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Physical properties of active biopolymer films based on chitosan, sodium caseinate, and rosemary essential oil

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ABSTRACT

To develop an active biopolymer film based on chitosan, sodium caseinate, and rosemary essential oil (REO), the objective of this work was to investigate the effect of REO on the structure and physical properties of biopolymer films. Fourier transform infrared spectroscopy was conducted for fingerprinting to identify the REO addition. The thermal behaviour of each film was investigated using a thermogravimetric analysis. Furthermore, the optical properties, surface hydrophobicity, solubility, mechanical properties, and water vapour permeability were investigated. The results showed that the presence of REO changes the structural organisation of the blended sodium caseinate and chitosan film, thereby inducing an increase in the surface hydrophilicity of the film. However, the solubility and water vapour permeability of the film were not affected by the structural modification induced by REO. Moreover, a mild effect of the REO on the mechanical properties of the sodium caseinate/chitosan film was observed. A thermal analysis confirmed that the incorporation of REO did not influence the degradation temperature of the films. Overall this studying has demonstrated that caseinate/chitosan film enriched in REO, related to his thermal and chemico physical properties can be applied for food packaging application.

1. Introduction

Biopolymers, coatings, and natural additives are currently considered sustainable alternatives for food packaging applications as they enhance resource efficiency and reduce negative environmental effects associated with packaging wastes after their useful life (Mohamed, El-Sakhawy, & El-Sakhawy, 2020). Biopolymers, such as proteins and polysaccharides, have been extensively studied as potential edible films or coatings for food application (Calva-Estrada, Jiménez-Fernández, & Lugo-Cervantes, 2019; Elsabee & Abdou, 2013; Giancone, Torrieri, Pierro, Cavella, Giosafatto & Masi, 2011; Volpe, Cavella, Masi & Torrieri, 2017; Yousuf, Qadri, & Srivastava, 2018).

Aiming to prolong shelf life, maintain food quality, and reduce the use of synthetic food additives, many studies have evaluated the incorporation of natural substances, such as essential oils, in biopolymer films or coatings in order to develop active films (Perdones, Chiralt, & Vargas, 2016; Ghadermazi, Keramat, & Goli, 2017). Essential oils are mainly composed of terpenes, terpenoids, and aromatic and aliphatic constituents, all of which are characterised by a low molecular weight (Burt, 2004). These phenolic compounds are part of the secondary metabolites of plants and contribute to their protection against ultraviolet (UV) radiation (antioxidant activity) and against pathogens, parasites, and predators (antimicrobial activities). The antioxidant activity of phenols occurs because of their capacity to release hydrogen molecules captured by free radicals, which are responsible for spoiling the quality of food products (Balasundram, Sundram, & Samman, 2006).

When used as active compounds in a film, it is also important to evaluate their effects on the physical and structural properties of the resulting film (Sánchez-González, González-Martínez, Chiralt & Cháfer, 2010; Torrieri, Cavella, & Masi, 2015). In particular, essential oil incorporation can affect the structure of the polymer matrix, leading to physical changes depending on the specific polymer-oil components interactions. Moreover, the oil composition and specific interactions with the polymer network determine its effectiveness as an active ingredient (Sánchez-González, Vargas, González-Martínez, Chiralt & Cháfer, 2011). Rosemary essential oil (REO) has been successfully used as an active food packaging compound to increase the antioxidant and antimicrobial properties of gelatin (Yeddes et al., 2019), gelatin-chitosan-pectin (Yeddes et al., 2020) chitosan-montmorillonite bionanocomposite (Pires, de Souza, & Fernando, 2018), and chitosan

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based biofilms (Abdollahi, Rezaei, & Farzi, 2012). The influence of essential oils on the physical properties of the film has been studied for corn and wheat starch films (Song, Zuo, & Chen, 2018a), chicken meat protein-based films (Sarıcaoglu & Turhan, 2020), and sodium caseinate films (Sani, Marand, Alizadeh, Amiri & Asdagh, 2020; Alizadeh-Sani, Moghaddas Kia, Ghasempour & Ehsani, 2020). On the one hand, it has been reported that essential oils can have a detrimental effect on film barrier properties owing to discontinuities in the structure of the film associated with the formation of lipid droplets embedded in the continuum polymer network (Bonilla, Atarés, Vargas & Chiralt, 2012; Torrieri, Cavella, & Masi, 2015; Dashipour et al., 2015). On the other hand, no effect on the water barrier properties has been reported as a function of the film composition or its structure (Bahram, Rezaei, Soltani, Kamali, Ojagh & Abdollahi, 2014; Chen & Liu, 2016; Hashemi, Mousavi Khaneghah, Ghaderi Ghahfarrokhi & Eş, 2017; Nisar, Wang, Yang, Tian, Iqbal & Guo, 2018; Perone, Torrieri, Cavella & Masi, 2014; Rocca-Smith et al., 2016). In a previous study, a chitosan-and caseinate-based blend film was optimised to improve the mechanical and water vapour barrier properties of chitosan films (Volpe et al., 2017). On this basis, we decided to develop an active film based on a chitosan and caseinate blend by incorporating REO. Therefore, in this study, the effect of oil on the structure and functional properties of the film was studied using a chitosan and caseinate film as a control to investigate the effect of the oil and biopolymer interaction on the matrix structure.

