



Evaluation of easy-removing antioxidant films of chitosan with *Melaleuca alternifolia* essential oil

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ABSTRACT

Chitosan - tea tree essential oil (TTEO) films were obtained as a new biodegradable material. Malic acid or lactic acid solvents were evaluated to obtain easy-removing films. The microstructure by SEM and FT-IR, the thermal properties by TGA/DSC, the mechanical properties, the water vapor permeability, the antioxidant (DPPH[•] and ABTS^{•+}) activity and the optical properties of the formulated films were evaluated. A complete dissolution of the film in water was obtained. The elongation to break was higher in the films with malic acid (145.88–317.33%), comparing with those with lactic acid (25.54–44.08%). Chitosan film obtained in malic acid with TTEO showed the highest antioxidant activity. The colour and transparency of the samples did not suffer significant variations by TTEO addition. Films showed good UV-barrier properties, with a slightly improvement by TTEO addition. The films obtained showed a great potential for food packaging applications.

1. Introduction

Polysaccharides such as chitosan can be a great alternative as polymer to reduce the presence of non-biodegradable synthetic packaging [1–3].

Chitosan is a derivate of chitin, one of the most abundant biological materials in the world. It is a high-molecular-weight cationic natural polymer composed of randomly distributed chains of β -(1–4)D-glucosamine and *N*-acetyl-D-glucosamine [1]. Most of the studies on chitosan highlight three main active features of chitosan relevant for packaging purpose: antimicrobial, antioxidant, and UV-barrier properties [4–7].

Most studies on chitosan have used acetic acid as solvent to obtain a film-forming solution [8–11]. Nevertheless, the properties of the chitosan matrix strongly depend on the type of solvent used. Therefore changing the acid used for the elaboration of these films will allow to develop novel matrix with desirable properties for certain applications [12–14]. For example, the solubility of the film can be modified using other types of solvents such as malic or lactic acid to obtain a chitosan-based film which are easy to remove from foodstuff. This is because acetic acid is volatile and lactic and malic acids are not. They remain in the film matrix and improve solubility in water. Note chitosan is soluble in acid solutions.

On the other hand, natural extracts from plants, herbs or fruits are a promising alternative to synthetic preservatives. They can be included in active packaging. Besides, these natural extracts can satisfy consumer demand for natural additives follow the new trend called “green consumerism” [8].

The essential oil of *Melaleuca alternifolia*, also named as tea tree essential oil (TTEO), is a complex mixture with a major share of monoterpenes, sesquiterpenes, and their alcohol derivatives. The main compounds of TTEO are terpinen-4-ol, γ -terpinene, α -terpinene, α -terpineol, α -terpinolene and 1,8-cineole [15]. TTEO presents powerful antioxidant properties with wide applicability in the food industry. However, the direct addition of these kind of natural antioxidants into foodstuff are limited since the high hydrophobic nature and intensity of the flavour associated to the volatile compounds [16]. The antibacterial properties of tea tree oil are well documented with identification of active compounds against bacteria and yeast. For example, TTEO presented antimicrobial activity in ground beef at the concentration 1.5% *v/w* against *Listeria monocytogenes* [17].

The use of polysaccharide matrices that can be applied as film or coating in food are an interesting alternative to serve as vehicles for antioxidant compounds such as TTEO [8].

Previous study indicated the antimicrobial effectiveness of chitosan-

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Table 1
Chitosan films formulation.

Film samples	Chitosan	Organic acid	Lecithin	TTEO
	% (w/w)	M: % (w/v) L: % (v/v)	% (w/v)	% (v/v)
Chitosan in malic acid solution				
CH_M1	1	2	0	0
CH_M2	1	2	0.1	0
CH_M3	1	2	0.1	0.5
CH_M4	1	2	0.1	1.0
Chitosan in lactic acid solution				
CH_L1	1	2	0	0
CH_L2	1	2	0.1	0
CH_L3	1	2	0.1	0.5
CH_L4	1	2	0.1	1.0

CH - chitosan; M - malic acid; L - lactic acid; TTEO - tea tree essential oil.

based films obtained in acetic acid and enriched with TTEO against *Listeria monocytogenes*, showing a complete inhibition of the microbial growth during the first fifth days at 10 °C [8].

Despite the potential applications of chitosan films with TTEO, no studies have been found that evaluate the interaction of these compounds as an antioxidant active matrix, or the investigation of its functionalities using solvents other than acetic acid. A lack of a complete characterization of this material is evident. The water solubility properties to apply as easy-removing antioxidant food packaging can be a potential application for TTEO-chitosan films.

Therefore, the present study was focused on evaluating the physicochemical and functional properties of essential oil of *Melaleuca alternifolia*-chitosan films using malic or lactic acid as solvents. Soy lecithin was evaluated as natural emulsifier to achieve homogeneous dispersion of essential oil in aqueous solution. The water solubility, equilibrium moisture content, tensile properties (tensile strength, percentage of elongation to break, Young's modulus and toughness), puncture properties (burst strength and distance to burst), water vapor permeability and antioxidant properties (DPPH[•] and ABTS^{•+}) were evaluated. The microstructure, thermal and optical properties (UV barrier, transparency, opacity and colour properties) of the formulated biopolymer were assessed by scanning electron microscopy, infrared spectroscopy, thermogravimetric, differential scanning calorimetry and UV-Vis spectroscopy.

