed in triplicate.

2.7. Water vapor permeability

Water vapor permeability (WVP) was determined gravimetrically according to ASTM E96-95 method (Guillard et al., 2003). Briefly, the top of permeation cells containing 50 mL distilled water (at 100% RH; 2.337×10^3 Pa vapor pressure at 20 °C) were sealed by the films and then placed in a desiccator with silica gel (0% RH and 0 Pa water vapor pressure at 20 °C). The cells were periodically weighted (every 2 h) and the weight loss was measured over time until steady state was reached (approx. 10 h). Water vapor transmission rate (WVTR) was obtained through the ratio between the slope of the linear regression of weight loss versus time and the area of the film, and WVP ($\text{g m}^{-1} \text{s}^{-1} \text{Pa}^{-1}$) was calculated according to equation (1):

$$\text{WVP} = \frac{(\text{WVTR} \times L)}{\Delta P} \quad 1$$

where WVTR is the water vapor transmission rate ($\text{g m}^{-2} \text{s}^{-1}$) through the film, L is the mean film thickness (m), and ΔP is the partial water vapor pressure difference (Pa) across the two sides of the film. All measurements were performed in triplicate.

2.8. Contact angle

Optical contact angle meter (OCA 20, Dataphysics, Germany) was used to measure the contact angle (θ) of the film surface, through the sessile drop method (Newman and Kwok, 1999). A 2 μL droplet of ultra pure water was placed on the film surface using a 500 μL syringe (Hamilton, Switzerland) and a needle (0.75 mm diameter). Measurements were performed at 1 s. Three replicates were performed with at least seven measurements each at 22.4 °C. Circle (height/width) fitting method was used to determine the contact angle.

2.9. Thermal analyses

Thermogravimetric analyses (TGA) were performed using a TGA 4000 equipment (PerkinElmer, Waltham, Massachusetts, EUA). Approximately 5 mg of the film sample were placed into an aluminum pan and heated from 20 °C up to 800 °C at a rate of 10 °C per min under nitrogen atmosphere. Analyses were performed in duplicate.

Differential scanning calorimetry (DSC) analyses were performed with a PerkinElmer DSC 6000 (PerkinElmer, Waltham, Massachusetts, EUA) calibrated with Indium as standard. Approximately 5 mg of the film sample were placed into an aluminum DSC pan and sealed with the aluminium covers (PerkinElmer, DSC, B0143016/B0143003, respectively). The measurements were performed from 20 °C to -45 °C and from -45 °C to 250 °C at a heating rate of 10 °C per min under a nitrogen atmosphere. Analyses were performed in triplicate.

2.10. Mechanical properties

Texture analyzer (TA.HD plus, Stable Micro Systems, United Kingdom), with the software Exponent, was employed for the mechanical analyses of the films, according to ASTM D882-10 methodology. Film samples (70 mm \times 20 mm) were trapped between claws with an initial distance of 50 mm. The force and deformation were recorded during extension at 50 mm min^{-1} . Tensile strength (TS, MPa), elongation-at-break (EB, %), and Young's modulus (YM, MPa) were

determined from stress-strain curves. At least six measurements were performed for each film.

2.11. DPPH radical scavenging assay

The radical scavenging activity (RSA) of films was determined using the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH), according to the modified method of Blois (1958). Briefly, 10 and 100 mg of films were placed into 15 mL Falcon flasks and 10 mL DPPH in ethanol at a concentration of 50 mg/L were added. The flasks were stirred (150 rpm) for until 24 h at room temperature in the dark and the absorbance was determined at 515 nm. Control corresponded to DPPH solution without the presence of the film. A sample control (film samples in ethanol) was also performed due to the dark color of lignin. The absorbance of this control was discounted of the absorbance of the sample. The radical scavenging activity (RSA) of the lignin films was calculated according to equation (2):

$$\text{RSA (\%)} = 1 - \left(\frac{A_{\text{sample}}}{A_{\text{control}}} \right) \times 100 \quad 2$$

where A_{sample} is the absorbance measured for the DPPH solution containing the film samples and A_{control} is the absorbance of DPPH solution without the addition of the film. Analyses were performed in duplicate.

2.12. Statistical analysis

Statistical analysis was performed using VassarStats website. One-way analysis of variance and Tukey test were performed to determine the significant differences. Significance was defined at a level of $p < 0.05$.