2. Materials and methods

2.1. Materials

Medium molecular weight chitosan (deacetylation degree 75–85%), sodium caseinate salt from bovine milk, glacial acetic acid, REO, Tween 80, sodium hydroxide (1 M), tris hydrochloride buffer, and glycerol were purchased from Sigma Aldrich.

2.2. Film-making procedure

Film-forming solutions containing chitosan (CH; 1%), sodium caseinate (SC; 4%), and CH (2%)/SC (4%) in a proportion of 1:1 (weight (w)/volume (v)) were obtained as reported by Volpe et al. (2017). Glycerol (GLY) was added as a plasticizer to all the solutions to achieve a GLY/SC, GLY/CH, and GLY/CH/SC weight ratios of 0.1. A mixture of REO and Tween 80 (4:1) was added to the solutions to obtain a final concentration of 1.5% (v/v). The film-forming solutions were homogenised at 15,500 rpm for 4 min at 20 °C using a rotor-stator homogeniser ultra-turrax R, T 18 (IKA, Italy), and degassed under vacuum for 15 min to prevent pinhole formation. Films were made by casting, in which 10 mL of each solution was poured into a Petri dish (area = 56.7 cm^2) immediately after preparation and left to dry at 40 °C and 50% relative humidity (RH) for 16 h in a circulating air system chamber (MMM Medcenter Einrichtungen GmbH, Germany). Then, the dried films were peeled from the Petri dishes and stored at 50% RH until testing. The composition and solid surface density of the films were calculated as reported by Giancone et al. (2011) and are listed in Table 1.

2.3. Film characterisation

2.3.1. Film thickness

The film thickness (x) was measured using a micrometer model H062 (Metrocontrol Srl, Italy) with a sensitivity of ± 2 μm. Five replications were conducted for each sample, and five measurements were taken at random positions around the film.

2.3.2. Moisture content and solubility

The moisture content (MC) of the films was evaluated by the gravimetric method, in which the films were dried in an oven at 105 °C for 24 h until they achieved a constant weight. The MC values were determined as the fraction of weight lost during drying as compared to the initial weight, and were reported on a wet basis (ASTM, 1994). The following equation was used for the MC (%) calculation:

$$MC \quad (\%) = \frac{Mi - Mf}{Mi} \times 100, \tag{1}$$

where Mi and Mf are the initial and final masses of the film, respectively.

The film solubility at different pH values was determined according to the method described by Giancone et al. (2011), as follows:

$$FS \quad (\%) = \frac{wi - wf}{wi} \times 100, \tag{2}$$

where "w" is the dry matter, and subscripts "i" and "f" correspond to the initial and final dry matter, respectively.

The determinations of MC and solubility were performed in triplicate.

2.3.3. Surface hydrophobicity

The sessile drop method, which is based on the optical contact angle (°), was used to estimate the surface hydrophobicity of the films. Specifically, a 0.2 μL drop of ultrapure water was deposited on the film surface, and the image of the drop was recorded with a digital microscope M.PG-2 (TQC sheen, China). The optical contact angle was calculated as reported by Wu et al. (2014) using the ImageJ free software. The measurements were performed on the upper side of the film, which was in contact with the air during the drying step.

2.3.4. Optical and colour properties

The film opacity was determined according to Park and Zhao, (2004) by measuring the absorbance at 600 nm using a V-550 UV/VIS spectrophotometer (Jasco, Japan). The films were cut into rectangular pieces 3 cm high and 0.4 cm wide and placed directly into the cuvette of the spectrophotometer. The results were reported as absorbance divided by film thickness (mm) based on five replicates. Three film specimens were used for each replicate. The colour of the film was evaluated using a portable colourimeter CR-300 (Konica Minolta, Japan). A CIE-Lab (Commission Internationale de l'Eclairage) colour scale was used to measure the degree of lightness (L*), redness (+a*) or greenness (-a*),

Table 1
Composition, solid surface density, Young's modulus (E), tensile strength (TS), and elongation at break (Ɛ %) of chitosan (CH), sodium caseinate (SC), and blend film (CH/SC) enriched with rosemary essential oil (REO). Letters indicate differences between groups ($p < 0.05$) (each group is symbolised by a different biopolymer matrix, with and without REO).

	Film Forming Composition (%w/v)					Mechanical properties		
Sample code	Chitosan	Sodium caseinate	REO:tTween80	Glycerol	solid surface density ρ (mg cm^{-2})	E (Mpa)	TS (Mpa)	Ɛ %
CH	1	0	0	0.1	1.9	n.a.	n.a.	n.a.
CH/REO	1	0	1.5	0.1	4.64	n.a.	n.a.	n.a.
SC	0	4	0	0.4	7.7	381 ± 90^a	6 ± 3^a	4 ± 2^a
SC/REO	0	4	1.5	0.4	10.4	889 ± 235^b	12 ± 3^a	3 ± 1^a
CH/SC	1	2	0	0.3	5.86	232 ± 43^a	10 ± 5^a	16 ± 6^b
CH/SC/REO	1	2	1.5	0.3	8.33	545 ± 219^b	11 ± 6^a	14 ± 9^b

and yellowness (+b*) or blueness (-b*) of the films. The total colour difference (ΔE) was calculated as follows (Rhim, Gennadios, Weller, Carole and Hanna, 1998):

$$\Delta E = \sqrt{[(L^*_{film} - L^*_{standard})^2 + (a^*_{film} - a^*_{standard})^2 + (b^*_{film} - b^*_{standard})^2]}, \quad (3)$$

where the standard colour parameters used as background values were L* = 96.94, a* = + 0.23, and b* = + 1.85. Five replicates were used for each sample.