2. Materials and methods

Chitosan (M_w 100,000–300,000) were purchased from Acros organics (Geel, Belgium), DL- malic acid extra pure and L(+)-lactic acid 88–92% extra pure provided by Scharlau Microbiology (Barcelona, Spain), soy lecithin and pure tea tree essential oil were supplied by Korott S.L. (Alicante, Spain), were used to prepare the chitosan film-forming solution.

2.1. Preparation of films

Chitosan films (Ch-films) were made by dissolving 1% (w/w) chitosan in an aqueous solution of 2% (w/v) malic acid or 2% (v/v) lactic acid (Table 1). The acid concentration was adjusted to achieve the complete dissolution of chitosan, giving a crystalline solution. Soy lecithin (0.1% w/v) was added into chitosan solution as natural emulsifier agent. Chitosan solution - soy lecithin was homogenized at 15000 rpm for 5 min (Ultra Turrax®, IKA, Staufen, Germany). The mixture was degassed using an ultrasonic bath for 15 min, time enough to remove the foam. The TTEO was added until a concentration ranged between 0 and 1.0% v/v, and homogenized at 15,000 rpm for 5 min following by a degassed step with ultrasonic bath for 15 min.

The resulting film-forming solution was casted in Petri dish and dried for 48 h at room temperature. The Ch-films were cut to a specific size

and stored at determined conditions depending on the test performing. The thickness (mm) was measured at five random locations using a Thickness Meter ET115S (Etari GmbH, Stuttgart, Germany).

2.2. Analysis of volatile compounds of TTEO

Volatile compounds of TTEO were determined by headspace solid phase micro-extraction (SPME) coupled with gas chromatography/mass spectrometry (GC/MS) (6890N GC, 5975 MS Agilent, USA) with using SPME fiber 50/30 μm (DVB/CAR/PDMS) (Supelco, Bellefonte, PA, USA). Details of the chromatographic separation and quantitative analysis were previously described [18].

2.3. Scanning electron microscopy (SEM)

The morphology of the Ch-films manufactured in malic or lactic acid solutions were observed and analysed by SEM images. Dried and gold-coated samples were photographed with a high-vacuum microscope (JEOL JSM-6360LV, Jeol Ltd., Tokyo, Japan) at an accelerating voltage of 20 kV. Samples were attached on slides using conductive double-sided carbon tape.

2.4. Fourier transform infrared spectroscopy (FT-IR)

FT-IR was carried out using a FT-IR ABB Bomen 102 (ABB Ltd., Zurich Switzerland) equipped with a Universal Attenuated Total Reflectance (UATR) accessory (SPECAC Golden Gate) with diamond crystal. Prior to the test, the selected samples were conditioned at 65 ± 2% relative humidity and 21 ± 1 °C for 48 h. FTIR spectra were recorded at a spectral resolution of 4 cm⁻¹ in the range (400–4000) cm⁻¹.

2.5. Water solubility, equilibrium moisture content and water vapor permeability

Water solubility of the samples was confirmed using the gravimetric method of immersion in a determined volume of distilled water as reported elsewhere [19]. The equilibrium moisture content (%W) was calculated following the gravimetric method by measuring the differences between the weights of conditioned (at 57% relative humidity, % RH) and dried samples as reported elsewhere by Cazón et al. [20]. Water vapor permeability (WVP) were measured following the ASTM Standard Test Method E96 as described elsewhere [20]. The WVP of the samples were measured at 30 °C and considering an intermediate water activity value between the two levels which define the relative humidity (RH) gradient used in WVP determination [21]. The test was run at least 8 h, enough time to reach a dynamic equilibrium in the water flux. Each test was performed by triplicate.

2.6. Mechanical properties analysis

The mechanical properties of the developed films were analysed by tensile and puncture tests using a texturometer (TA-XTplus, Stable Micro System, UK) with the accessories indicated for each test. Tensile strength (TS, MPa), percentage of elongation at break (%E, %), Young's Modulus (YM, MPa), toughness (T, MJ/m³), burst strength (BS, g) and distance to burst (DB, mm) were measured as described elsewhere [20,22]. The tensile test was carried out following the standard method D-882 (ASTM) and setting the jaw separation in 20 mm and the rate of loading in 1 mm/s.

2.7. Antioxidant properties

The antioxidative capacity of the developed films and its improvement by the addition of TTEO was determined by two methods: 1,1-diphenyl-2-picrylhydrazyl radicals (DPPH[•]) and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS^{•+}) free radical scavenging