3. Results and discussion

3.1. Color and opacity

CMC-based films showed a visually homogeneous lignin distribution into the film (Fig. 1), but also an intense brown color that significantly influenced the optical properties of films. Although the CMC-based film is transparent, the incorporation of lignin into the film led to a change in the film's color from transparent to brown. Lignin itself had an intense brown color, and when it is dissolved in a solvent, the solution color became dark as lignin concentration increases. Thus, the concentration of lignin had a significant influence on the color of CMC-based films. This reduction in the transparency (or increase in the opacity) of the films by the incorporation of lignin is mainly due to the chromophore nature of lignin, which presents high capacity for UV-shielding (Ban et al., 2007). From the consumer's perspective, darkening of films and reduced brightness can be an issue, since most of the consumer prefer transparent packaging, however for some products (e.g. cheese and minimally processed vegetables) where the degradation by light is an issue, suffering from lipid oxidation or color change, this opacity can be an advantage (Dalsgaard et al., 2010; Zappia et al., 2018).

The color results of the produced films are presented in Table 1. Results showed that L^* value (lightness) markedly decreased with the increase of lignin concentration, while a^* and b^* values present higher values in relation to ones obtained for the neat CMC-based films. The increasing a^* values indicates more reddish films, while increasing b^* values indicates a more yellow color of the films. Similar results were observed by Kovalcik et al. (2015) for PHBHV/lignin films. It is important to highlight that the PHBHV film with 10 wt% lignin presented similar color to that of CMC-based film with 50 wt% lignin (current work). This can be related to the type of lignin, since the current work used organosolv lignin that is a lighter brown than the kraft lignin used by Kovalcik and co-authors.

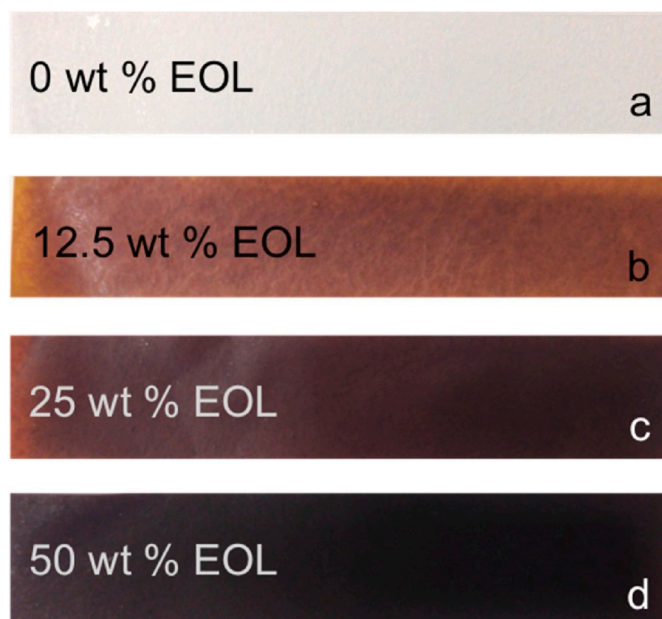


Fig. 1. CMC-based films images with (a) 0 wt% EOL, (b) 12.5 wt% EOL, (c) 25 wt% EOL, and (d) 50 wt% EOL.

3.2. Microstructure and morphology

The films produced with different lignin ratios were visually homogeneous, flexible, easily handled and non-sticky. SEM images showed the surface microstructure of the films and how lignin dispersed in the CMC matrix (Fig. 2). The images showed clear differences between films with and without lignin, where the CMC/lignin-based films (Fig. 2b–d) showed higher roughness (aggregate structures) compared to the neat CMC-based films (Fig. 2a) that presented a smooth and compact surface. A similar observation was reported by Shankar et al. (2015) on agar/lignin films containing 10 wt% alkali lignin. The small aggregate structures on CMC/lignin-based films can be explained by the evaporation-induced self-assembly process (Brinker et al., 1999). In theory, lignin display self-assembly behavior due to its amphiphilic nature composed of hydrophobic aromatic rings and hydrophilic hydroxyl groups (Bartzoka et al., 2016), leading to the appearance of these structures. Lignin could be modified chemically, or a compatibilizer could be added to enhance its dispersibility in the polymer blends or compatibility with biopolymers (Kun and Pukánszky, 2017).