2.3.5. Fourier transform infrared spectroscopy – attenuated total reflectance (FTIR–ATR)

To elucidate the effect of REO on the structure of the CH, SC, and CH/SC films, FTIR-ATR spectrometry was conducted using a spectrometer Avatar 330 (Thermo Scientific, USA). The FTIR-ATR spectra were recorded from 2000 cm^{-1} to 650 cm^{-1} via 32 scans at a resolution of 1 cm^{-1}. The data were analysed using OMNIC software (Thermo Fisher Scientific, USA), and were then normalised to ensure the results were reported as arbitrary units (a. u.).

2.3.6. Thermogravimetric analyser

The thermal stability of the films was investigated using thermogravimetric analysis (TGA) with a thermogravimetric analyser (TGA 7/DZ, Perkin Elmer, Japan). CH powder (CH-P), SC powder (SC-P), GLY, and REO were also characterized using a TGA. To investigate the effect of REO, the initial (T_i) final degradation temperatures (T_f), and the maximum decomposition temperature (DTG_{max}) were determined from the derivative curves (DTG). The final residual weight after 500 °C ($Ress_{500\,°C}$) was evaluated from the TGA curves as the percentage of weight loss. The samples were packed in an aluminium pan inside the thermogravimetric balance and heated from room temperature to 500 °C at a rate of 10 °C/min under a dry nitrogen atmosphere (20 mL min^{-1}) in order to avoid thermo-oxidative reactions. All analyses were performed in triplicate.

2.3.7. Mechanical properties

The tensile properties of each film were measured according to the standard method ASTM D882–00 (ASTM, 2002) using a universal testing machine (Instron 5900R-4467; Instron, USA). The load cell was 1 kN, and the speed of the mobile crosshead was set to 50 mm min^{-1}. Then, the stress-strain curves were recorded, of which the tensile strength (TS, MPa), elongation at break (ε %), and elastic modulus (E, MPa) were derived according to ASTM (2002). Ten replicates were performed.

2.3.8. Water vapour permeability

The water vapour permeability (WVP) of the films was evaluated using a gravimetric test according to ASTM (1993) using a payne permeability cup (Carlo Erba, Italy). Briefly, 9.89 cm^2 of the film surface was exposed to vapour transmission by being placed on the top of the permeability cup containing silica gel. All the cups were placed into a desiccator at an aw of 0.85 and stored in a thermostatic incubator KBF240 (Binder, Italy) at 20 °C. The cups were weighed at scheduled times, and the water vapour transmission rate (WVTR) through the film was estimated according to the linear portion of the diagram obtained by plotting the weight increment of the cup as a function of time.

A steady state was assumed to be reached once the regression analysis (R2) based on the last four data points resulted in an R2 ≥ 0.998. The WVP was calculated as:

$$WVP = \frac{WVTR \quad x \quad L}{\Delta p}, \quad (4)$$

where WVTR is the water vapour transmission rate (g m^{-2} s^{-1}), L is the average film thickness (m), and Δp is the partial water vapour pressure difference between the two sides of the film (Pa). The results were reported as the average of three replicates for each sample.

2.4. Data analysis

The results were reported as the mean ± standard deviation. Analysis of variance (ANOVA) was used to evaluate the effect of the film composition on the film properties. A Duncan's test was performed to determine the source of the significant differences between the samples. Differences were considered significant at $P < 0.05$. All statistical analyses were performed using statistical package for social science (SPSS) software (SPSS Inc. 17.0, Chicago, 2002).

3. Results and discussion

3.1. Thickness, MC, solubility, and contact angle

The thickness, MC, solubility, and contact angle results of the tested films are listed in Table 2. The film thickness changed from 27 ± 9 μm for the CH-based film to 76 ± 12 μm for the CH/SC film enriched with REO. This is consistent with previous studies, which have shown that REO affects the thickness of the film ($p < 0.05$) (Giancone et al., 2011; Nogueira, Fakhouri, & Velasco, 2019; Ortega, Giannuzzi, Arce & García, 2017). Further, as the interaction of CH and SC into a blend film formed a more complex network (entanglements) than a single polymer, as reported by Volpe et al. (2017), it can be assumed that REO in the blend film interacts with both polymers, thereby increasing the volume of the film.

The addition of REO only influenced the MC of the CH/SC blend film, which increased from 12.1% ± 0.3–17% ± 2%. Hence, REO mainly interacts with SC, leaving free bonding sites of CH available to link with water molecules, causing additional structural swelling. Similar behaviour was observed for CH-gelatin blend films containing thyme, pink clove, citronella, nutmeg, or cinnamon essential oils (Haghighi et al., 2019). However, the addition of essential oils to biopolymers does not always result in an improvement in the water-holding capacity of the resulting film. For example, Song, Zuo, and Chen (2018b) reported that the addition of lemon essential oil to wheat-corn starch films reduced the MC.