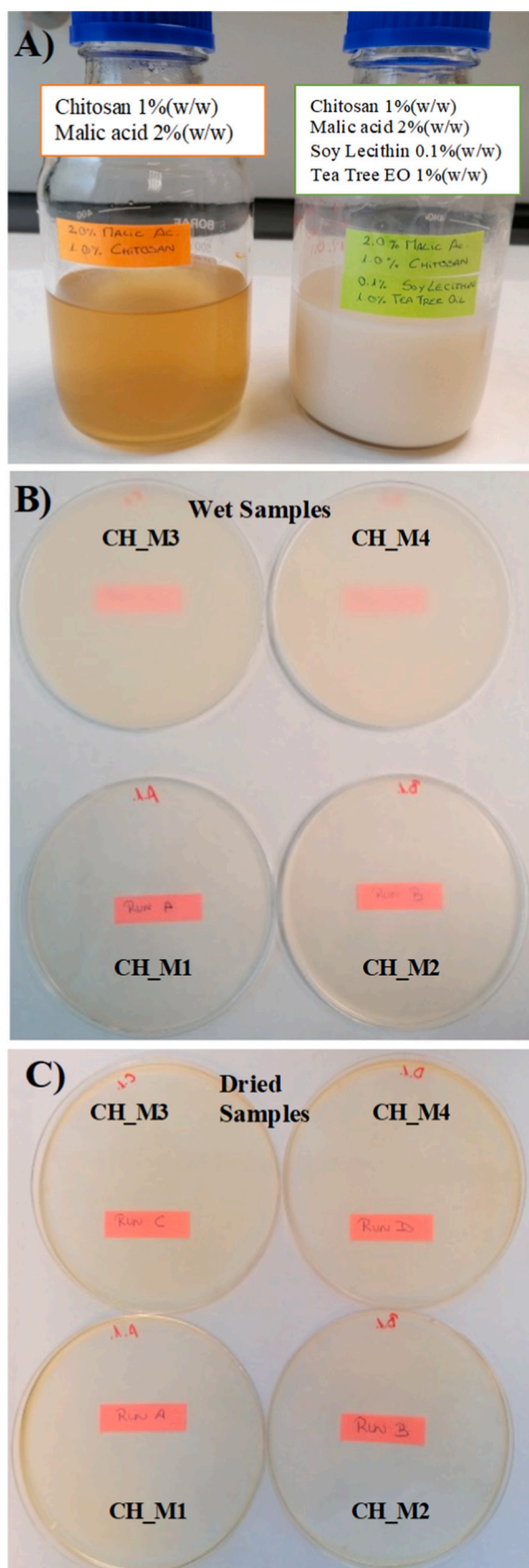


Fig. 1. Visual appearance of the chitosan film-forming solution with and without soy lecithin and tea tree essential oil (A) of the wet films (B) and dried films (C) with malic acid.

assay. The aliquots were obtained by the methanolic extracts of the films, using 0.4 g of sample in 24 mL of ethanol and left overnight in darkness.

Radical scavenging activity of the Ch-films on DPPH[•] was analysed using a UV–Vis spectrophotometer (Specord 40, Analytik Jena AG, Germany) at 515 nm following the method described elsewhere [23]. The ABTS^{•+} radical cation scavenging activity of the Ch-film samples was measured spectrophotometrically measuring the absorbance at 734 nm following the method described elsewhere [24]. The results were expressed as %DPPH[•] and %ABTS^{•+} scavenging activity.

2.8. Optical and thermal properties of films (TGA/DSC)

The UV–Vis spectra of the samples were performed by spectrophotometer V-670 (Jasco Inc., Japan) in the UV–Vis light regions (190 nm–800 nm), settled 2 nm intervals. The films were cut in rectangular pieces and placed inside the test cell of the spectrophotometer. The test was run in duplicate for each sample. The transparency and opacity values of the samples were calculated from the transmittance and absorbance values of the samples at 500 nm and 600 nm of wavelength, as described elsewhere [19]. The CIE L*a*b* coordinates were determined by the Spectra Manager software (Jasco Inc., Japan) established on the second standard observer with light source D65.

A thermogravimetry and differential scanning calorimetry equipment TGA/DSC (Mettler Toledo, Switzerland) were used to analyse the thermal properties and stability. The samples were placed in hermetic aluminium pans and the test was carried out at a heating rate of 10 °C/min from 50 to 400 °C, in atmosphere of N₂ (50 mL/min).

2.9. Statistical analysis

Result obtained were statistically analysed by one-way analysis of variance (ANOVA) employing Microsoft Excel® software. Differences between pairs of means were assessed based on confidence intervals using the Tukey Post Hoc test. The least significance difference was $p < 0.05$.

3. Results and discussion

3.1. Volatile composition of TTEO

The analysis of volatile compounds in the headspace of TTEO using SPME yielded a total of 36 identified and quantified compounds (Table S1). Monoterpenes were the most abundant (87.3%) with 14 compounds identified. With accordance to literature, terpinen-4-ol was a predominant compound and accounted for 25.5% of the total peak area [25]. The other quantitatively important compounds also included in the monoterpene group: γ -terpinene (18.5%), α -terpinene (17.1%), α -pinene (8.04%), α -terpinolene (5.7%), *m*-cymene (4.7%) and sabinene (4.5%). All identified monoterpenes are known as TTEO marker compounds and contributed to antioxidant and antimicrobial activity of TTEO [15]; [25]. Sixteen sesquiterpenes were identified in minor share (9.4%).