3.3. FTIR study

Fig. 3 shows the FTIR spectrum of CMC-based films from 4000 to 500 cm^{-1} . Results show that the incorporation of lignin in the films leads to a slight change in the structural characteristics of them. A new peak appeared at 1513 cm^{-1} due to the incorporation of lignin in the CMC-based films, that corresponds to aromatic skeletal vibrations of lignin (Faix, 1991). Regarding the other bands, no differences were observed between samples. It was observed a broad absorption band around 3265

cm^{-1} that is attributed to the stretching of the –OH group and a peak at 2924 cm^{-1} that is attributed to the C–H stretching. The presence of –COO^- , assigned to stretching of the carboxyl group, was confirmed by the band at 1590 cm^{-1} . The bands at 1415 cm^{-1} and 1320 cm^{-1} are assigned to –OH stretching in-plane and C–H stretching in symmetric of CMC, respectively. The band at 1024 cm^{-1} is due to CH–O–CH₂ stretching (Chai and Isa, 2013). Additionally, a small band at 920 cm^{-1} is presented, which is related to the presence of glycerol and corresponds to symmetric (C–O–C) stretching vibrations bands (Cerqueira et al., 2012). Plasticizers can decrease the strong intermolecular interactions of the molecules of lignin that leads to its poor miscibility with other polymers, resulting in improved lignin dispersion in the film. However, plasticizer's efficiency depends on the lignin structure (Kun and Pukánszky, 2017), which is related with the source (hard wood/soft wood/grass) and the extraction process (Thakur et al., 2014).

3.4. Moisture content and water solubility

Moisture content values of films are presented in Table 2. CMC-based films present a high moisture content percentage (30.7%), which can be related to their hydrophilic nature and with the presence of glycerol. The presence of plasticizers, such as glycerol, is one of the factors that most influence the water sensitivity on this kind of films, once they can form hydrogen bonding with water molecules (Da Silva et al., 2009). Their presence is important to guarantee the processability of the films (i.e. films without glycerol are brittle and difficult to handle) but they also increase the water affinity (due to the available –OH groups) and thus, normally, their presence (and increasing concentrations) lead to higher values of moisture content, solubility, water vapor permeability, as well as elongation at break for polysaccharide-based films (Cerqueira et al., 2012).

On the other hand, with the incorporation of lignin into CMC-based films the molecules of lignin can interact with free CMC hydrophilic groups via hydrogen and hydrophobic interaction, thereafter, leading to a decrease of water molecules adhesion (Aadil et al., 2016b). Table 2 shows that CMC/lignin-based films present lower moisture content values (20.4%–23.1%) than CMC films, thus affecting the way that the films are absorbing water molecules (films were stored at room temperature and controlled 54% RH). However, no effect ($p > 0.05$) on the moisture content was observed when the lignin's concentration increased from 12.5 to 50% (wt% EOL).

Regarding the solubility of the films, the incorporation of lignin into CMC-based film led to a pronounced decrease ($\approx 60\%$) in film solubility, from 57% (control) to 23.5% (50 wt% EOL) (Table 2). In this case, the lignin concentration had a significant influence ($p < 0.05$), considering that the solubility values obtained for CMC-based films with 50% EOL were lower than the ones obtained for 12.5% EOL content. The high water resistance of CMC/lignin-based films is explained by the high hydrophobicity and insolubility of EOL. High water solubility values are a sign of low water resistance, which is an important parameter for commercial applications; nevertheless, this is a case-by-case decision dependent on the purpose of the application.

Similar results were observed for starch/lignin films (Bhat et al., 2013), gelatin-lignosulfonate films (Núñez-Flores et al., 2012), gelatin/lignin films (Aadil et al., 2016a) and alginate/lignin films (Aadil et al.,

Table 1

Color parameters of CMC-based films.

Lignin concentration in CMC films ($w_{\text{lignin}}/w_{\text{CMC}}$, wt%)	L*	a*	b*	Opacity (%)
0 EOL	97.05 \pm 0.25 ^a	–0.09 \pm 0.05 ^a	2.67 \pm 0.12 ^a	4.81 \pm 1.44 ^a
12.5 EOL	33.60 \pm 1.13 ^b	20.59 \pm 0.85 ^b	17.98 \pm 1.94 ^b	62.14 \pm 5.53 ^b
25.0 EOL	26.68 \pm 0.81 ^c	12.62 \pm 1.74 ^c	8.74 \pm 0.97 ^c	88.55 \pm 4.99 ^c
50.0 EOL	22.79 \pm 0.54 ^d	6.18 \pm 0.40 ^d	5.36 \pm 0.39 ^d	100.63 \pm 2.69 ^d

Values reported are the mean \pm sd. Different letters (a–d) in the same column indicate a statistically significant difference ($p < 0.05$).