To test the behaviour of the film in contact with a variety of foodstuffs, ranging from fruit and vegetables to dairy and meat products, the solubility of the film was tested under different pH conditions, as summarized in Table 2. Overall, the film composition affected the film solubility, but the presence of REO only had a significant effect on the solubility of the SC film at a pH of 8, at which it increased from 33% ± 3–50% ± 9% (Table 2). For all other samples, no REO effect was observed on film solubility, revealing average values of approximately 30% at a pH of 4, an average of 31% for the blend and SC films, and 16% for the CH film at pH values of 6 and 8. Note that the CH and SC films showed higher solubilities at pH values of 4 and 8, respectively. Meanwhile, the combination of SC and CH reduced the solubility of the film at both acidic and basic pH values, allowing the film to be in contact with different food matrices. Similar results were reported by Wu et al. (2014), where gelatin /chitosan film enriched in oregano essential oil shows not effect on the solubility.

The optical contact angle values ranged from 75° to 12° (Table 2). The ANOVA showed that the film composition and REO had a significant effect on the contact angle. Specifically, the blend film had the highest contact angle, confirming the less hydrophilic nature of the film owing to the interaction between CS and CH. The presence of REO caused an increase in the surface hydrophilicity of the blend film. This result was also confirmed for the CH and SC films. These can be justified by the discontinuities of the REO droplets, which give rise to a more open film structure (Atarés & Chiralt, 2016; Bonilla *et al.* 2012).

Table 2
Thickness (x), moisture content (MC), solubility, and contact angle of chitosan (CH), sodium caseinate (SC), and blend film (CH/SC) films incorporated with rosemary essential oil (REO).

Code Sample	x (μm)	MC%	solubility % pH 4	solubility % pH 6	solubility % pH 8	Contact angle °
CH	27 ± 9^{a}	23 ± 2^{b}	30 ± 8^{a}	18 ± 2^{a}	19 ± 5^{a}	32.7 ± 0.3^{a}
CH/REO	27 ± 11^{a}	21 ± 5^{c}	33 ± 7^{a}	13 ± 4^{a}	16 ± 2^{a}	$15 \pm 4^{a*}$
SC	54 ± 7^{b}	11 ± 1^{a}	31 ± 4^{a}	30 ± 6^{b}	33 ± 3^{b}	25 ± 5^{a}
SC/REO	59 ± 19^{b}	11 ± 1^{a}	25 ± 6^{a}	38 ± 5^{b}	$50 \pm 9^{b*}$	$12 \pm 2^{a*}$
CH/SC	61 ± 13^{c}	12.1 ± 0.3^{a}	27 ± 10^{a}	26 ± 1^{b}	22 ± 2^{a}	75 ± 10^{b}
CH/SC/REO	$76 \pm 12^{c*}$	$17 \pm 2^{b*}$	25 ± 3^{a}	31 ± 12^{b}	20 ± 6^{a}	$12 \pm 1^{a*}$

The lowercase letters indicate differences among the samples (CH, SC, and CH/SC) with or without REO ($p < 0.05$); * indicates the effect of oil on each sample (CH, SC, or CH/SC) ($p < 0.05$)

3.2. Optical and colour properties of the film

Opacity is a fundamental parameter for packaging films because it influences the appearance of the products. Moreover, light can affect food quality because it affects the rate of lipid oxidation. The presence of REO increased the opacity of the CH/SC film. This result was also observed in the CH and SC control films ($p < 0.05$) (Table 3). Ojagh, Rezaei, Razavi, and Hosseini (2010) reported the same effect when ginseng extract was added to an alginate film. As the film transparency depends on its internal structure (Sánchez-González *et al.* 2010), the increased opacity of the film in the presence of REO may depend on the interaction of REO with the polymers used (Aguirre et al., 2018). The colourimetric parameters of the films are listed in Table 3. The ANOVA showed that the film composition also had a significant effect on the colour parameters ($p < 0.05$). Conversely, the REO only had a significant effect on the colour parameters of the CH/SC films ($p < 0.05$), causing an increase in the L* and b* values, and consequently, the ΔE. A similar behaviour was observed by Ekthamasut and Akesowan (2001) when corn oil and sunflower seed oil were added to edible Konjac films.

3.3. FTIR–ATR

The FTIR-ATR spectra of the CH and CH/REO, SC and SC/REO, CH/SC and CH/SC/REO films are shown in Fig. 1. For a better understanding, pure REO was also analysed, as shown in Fig. 1. In the CH spectra, the amide I and amide II bands were at 1640 cm^{-1} and 1560 cm^{-1}, respectively (Kam, Khor, & Lim, 1999). With regard to SC, the absorption peaks in the area between 1631 cm^{-1} and 1516 – 1532 cm^{-1} were attributed to amide I and amide II, respectively. For the CH/SC film, the absorption peaks dominated by the amide I band of CH at 1640 cm^{-1} and 1560 cm^{-1} decreased to 1635 cm^{-1} and 1538 cm^{-1}, confirming that there were electrostatic interactions between the amino groups of CH and the negatively charged carboxyl groups of the CS in the complex films (Volpe et al., 2017; Khwaldia, Basta, Aloui & El-Saied, 2014). Regarding REO, an evident peak at 1744 cm^{-1} was related to a keto group of camphor, and two peaks at 1214 cm^{-1} and 984 cm^{-1} were related to the epoxy ring of 1.8-cineole, as confirmed by Stramarkou, Oikonomopoulou, Missirli, Thanassoulia, and Krokida (2020).