3.2. Film characterization

The film-forming solution was prepared at room temperature obtaining a low energy demand process. An acid concentration up to 2% was needed to reach a complete dissolution of chitosan. In the lactic acid solutions, a significant amount of foam was formed during the stirring process of chitosan and during the homogeneous dispersion of soy lecithin and essential oil. The foam was easily removed under ultrasound treatment, giving a transparent solution free of air bubbles. The addition of soy lecithin and essential oil resulted in solutions with a milky appearance, as shown Fig. 1a. Despite the whitish appearance of the chitosan-lecithin-essential oil solution (Fig. 1b), once the water has

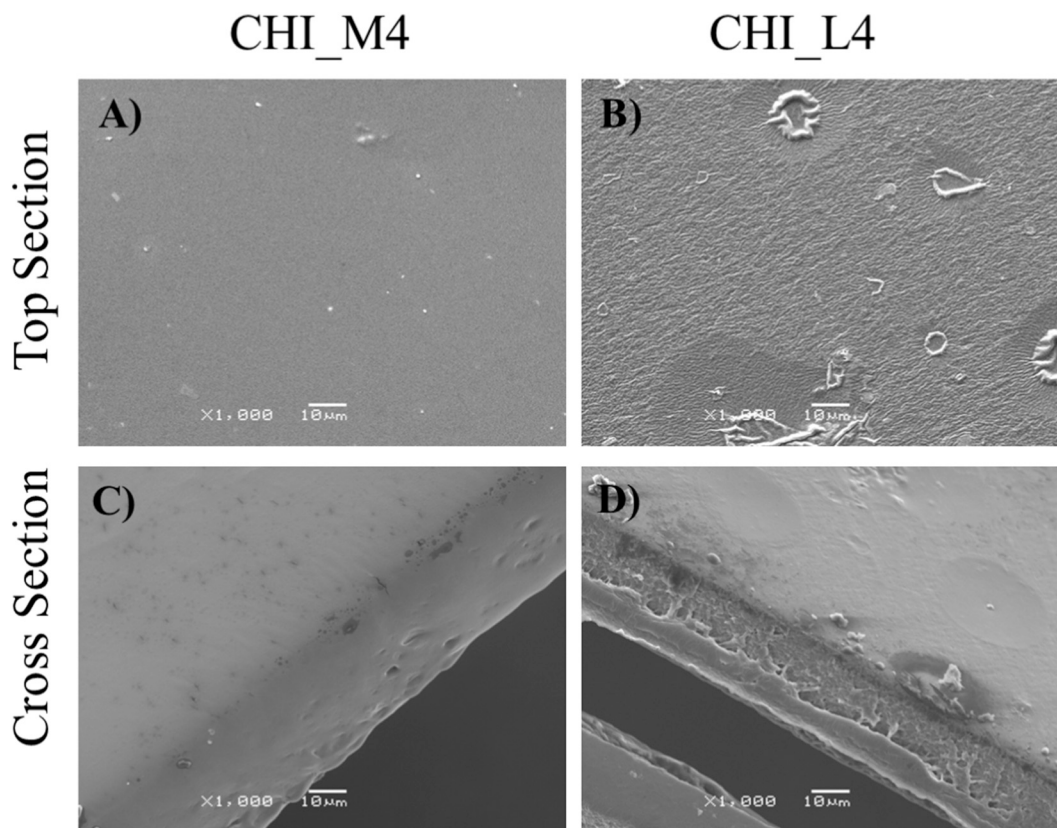


Fig. 2. Scanning electron microscopy images of chitosan films with tea tree essential oil 1.0% (v/v). Experiments are defined in Table 1.

evaporated during the casting process, the final film obtained did not have the whitish colour of the solution and shows the transparent colour of pure chitosan samples, as shown Fig. 1c.

The films formulated from lactic acid were peeled off and handle easily after drying. The films obtained from malic acid were more adhesive. This adhesive characteristic of chitosan-based films from malic acid could be a great advantage to be applied easily as film or coating on food.

The formulations of the experiments are shown in Table 1. The average thickness of the samples ranged between $4.31 \cdot 10^{-2}$ and $5.18 \cdot 10^{-2}$ mm.

3.3. Scanning electron microscopy (SEM)

The SEM images of the surface and cross section (Fig. 2) of the developed Ch-films showed a homogeneous dispersion of the soy lecithin and TTEO. The SEM images showed a smooth surface without the presence of cracks or disruptions due to the presence of TTEO throughout the matrix. In addition, the absence of cracks in the films from lactic acid solutions (Fig. 2b–d) confirms that the ultrasound treatment was sufficient to remove the excess bubbles produced under agitation.

The cross-section SEM images (Fig. 2c–d) showed a compact structure of the Ch-films produced from both acid solvents. Note the difference in cut-off between films from the malic acid solutions (Fig. 3c) and lactic acid (Fig. 3d). SEM images of films with lactic acid showed a clean cut, unlike the cross section of films with malic acid. The cross section of samples with malic acid showed a rubbery behaviour. This behaviour of the films with malic acid concurred with the adhesive behaviour observed during the handling of the samples.

In literature, SEM images of chitosan-based films elaborated from acetic acid solutions and enriched with TTEO without any emulsifier showed a discontinuous structure formed by lipid droplets embedded in

the continuous chitosan network [8]. Therefore, the addition of small amounts of soy lecithin (0.1% w/v) achieved a homogeneous dispersion of the essential oil in the matrix. Soy lecithin prevented the agglomeration of oil molecules into larger droplets that could cause a greater disruption in the chitosan network.

3.4. Fourier transform infrared spectroscopy (FT-IR)

The FT-IR spectra of pure chitosan samples obtained from malic acid and lactic acid solutions in the range between 4000 and 800 cm^{-1} are shown in Fig. 4a. FT-IR spectra of chitosan-based films with soy lecithin and TTEO using malic acid and lactic acid are also shown in Fig. 3.