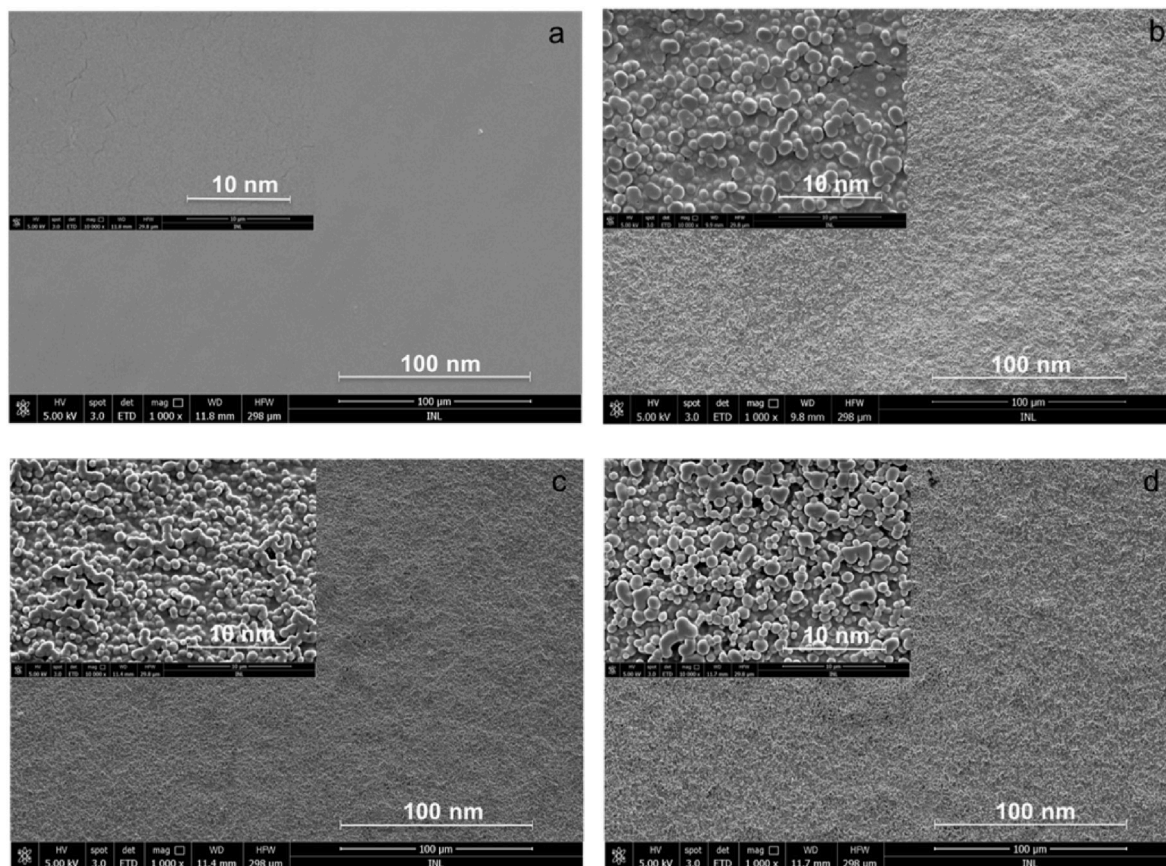


Fig. 2. SEM micrographs of CMC-based films with (a) 0 wt% EOL, (b) 12.5 wt% EOL, (c) 25 wt% EOL, and (d) 50 wt% EOL. Regular images were taken at 1000× magnification, and the insert images at 10,000× magnification.

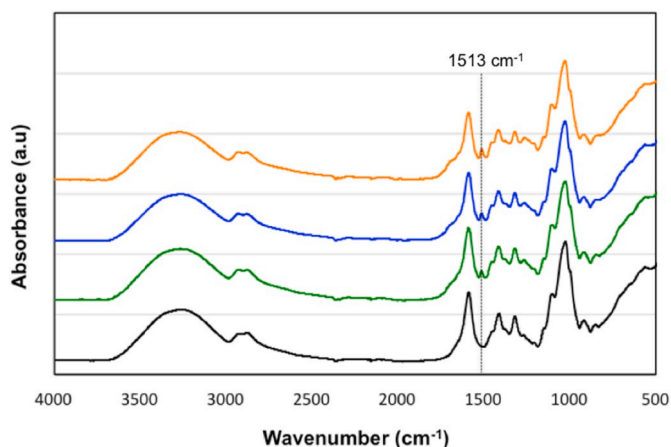


Fig. 3. FT-IR spectrum of CMC-based films with 0 wt% EOL (black line), 12.5 wt% EOL (green line), 25 wt% EOL (blue line), and 50 wt% EOL (orange line). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

2016b), being the film solubility approximately 33%, 41%, 65% and 45% lower, respectively, than the control films. On the other hand, Shankar et al. (2015) obtained agar/lignin films with higher solubility than agar film (control). The increase in lignin content increased the solubility, which might be related to the type of lignin, since the alkali lignin is highly soluble in water.