Only one new peak at 1744 cm^{-1} was identified in the SC/REO film (Fig. 1). This peak was not clear in the CH/REO and CH/SC/REO films because of the noise in the wavelength regions. For the CH/SC film, the addition of REO led to a reduction in the peak intensity, suggesting a reduction in the hydrophilic character of the films. This result is in agreement with the findings of Pereda, Aranguren, and Marcovichs (2010).

3.4. TGA

The TGA results allowed us to gain information regarding the effect of REO addition on the thermal stability of the films, as listed in Table 4. Generally, the addition of REO did not affect the thermal properties of the film. To better understand this behaviour, each film component was analysed separately. In detail, the REO showed a low thermal stability, as compared with the biopolymers and plasticizers (SC-P, CH-P, and GLY). In particular, within the temperature range of 32 ± 3.17 °C – 148 ± 1.6 °C, REO completely volatilized, making the final residual weight 0%.

SC-P and CH-P exhibited similar behaviours, reaching a DTG_{max} of approximately 300 °C. However, the thermal degradation of the SC-P started at a lower temperature and ended at a higher temperature, and its residual weight was higher than that of CH-P. GLY also had a single transition between 100 °C and 304 °C, with a peak at 258 °C. The concentration of GLY used for the preparation of the film did not affect the thermal stability of the films. This result is consistent with Pereda, Aranguren, Marcovich, and Plata (2007), who proved that the thermal properties of SC/CH film enriched in GLY up to 28% were not affected by the presence of GLY. According to the TGA curves of the film samples (Fig. 2 A, B, and C), it can be assumed that the individual components did not interact. Specifically, the DTG curves (not shown) were characterised by a maximum peak accompanied by shoulders or relative peaks, as a result of the simultaneous degradation of CH, SC, and CH/SC, resulting in the overlap of their individual peaks. This confirmed that the incorporation of REO into the CH, SC, and CH/SC films at the studied concentration did not influence the T_i and T_f degradation values of the active films. Similar results were reported by Dong, Xu, Ahmed, Li, and Lin (2018) and Shen and Kamdem, (2015), who found that the addition of essential oil up to 30% did not affect the thermal stability of the active films. Moreover, for all the films, a residual weight loss of approximately 30% was observed at temperatures higher than 500 °C. Further, the results indicate that all the samples were thermally stable in the range of

Table 3
Opacity and colorimetric parameters (lightness (L*), redness (+a*) or greenness (-a*), yellowness (+b*) or blueness (-b*), and total colour difference (ΔE)), of chitosan (CH), sodium caseinate (SC), and blend film (CH/SC) films incorporated with rosemary essential oil (REO).

Code Sample	Opacity (Abs/mm)	L	a*	b*	△E
CH	3.84 ± 0.02^{a}	92.24 ± 2^{a}	-0.2 ± 0.9^{b}	2 ± 1^{a}	4 ± 1^{a}
CH/REO	$4.3 \pm 0.3^{a*}$	92.26 ± 2^{a}	-0.2 ± 0.2^{b}	3 ± 2^{a}	4 ± 1^{a}
SC	3.6 ± 0.9^{b}	90.1 ± 0.4^{a}	-1.0 ± 0.1^{a}	5.3 ± 0.8^{b}	6.1 ± 0.6^{b}
SC/REO	7 ± 1^{b}	$92.9 \pm 0.5^{ab*}$	-1.5 ± 0.8^{a}	$8.5 \pm 0.8^{b*}$	$6.8 \pm 0.9^{b*}$
CH/SC	3.1 ± 0.7^{a}	90.4 ± 0.5^{a}	-0.7 ± 0.6^{a}	4.9 ± 0.9^{b}	5.7 ± 0.9^{b}
CH/SC/REO	$4.8 \pm 0.3^{a*}$	$93.3 \pm 0.5^{b*}$	-1.0 ± 0.8^{a}	$10 \pm 1^{c*}$	$7.8 \pm 0.9^{c*}$

Lowercase letters indicate differences between groups ($p < 0.05$) (each group is symbolised by a different biopolymer matrices, with and without oil); * indicates the effect of oil on the same film ($p < 0.05$).