All spectra showed the characteristic absorbance peaks of the pure Ch-films. The broad absorption band between 3500 and 3200 cm^{-1} corresponded to the overlapping of the stretching vibration of hydroxyl groups ($-\text{OH}$) and the asymmetric and symmetric stretching of the $\text{N}-\text{H}$ bonds in the amino group of the chitosan [11]; [14]. The absorption peaks at 2928 cm^{-1} corresponded to the stretching vibrations of the $\text{C}-\text{H}$ bond in $-\text{CH}_2$ group [26]. The absorption peaks at 1647 cm^{-1} and 1554 cm^{-1} were attributed to $\text{C}=\text{O}$ stretching (amide I) and $\text{N}-\text{H}$ bending (amide II), respectively [27].

The main differences between the spectra of samples from malic or lactic acid were found in the region from 2000 to 1000 cm^{-1} . Similar result was observed in samples elaborated from lactic acid and acetic acid [28]. The higher peaks shifted in films from malic acid in the $2000-1000$ cm^{-1} range were due to the variations in the protonated amine concentration of the samples. The interaction between the solvent acid and chitosan increases as the pK_a of the acid decreases. The pK_a of malic and lactic acid are 3.40 and 3.86 , respectively, observing lower absorbance peaks for samples with lactic acid [28].

The spectra of the samples enriched with TTEO showed the characteristic peaks of the pure Ch-films with slight shifts and lower adsorption. These changes were probably due to the formation of new

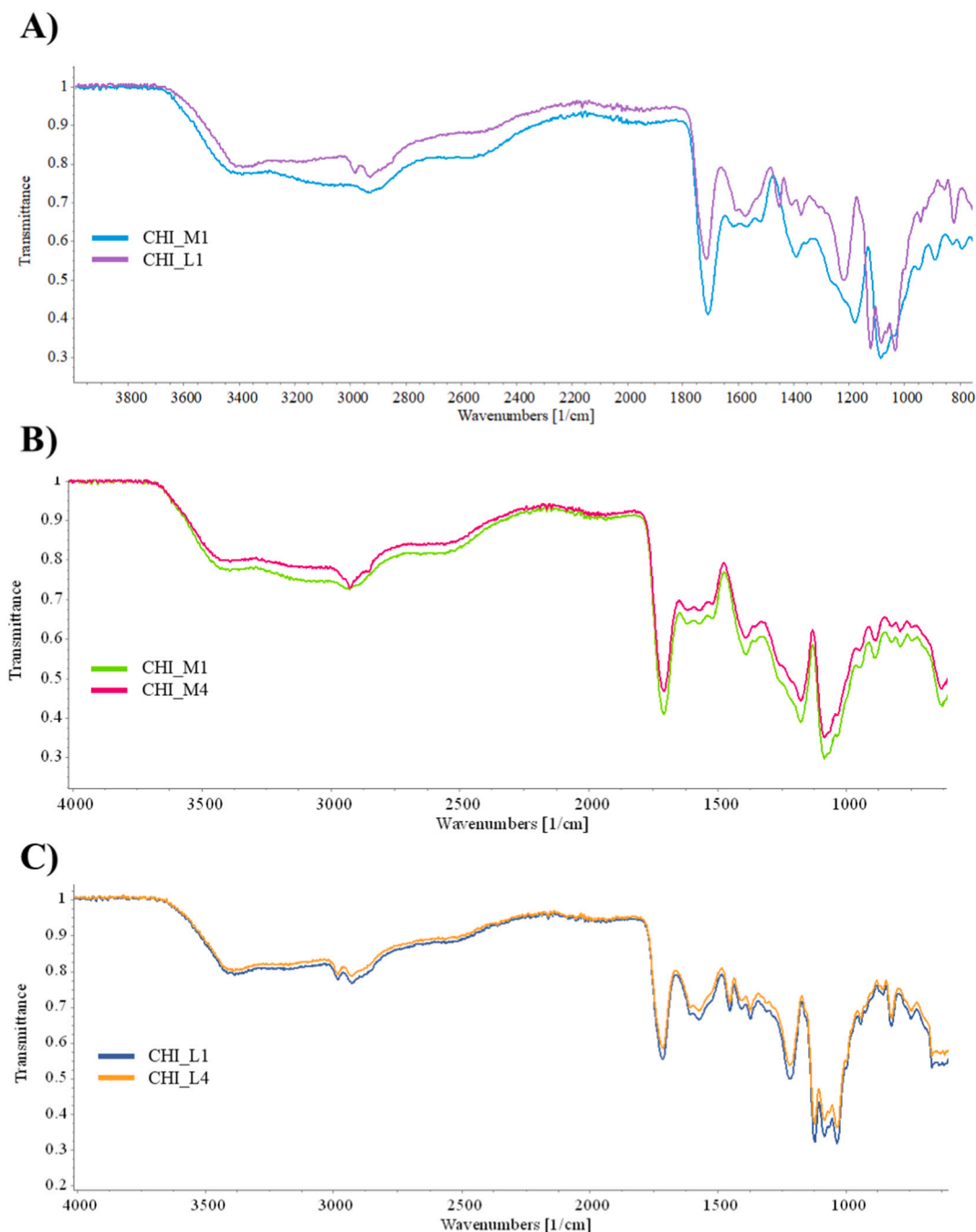


Fig. 3. FT-IR spectra of chitosan-based film with tea tree essential oil. A) FT-IR spectra of chitosan (CHI) samples using malic acid (CHI_M1) and lactic acid (CHI_L1) as solvent. B) FT-IR spectra of pure chitosan (CHI_M1) and with 1% tea tree essential oil (CHI_M4) samples using malic acid as solvent. C) FT-IR spectra of pure chitosan (CHI_L1) and with 1% tea tree essential oil (CHI_L4) samples using lactic acid as solvent.