3.5. Water vapor permeability (WVP)

The results presented in Table 2 revealed that lignin affected the WVP values in the CMC-based films, which decreased with increasing lignin concentration. The reduction in WVP values of the CMC/lignin-based films is explained by the hydrophobicity of lignin, that works as a barrier in the polymeric matrix and increasing the tortuous path for the diffusion of water vapor, resulting in lower permeation of water molecule through the polymer film.

In fact, there is a concentration effect on the reduction of the WVP values, being observed that the values obtained for 25 and 50 wt% of EOL were lower than that for 0 and 12.5 wt% of EOL. In the case of the CMC-based films with 25 and 50 wt% of EOL, the reduction was around 20%. Similar results have been reported by Shankar et al. (2015) in

Table 2
Physical properties of CMC-based films.

Lignin concentration in CMC films ($w_{\text{lignin}}/w_{\text{CMC}}$, wt%)	Thickness (μm)	Moisture content (%)	Water solubility (%)	WVP $\times 10^{-10}$ ($\text{g m}^{-1} \text{s}^{-1} \text{Pa}^{-1}$)	Contact angle ($^\circ$)
0 EOL	41.77 ± 0.72^a	30.70 ± 0.85^a	57.04 ± 3.24^a	3.61 ± 0.29^a	40.96 ± 2.15^a
12.5 EOL	48.86 ± 0.74^b	21.05 ± 0.77^b	33.97 ± 1.16^b	3.30 ± 0.06^a	51.72 ± 3.43^b
25.0 EOL	50.96 ± 1.00^c	23.08 ± 1.10^b	28.68 ± 1.98^{bc}	2.85 ± 0.12^b	49.70 ± 3.74^b
50.0 EOL	67.92 ± 0.56^d	20.43 ± 1.62^b	23.55 ± 1.87^c	2.93 ± 0.11^b	50.89 ± 3.63^b

Values reported are the mean \pm sd. Different letters (a-d) in the same column indicate a statistically significant difference ($p < 0.05$).

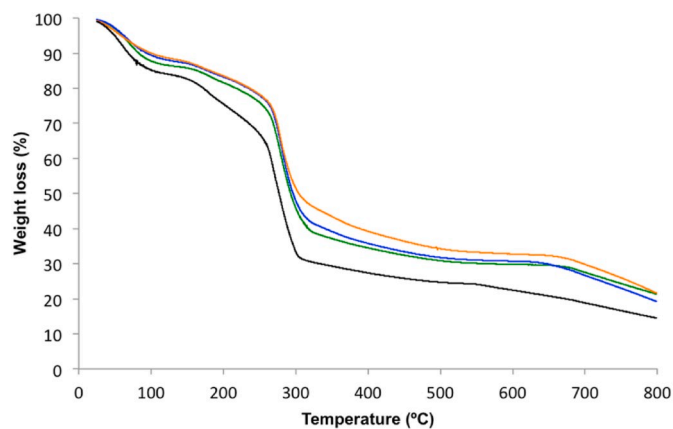


Fig. 4. TGA of CMC-based films. TG curve of CMC-based films with 0 wt% EOL (black line), 12.5 wt% EOL (green line), 25 wt% EOL (blue line), and 50 wt% EOL (orange line). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

agar/lignin films, with a reduction of 27.5%. On the other hand, other materials have been used to improve the barrier properties on CMC-based films, such as cellulose nanocrystals (CNC). For example, Oun and Rhim (2017) reported a reduction of 25% on WVP in CMC films reinforced with CNC, showing that lignin can reach similar reduction values to these promising nanomaterial.

3.6. Contact angle

The contact angle gives information regarding the surface hydrophobicity of a film. Results presented in Table 2 show that the incorporation of lignin in CMC-based films induced an increase of the water contact angle value (from 41° to 50–52°). Thus, lignin slightly improved the surface hydrophobicity of the films. This can be explained by the hydrophobic characteristics of the lignin but also can be related to the morphology of the surface. According to Crouvisier-Urión et al. (2017) higher values of contact angle may be related to a change in the surface hydrophobicity (surface chemistry) or surface roughness (surface physics) or both. In this case, the surface roughness is higher for films with lignin, as can be seen in Fig. 2, and as reported by Crouvisier-Urión et al. (2017) this showed to be strongly related to the homogenization process during film production.