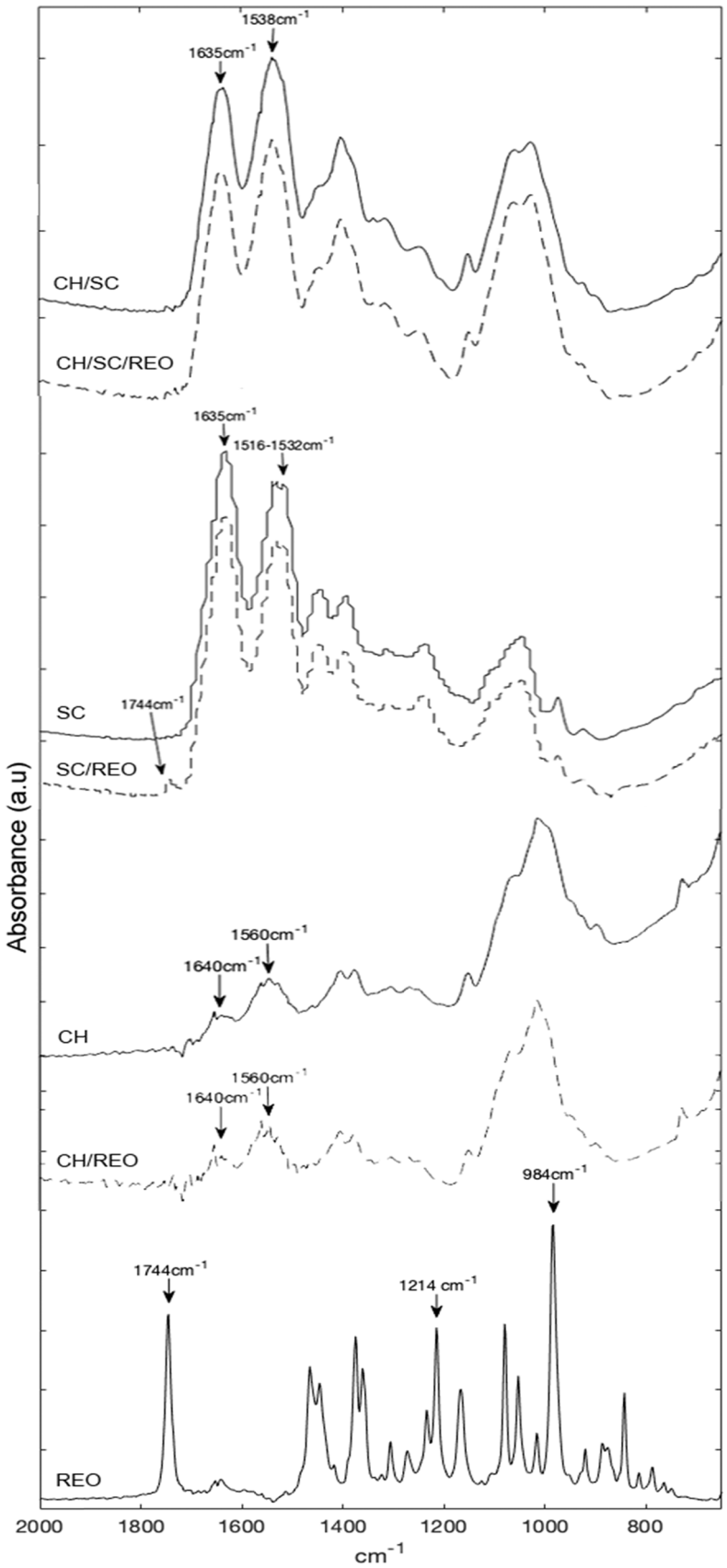

Fig. 1. Fourier transform infrared spectroscopy – attenuated total reflectance (FTIR-ATR) spectra of (A) chitosan (CH) and CH/rosemary essential oil (REO); (B) sodium caseinate (SC) and SC/REO; (C) CH/SC and CH/SC/REO films; and (D) REO. All films were equilibrated at 50% relative humidity (RH) and 25 °C. Control films and REO are represented with a straight line and films enriched in REO have a dotted line.

Table 4
Initial and final decomposition temperatures (T_i and T_f, respectively) maximum decomposition temperature (DTG_{max}), and percentage of residue at 500 °C ($Res_{500\,°C}$) of films incorporated with rosemary essential oil (REO) and their components: glycerol (GLY), REO chitosan powder (CH-P), and sodium caseinate powder (SC-P).

Code Sample	T_i (°C)	DTG_{max} (°C)	T_f (°C)	Res $_{500\,°C}$ (%)
CH-P	185 ± 10	296 ± 1	408 ± 13	20 ± 8
SC-P	165 ± 5	300 ± 2	475 ± 5	35 ± 1
REO	32 ± 3	93 ± 6	148 ± 2	0 ± 1
GLY	100 ± 1	258 ± 1	304 ± 5	0 ± 0
CH	119 ± 15	282 ± 25	426 ± 16	32 ± 1
CH/REO	121 ± 20	307 ± 35	490 ± 14	23 ± 1
SC	151 ± 1	340 ± 2	494 ± 6	29 ± 0
SC/REO	136 ± 1	329 ± 4	499 ± 1	30 ± 1
CH/SC	149 ± 7	274 ± 0	472 ± 10	33 ± 2
CH/SC/REO	141 ± 2	277 ± 1	477 ± 5	28 ± 1

120 – 150 °C and are thus suitable for industrial applications in this temperature range.