hydrogen bonds between the -OH groups from compounds in TTEO (mostly from terpinen-4-ol) and the -NH^{3+} and -OH groups in chitosan. This observation leads to the assumption that there could be an arrangement and interaction in the film. Ch-films enriched with turmeric extract [26], citronella essential oil and cedarwood oil [27] and rosemary essential oil [11] showed a similar pattern.

3.5. Evaluation of the water solubility and equilibrium moisture content

The soluble matter of the samples was analysed to determine the ease of removal of TTEO-chitosan films or coatings. When the samples were submerged in the containers with distilled water, an instantaneous swelling of the sample was observed followed by the complete loss of the

structure. The results indicated 100% dissolution of the Ch-films made with malic acid or lactic acid. Addition of TTEO did not decrease the solubility of the samples.

Ch-films with lactic acid solvent dissolved completely in water within 24 h. The high solubility of Ch-films with malic acid was not previously reported. The complete dissolution of chitosan-glycerol samples using lactic acid as solvent was also observed [14]. These results compared very well with that for chitosan films using acetic acid as solvent were a water solubility of only 11.39% was obtained [29].

Moisture content (%W) of the equilibrated films at standard conditions was analysed. Previous studies demonstrated the relevant plasticizing effect of the water molecules on the inner structure of polysaccharide-based polymers, and consequently on their functional

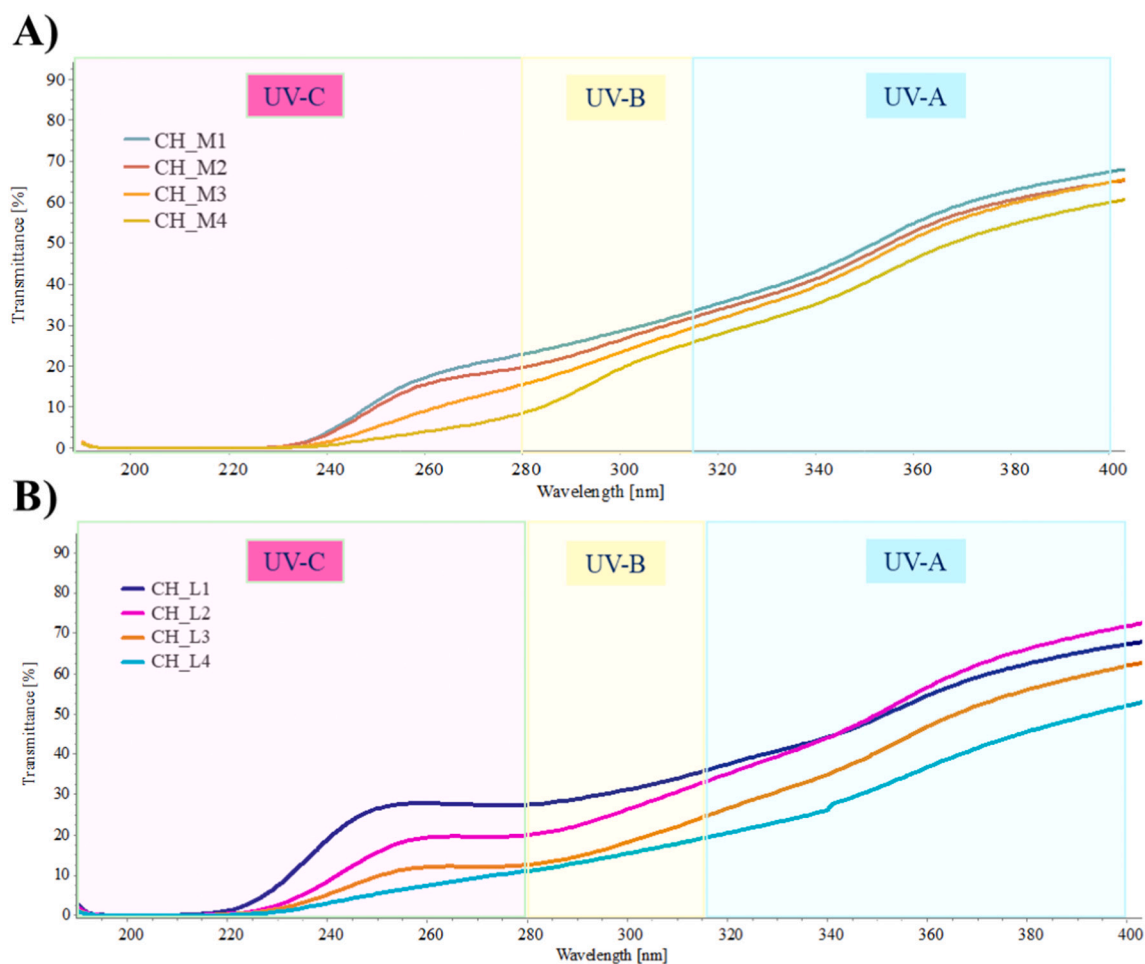


Fig. 4. UV-VIS spectra profile of A) Chitosan films with tea tree essential oil, using malic acid as solvent. B) Chitosan films with tea tree essential oil, using lactic acid as solvent. CHI, chitosan; L, lactic acid and M, malic acid.

properties [30,31].