3.7. Thermal behavior

Thermogravimetric analysis (TGA) is a process that decomposes the material by the heating, breaking the bonds within the molecule. It is an important methodology to determine the thermal stability of the materials. The thermal behavior of CMC-based films and CMC/lignin-based films are presented in Fig. 4.

TG curves showed a similar behavior, presenting 3 events of thermal degradation. The first event is related to moisture content, the second event is due to the glycerol degradation (Bhat et al., 2013; Cerqueira

et al., 2012) and the third event refers to the degradation of CMC and lignin. Since lignin degradation occurs slowly over a wide temperature range, its degradation curve overlaps the CMC degradation curve. This occurs because lignin presents a complex structure, that is composed of phenolic hydroxyl, carbonyl groups, and benzylic hydroxyl, that present different thermal stability. So, its degradation occurs in temperatures ranging from 200 to 500 °C where the weight of the sample is reduced ~50 wt% (Domínguez et al., 2008) On the other hand, CMC degradation occurs in a narrow temperature range (200–340 °C) (Li et al., 2018).

The addition of lignin to CMC-based films leads to an increase of the thermal stability of the material, as well as to a displacement of the third event to a higher degradation temperature (Table 3). At a temperature around 800 °C the CMC-based film (control) and CMC-based films with 12.5 wt%, 25 wt% and 50 wt% EOL are decomposed at 83.5%, 78.6%, 79.8% and 77.7%, respectively. Moreover, T_{onset} and T_{max} increase as the amounts of EOL increase. This is an indication that incorporation of lignin into CMC-based films can improve thermal stability. Similar results were observed by Spiridon et al. (2011) in starch/lignin films and according to them, can be assigned to the inter-component H-bonding, which may, in turn, improve the thermal stability of the films. Aadil et al. (2016a) also reported enhanced thermal stability of the lignin/gelatin films. On the other hand, the thermal stability of alginate films decreased with the incorporation of lignin (Aadil et al., 2016b).

No significant differences were observed in the T_g values of CMC-based films and CMC/lignin-based films, probably due to values close to T_g of CMC (El-Sayed et al., 2011) and this type of lignin (65 ± 0.36 °C). The T_g could have a significant influence in the films' properties, being related to the molecular mobility of the materials. In general, T_g varies widely according to the extraction method, molecular weight, water content, chemical modification, and thermal history of lignin (Laurichesse and Avérous, 2014). Overall, organosolv lignin presents low T_g and is easy to process thermally. It is a lignin of high purity, with a more oxidized structure and a lower molecular weight that contributes to its lower T_g (Gordobil et al., 2016). El-Zawawy et al. (2011) verified a decrease of T_g in the polymer matrix with the incorporation of lignin and attributed this behavior to the poor miscibility between the polymeric matrix and lignin. On the other hand, Núñez-Flores et al. (2012) reported that gelatin films incorporated with lignosulphonate (concentration as high as 30–35% of LS) presented an increase in the T_g values.

3.8. Mechanical properties

Mechanical properties of biopolymer films are an indication of the integrity of films under stress conditions. Some works have used lignin as filler in different matrices to improve the mechanical properties of the films, since lignin by itself is not able to form a film. They have reported improvement or no significant difference in some mechanical properties (Kovalcik et al., 2015; Shankar et al., 2015). In this work, the TS value obtained for CMC-based film incorporated with 50 wt% EOL was significantly ($p < 0.05$) reduced, however for films with lower lignin concentrations no significant difference was observed compared with the neat CMC-based film (Table 4).

The same behavior was observed for the EB, however, in this case, both 25 and 50 wt% of EOL led to films with lower values of EB. On the

Table 3
Thermal properties of CMC-based films.

Lignin concentration in CMC films ($w_{\text{lignin}}/w_{\text{CMC}}$, wt%)	T_g (°C)	T_{onset} (°C)	T_{max} (°C)	Weight loss (%)	Char ₈₀₀ (%)
0 EOL	67.2 ± 0.85	259.0 ± 0.67	271.0 ± 0.28	69.2 ± 0.14	16.5 ± 1.75
12.5 EOL	63.3 ± 1.84	261.9 ± 0.57	277.9 ± 2.04	61.1 ± 2.12	21.4 ± 0.02
25.0 EOL	64.6 ± 2.18	265.0 ± 0.21	279.8 ± 1.76	60.2 ± 0.71	20.2 ± 1.20
50.0 EOL	65.8 ± 1.42	267.5 ± 0.42	279.9 ± 0.42	55.7 ± 1.56	22.3 ± 0.92

Values reported are the mean ± sd. T_g is the glass transition temperature; T_{onset} is the extrapolated temperature that denotes the temperature at which the weight loss of the polymer begins; T_{max} is the temperatures where the maximum weight loss rate occurs at the DTG curve; weight loss is the rate of degradation in the end of the main event; char₈₀₀ is the unvolatilized weight fraction at 800 °C (char residue).