3.5. WVTR and WVP

Acting as a barrier to water vapour transmission is one of the most important properties of films for application in food packaging. The WVP and WVTR of the films in this study are shown in Fig. 3 A and B. The ANOVA showed that the presence of REO did not significantly affect the WVTR or the WVP ($p > 0.05$) of the films. However, the water vapour barrier properties were found to depend on the nature of the biopolymers. For example, CH had a higher WVP value of 14.9 ± 0.4 g m^{-1} s^{-1} $Pa^{-1} \times 10^{-11}$, as compared with the SC and CH/SC films, which had WVP values of 8.6 ± 0.3 g m^{-1} s^{-1} $Pa^{-1} \times 10^{-11}$ and 8.6 ± 0.1 g m^{-1} s^{-1} $Pa^{-1} \times 10^{-11}$, respectively, (Fig. 3 A). Meanwhile, for the WVTR, the CH/SC film had the lowest value at 33 ± 1 g m^{-2} $s^{-1} \times 10^{-4}$, whereas the CH film had the highest value, at 99 ± 11 g m^{-2} $s^{-1} \times 10^{-4}$ (Fig. 3 B). Thus, the inclusion of REO in the films did not improve the barrier properties of the films. This is because, in addition to the hydrophobic nature of the REO, its presence results in a more open film structure (Perone et al., 2014; Torrieri et al., 2015). Thus, the increase in film hydrophobicity in the presence of REO is balanced by the more open structure formed in the presence of the oil. Similar results were reported by Ojagh *et al.* (2010). As suggested by Atares and Chiralt, (2016), we cannot assume that the WVP of the edible films is reduced simply by adding a hydrophobic component in the formulation because the impact of the lipid addition on the microstructure of the emulsified film is a determining factor in water barrier performance. Moreover, the microstructure of the emulsified film is affected by other factors, such as the physical state of the essential oil and its distribution in the polymer matrix. In a previous study we showed that the REO droplets had a monomodal particle size distribution in a CH solution, with a particle diameter between 0.13 μm and 0.6 μm. Further, the oil droplets were uniformly distributed along the film thickness, and their sizes were larger in the film-forming solution. Thus, the film processing condition is of paramount importance because it can affect the microstructure of the emulsified film as a result of possible droplet coalescence during the casting operation (Torrieri, Cavella, & Masi, 2015).

3.6. Mechanical properties

The E, TS, and Ɛ % of the films are shown in Table 1. Note that the CH films were fragile, and because of their small thickness, it was not possible to submit them to the uniaxial elongation test as they cracked while clamping before testing. Therefore, the tensile properties of this film were not considered. Nevertheless, the REO had a significant effect on the E of the CH/SC/REO films, leading to an increase in E. Similar results were observed for the SC/REO film. However, the REO addition

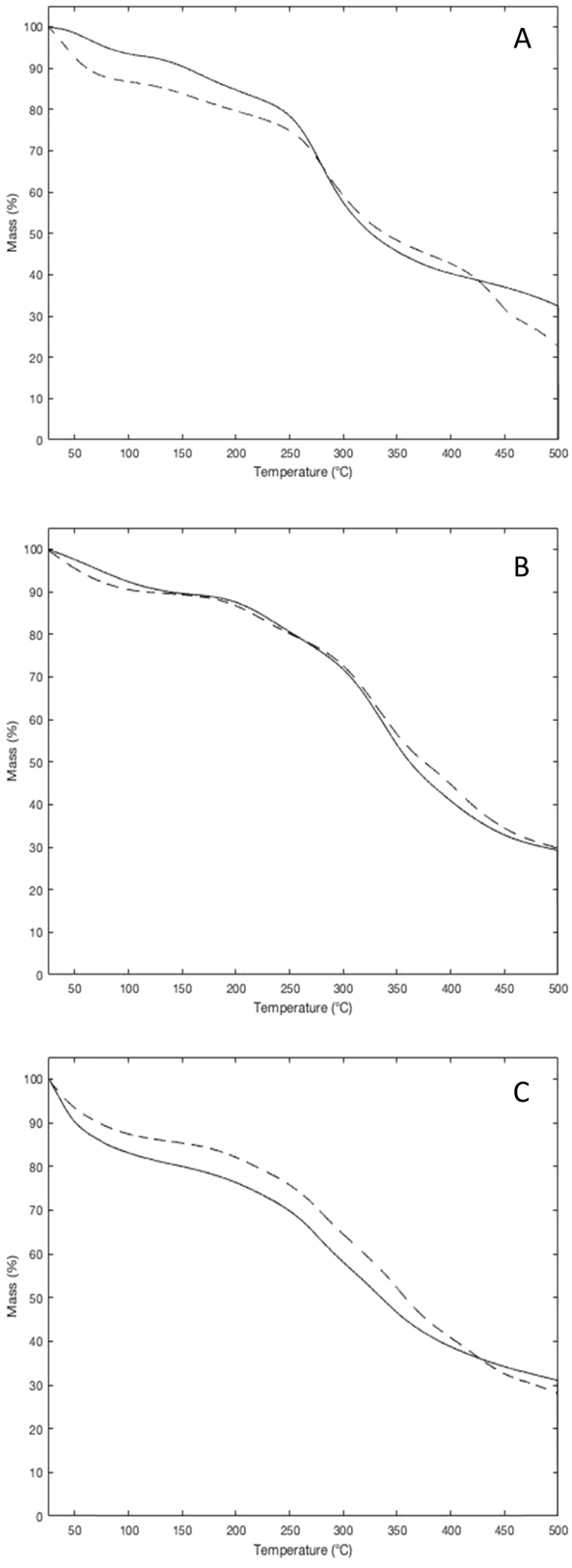

Fig. 2. Thermogravimetric analysis (TGA) curves of (A) chitosan (CH) and CH/rosemary essential oil (REO); (B) sodium caseinate (SC) and SC/REO; and (C) CH/SC and CH/SC/REO films obtained at a heating rate of 10 °C/min. Control films are represented with a straight line and films enriched in REO have a dotted line.