As shown in Table 2, inclusion of TTEO into CH-films did not showed a significant influence on the %W values ($p < 0.05$). Results indicated that supplementation CH-films with hydrophobic TTEO (up to 1%) does not reduce the water-chitosan interactions which would result in a disarrangement of film network. Unlike, adding rosemary essential oil up to 1.5% (w/w) to chitosan films produced a significant increase of moisture content of the samples [11].

The moisture content of the samples strongly depended on the type of acid used in the film-forming solutions ($p < 0.05$), which influenced other properties. The difference in the moisture was due to the joint effect of several factors. First, the chemical structure differences of the acids used [15]. The protonation degree of the amide group of the chitosan and the presence of more hydroxyl groups in lactic acid provided higher amount of available active sites for water molecule interactions [14]. Furthermore, the intermolecular arrangement of chitosan in an aqueous solution is influenced by ionic strength and degree of dissociation. The type of acid may affect both the density of the bonds and the topological limitations of the film. Different interactions between chitosan-acid are represented by the spatial configuration of the chitosan molecules during film formation. These structural changes will affect the final properties of the samples [13]. Rhim et al. [12] also observed higher moisture content on chitosan-based film from lactic acid solution than from malic acid solutions.

3.6. Evaluation of the water vapor permeability (WVP)

The WVP values calculated for Ch-films using malic acid as solvent were ranged between $3.88 \cdot 10^{-11}$ - $4.38 \cdot 10^{-11}$ g/m \cdot s \cdot Pa for samples using malic acid and between $4.86 \cdot 10^{-11}$ - $6.16 \cdot 10^{-11}$ g/m \cdot s \cdot Pa for samples using lactic acid as solvent, as shown Table 2. The addition of TTEO did not showed a significant influence on WVP values of films ($p < 0.05$). Lower values of WVP was observed in Ch-films combined with bacterial cellulose and glycerol [32]. The non-significant influence of the TTEO concentration on WVP behaviour could be related with the low levels used. The hydrophobic nature of the essential oil was not sufficient to reduce the permeability of the film, which was probably also favoured by the internal structural changes in the matrix promoted by the presence of TTEO molecules.

Previous works indicated that higher oil phase ratio or reduced oil particle size hindered the diffusion of water molecules through the matrix [33]. TTEO up to 2% (w/w) showed a significant decrease of the WVP values of chitosan film elaborated from acetic acid solutions [8]. Similar results of WVP values as in our study were assayed in the enriched with essential oils Ch-films formulated with acetic acid [33]. It should be also noted that Ch-films with lactic acid showed slightly higher WVP values than with malic acid (Table 2).

As aforementioned in the FT-IR spectra section, it can be related with the properties of malic acid with more interactions between chitosan-malic acid than chitosan-lactic acid. It was observed that a lower degree of interaction between the components of the matrix (glycerol and chitosan) causes easier migration of water vapor molecules through the

Table 2
Physical and mechanical properties of chitosan films.

Film samples	%W	TS	%E	YM
	%	MPa	%	MPa
CH_M1	20.34 ± 0.31	4.23 ± 0.69 ^a	158.80 ± 8.93 ^a	2.85 ± 0.21 ^a
CH_M2	19.98 ± 0.57	3.40 ± 0.24 ^a	145.88 ± 27.40 ^a	2.95 ± 0.39 ^a
CH_M3	19.41 ± 0.55	3.51 ± 0.72 ^a	150.55 ± 25.10 ^a	2.96 ± 0.28 ^a
CH_M4	19.26 ± 0.82	1.54 ± 0.30 ^b	317.33 ± 22.84 ^b	1.81 ± 0.22 ^b
CH_L1	59.88 ± 0.65	6.32 ± 2.29 ^c	38.30 ± 12.04 ^c	33.96 ± 5.06 ^c
CH_L2	60.14 ± 3.15	6.15 ± 0.91 ^c	44.08 ± 7.37 ^c	33.72 ± 6.94 ^c
CH_L3	59.37 ± 0.90	4.40 ± 1.11 ^d	25.54 ± 7.30 ^d	29.40 ± 3.04 ^{cd}
CH_L4	58.68 ± 2.28	4.09 ± 0.61 ^d	33.10 ± 3.08 ^{cd}	23.01 ± 1.95 ^d