Table 4
Mechanical properties of CMC-based films.

Lignin concentration in CMC films ($W_{\text{lignin}}/W_{\text{CMC}}$, wt%)	TS (MPa)	EB (%)	YM (MPa)
0 EOL	30.01 ± 2.10 ^a	15.31 ± 3.91 ^a	7.94 ± 1.66 ^a
12.5 EOL	29.02 ± 1.36 ^a	13.74 ± 2.71 ^{ab}	8.30 ± 0.68 ^{ab}
25.0 EOL	26.67 ± 2.31 ^a	9.41 ± 2.62 ^{bc}	9.38 ± 0.80 ^b
50.0 EOL	20.00 ± 3.89 ^b	5.92 ± 3.49 ^c	9.70 ± 0.81 ^b

Values reported are the mean ± sd. Different letters (a-d) in the same column indicate a statistically significant difference ($p < 0.05$). TS: tensile strength; EB: elongation-at-break; YM: Young's modulus.

other hand, an increase in YM was observed for CMC-based films with 25 and 50 wt% of lignin, where a statistically significant difference ($p < 0.05$) was observed in relation to the control film. This decrease in resistance may be related to the low compatibility between CMC matrix and lignin. Kovalcik et al. (2105) observed that TS of PHBHV/lignin films did not change regarding the neat PHBHV film. However, it is important to highlight that the lignin concentration used in this work (1–10 wt% lignin) was lower than the current work. They also reported reduced EB and increased YM in PHBHV film up to 10 wt% lignin (the highest lignin concentration tested). According to Kovalcik and co-authors (2015) this loss of EB occurred probably due to the aromatic structure of lignin and its higher rigidity regarding the polymer matrix. Núñez-Flores et al. (2012) also reported reduced EB in gelatin/lignosulphonate films.

On the other hand, other works have reported lignin acting as a reinforcing agent in some composite films. For instance, Shankar et al. (2015) observed that the TS of agar/lignin films increased with the incorporation of lignin (1%–10 wt% lignin). Chen et al. (2009) also reported an increment in TS of chitosan films with the incorporation of lignin. In general, the TS values can be related to the compatibility between the biopolymer and lignin, since the dispersibility of filler material in the polymer matrix have great influence in the TS of the composite film (Herrera-Franco and Valadez-Gonzalez, 2005).

So, it can be said that the higher filler loading and the low compatibility between the CMC matrix and lignin led to a low dispersion and lignin agglomeration. This can be easily seen in SEM images of CMC-based films with lignin, that showed small aggregated structures (see Fig. 2). This behavior could be changed by the use of compatibilizers, which can act by reducing interfacial tension and improving the lignin miscibility in the CMC matrix (Imre and Pukánszky, 2013) or as grafting and crosslinking agents (Olivato et al., 2012).

3.9. Antioxidant activity

Antioxidant activity of CMC-based films was assessed by the DPPH scavenging method and expressed as radical scavenging activity (RSA). Fig. 5 presents the RSA for different amounts of films, 10 and 100 mg, representing lignin concentrations ranging from 12.5 to 50 μg and from 125 to 500 μg , respectively (theoretical values). As expected, the control film did not show antioxidant activity. The highest RSA values were achieved in the CMC/lignin-based films with high lignin content (100 mg film containing 25 and 50 wt% lignin). The antioxidant activity is explained by the presence of the phenolic hydroxyl groups of lignin (An et al., 2017; Michelin et al., 2018). Maximal RSA of around 90% was achieved with 30 min of reaction in these films. Considering the lignin concentration in the evaluated CMC-based films, the IC_{50} was achieved with 50 $\mu\text{g}_{\text{lignin}}/\text{mL}$. These results show that the CMC-based films incorporated with lignin could be useful as packaging for the preservation of some food sensitive to oxidation, which may limit its self-life.