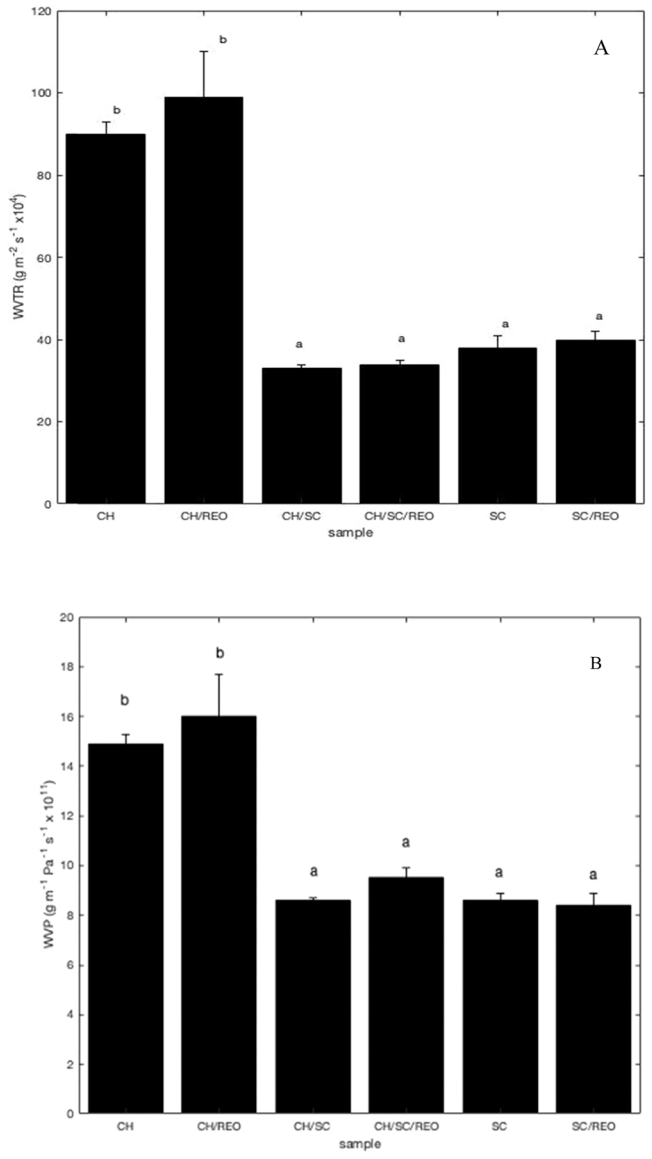

Fig. 3. Barrier properties: (A) Water vapour permeability (WVP) and (B) water vapour transmission rate (WVTR) of chitosan (CH), sodium caseinate (SC), and blend film (CH/SC) films enriched with rosemary essential oil (REO). Letters indicate differences between groups ($p < 0.05$) (each group is symbolised by a different biopolymer matrix, with and without REO).

did not affect the TS or ε %. These results are in good agreement with those of Fabra, Talens, and Chiralt (2010), who found that the addition of oleic acid to a SC film had a mild effect on its mechanical properties. Meanwhile, the blend film reached a higher ε % value, as compared with the SC film (Table 1), confirming that the interaction between protein and polysaccharide results in a more flexible structure than that obtained with only protein or polysaccharide (Volpe et al., 2017; Pereda et al., 2008).

4. Conclusions

The addition of REO to the CH/SC film modifies its structure, thereby causing some changes in the film properties of interest for industrial applications. Further, the addition of REO in active film preparation gives rise to films with a higher thickness than that of the control film. This behaviour is related to the interaction between REO and SC, which leaves free bonding sites of chitosan available for linkage with water molecules, resulting in a more swollen structure. As a consequence, the CH/SC films obtained in the presence of REO had a higher MC, solubility, and surface hydrophilicity than the CH/SC control films. However, the different structures of the films did not affect the water barrier properties, which were very similar to those of films prepared without the addition of an essential oil and assumed an average WVP value of 9 g m^{-1} s^{-1} Pa^{-1} × 10^{11}. In addition, the thermal properties were not affected by the presence of REO. Instead, the presence of REO affected the opacity of the film and the E, which increased in the presence of REO. In conclusion, active films based on CH/SC and REO exhibit physical properties suitable for potential application in food preservation as coatings in contact with food. However, further studies must be conducted to elucidate the effective antioxidant capacity of these films when used in direct contact with a food matrix.

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