RSA performed with 10 mg of CMC/lignin-based films increased with

the increment of the reaction time, achieving maximal RSA of 83.75% at 50 μg lignin after 24 h reaction. However, with 100 mg of CMC/lignin-based films no significant improvement in the RSA was observed with the increment of the reaction time. The antioxidant capacity can be attributed to some lignin groups presents in the surface with recognized antioxidant activity or to the migration of some of these free active molecules into the solution, such as monomers and oligomers, as well as other free phenols that could be present in the lignin samples, since lignins, as high molecular weight polymers, present low mobility (Crouvisier-Urien et al., 2016).

Aadil et al. (2016a) found that the free radical scavenging capacity of the gelatin/lignin films increased with the increase in lignin content, achieving 67.51% RSA (IC_{50} 111.10 $\mu\text{g}/\text{mL}$) in the water soluble fraction of film containing 40% lignin (the highest concentration). Nunez-Flores et al. (2012, 2013) measured the antioxidant activity of the ligno-sulphonates and soda lignin used in the production of lignin/gelatin films and reported IC_{50} of 83.4–97.5 $\mu\text{g}/\text{mL}$ and 36.5 $\mu\text{g}/\text{mL}$, respectively. Guo et al. (2019) have studied the antioxidant activity of cellulose-based films incorporated with several types of technical lignins. Results showed that soda/AQ lignin and organosolv lignins presented higher antioxidant activity than kraft, DES and hydrotrope lignins. The higher antioxidant activity of the organosolv lignin regarding the other ones has been reported to be related to its lower molecular weight, as well as with its high purity (Arshanitsa et al., 2013; Michelin et al., 2018). Additionally, Guo and co-authors (2019) showed that lignin with a higher content of syringyl units presents a higher antioxidant potential. On the other hand, Alzagameen et al. (2019) showed that lignin organosolv of different sources (Miscanthus, spruce/pine, and beech wood) have different antioxidant potential (from 50.9% to 74.3%) in HPMC-based films. Therefore, it can be concluded that the incorporation of EOL from corncob in CMC-based films, as well as in another type of polymer films, may be favorable (lower IC_{50} than other sources) in protecting against the oxidation process in some type of food packaging applications, such as cheese.

4. Conclusion

The biodegradable CMC-based films incorporated with organosolv lignin were successfully produced by the casting method. Organosolv lignin improved water resistance of CMC-based films, such as moisture, water solubility and WVP as well as their thermal stability. On the other hand, no significant improvement in the mechanical properties (TS, EB and YM) was observed, which may be related to the low compatibility between organosolv lignin and CMC polymer. Moreover, organosolv

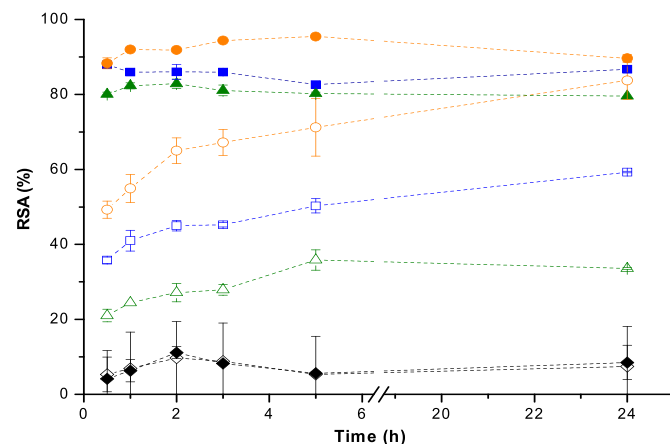


Fig. 5. Radical scavenging activity (RSA) of CMC-based films by the DPPH method. CMC-based films with 0 wt% EOL (diamond) 12.5 wt% EOL (triangles); 25 wt% EOL (squares); and 50 wt% EOL (circles). Open and closed symbols refer to analysis by using 10 mg and 100 mg of film, respectively.

lignin conferred excellent antioxidant capacity to the CMC-based films, boosting its use as antioxidant in food packaging films. This property combined with the high opacity of the films could be useful in food products where the oxidation is a problem, such as cheese and minimally processed vegetables, where the combination of antioxidant properties and opacity could help to maintain the shelf-life parameters during storage.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Michele Michelin: Conceptualization, Methodology, Investigation, Formal analysis, Writing - original draft. **Arlete M. Marques:** Methodology, Investigation, Formal analysis. **Lorenzo M. Pastrana:** Resources, Funding acquisition. **José A. Teixeira:** Resources, Funding acquisition. **Miguel A. Cerqueira:** Conceptualization, Methodology, Validation, Writing - review & editing, Supervision.

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