Film samples	T	BS	DB	WVP
	MJ/m ³	g	mm	g/m·s·Pa
CH_M1	3.12 ± 0.74 ^{ab}	351.84 ± 23.20 ^a	5.41 ± 0.31	3.88·10 ⁻¹¹ ± 9.58·10 ⁻¹³
CH_M2	2.30 ± 0.64 ^a	347.27 ± 46.57 ^a	5.72 ± 1.14	4.38·10 ⁻¹¹ ± 3.67·10 ⁻¹²
CH_M3	2.27 ± 0.99 ^a	175.64 ± 20.06 ^b	5.09 ± 0.11	4.42·10 ⁻¹¹ ± 4.73·10 ⁻¹²
CH_M4	4.07 ± 1.43 ^b	199.90 ± 39.51 ^b	5.62 ± 0.90	4.30·10 ⁻¹¹ ± 2.57·10 ⁻¹²
CH_L1	1.74 ± 0.90 ^c	434.58 ± 28.52 ^c	4.04 ± 0.20 ^c	5.11·10 ⁻¹¹ ± 6.04·10 ⁻¹²
CH_L2	1.94 ± 0.49 ^c	360.52 ± 0.05 ^d	3.56 ± 0.20 ^c	6.16·10 ⁻¹¹ ± 5.47·10 ⁻¹²
CH_L3	0.81 ± 0.31 ^d	438.80 ± 13.59 ^c	3.65 ± 0.18 ^d	4.89·10 ⁻¹¹ ± 1.19·10 ⁻¹¹
CH_L4	0.96 ± 0.11 ^d	358.05 ± 28.78 ^d	3.42 ± 0.22 ^d	4.86·10 ⁻¹¹ ± 2.82·10 ⁻¹³

CH - chitosan; M - malic acid; L - lactic acid; %W - equilibrium moisture content; TS - tensile strength; %E - percentage of elongation to break; YM - Young's modulus; T - toughness; BS - burst strength (puncture properties); DB - distance to burst (puncture properties); WVP - water vapor permeability properties. Values are expressed as mean ± standard deviation (SD). Different letters in the same column indicate significant differences ($p < 0.05$). Experiments are defined in Table 1.

film [29].

A slightly lower WVP values for Ch-films prepared with malic acid than with lactic acid was also reported [12,13]. The results obtained in these studies were 2 order of magnitude lower than those of the present study. The WVP values of Ch-films are affected by several factors, such as chitosan properties (molecular weight, deacetylation degree), film composition (chitosan, solvent type and/or plasticizing concentration), WVP measuring method and conditions (temperature, RH), correction of

Table 3
Antioxidant properties of the developed chitosan-based films.

Film samples	Radical scavenging activity on DPPH* and ABTS*+ ⁺	
	DPPH* (%)	ABTS*+ (%)
CH_M1	5.06 ± 1.59 ^a	1.31 ± 0.99 ^a
CH_M2	5.23 ± 0.79 ^a	1.93 ± 0.75 ^a
CH_M3	30.71 ± 1.35 ^b	11.02 ± 0.42 ^b
CH_M4	61.24 ± 0.64 ^c	25.41 ± 0.49 ^c
CH_L1	0.57 ± 0.41 ^d	3.30 ± 1.38 ^d
CH_L2	0.75 ± 0.40 ^d	2.18 ± 0.51 ^d
CH_L3	4.73 ± 1.62 ^e	3.39 ± 0.94 ^d
CH_L4	8.31 ± 2.12 ^f	7.57 ± 1.63 ^e

CH - chitosan; M - malic acid; L - lactic acid; TTEO - tea tree essential oil. Values are expressed as mean ± standard deviation (SD). Different letters in the same column indicate significant differences ($p < 0.05$). Experiments are defined in Table 1.

air gap effect and film thickness [12]. Thus, the WVP values in the literature should be compared with caution, taking into account all these factors.

3.7. Evaluation of the mechanical properties

The results of mechanical properties of Ch-films calculated by the tensile and puncture tests are presented in Table 2. The tensile test for Ch-films with malic acid (CH_M1 and CH_M2) showed values in the ranges 1.54–4.23 MPa for TS, 145.88–317.33% for %E, 1.81–2.96 MPa for YM and 2.27–4.07 MJ/m³ for T. Inclusion of the TTEO in the film formulation had a significant effect ($p < 0.05$) on the tensile parameters. Subsequently, the Tukey post hoc test detected significant differences of the means ($p < 0.05$) at the highest TTEO concentration.

Using lactic acid as solvent, Ch-films (CH_L1 and CH_L2) ranged 4.09–6.32 MPa for TS, 25.54–44.08% for %E, 23.01–33.96 MPa for YM and 0.81–1.74 MJ/m³ for T. Similarly like in the films with malic acid, addition of TTEO significantly influenced the tensile parameters of the films with lactic acid (Table 2).

The results showed the decrease in resistance to break and the increase in deformation, decreasing the TS and YM values and increasing the %E and T values in films contained 1% TTEO. This behaviour responded to a plasticizing effect, indicating that addition of TTEO up to 1% (v/v) promoted a plasticizing effect on the chitosan network.

The plasticizing effect could be explained by the partial replacement of stronger polymer-polymer interactions by weaker polymer-essential oil interactions in the film network. The new formed interactions may reduce the cohesion of the polymer network forces, decreasing the TS and YM values and increasing the %E and T values. The plasticizing effect is strongly determined by the essential oil ratio in the matrix and the solvent used to obtain the chitosan film-forming solution. The beneficial plasticizing result was achieved with using malic acid solution and higher TTEO concentrations.

The parameter that attracted the most attention was %E, giving values between 4 and 10 times higher for films made with malic than with lactic acid. This phenomenon is due to how chitosan chains interact and the acid solvent role. Chitosan network is formed by the hydrogen bonds between hydroxyl groups and amino groups in the polymer chains. These interactions increase with the increasing amount of amino and hydroxyl groups. For that reason, the intermolecular arrangement of chitosan in an aqueous solution is influenced by the properties of the acid solutions, such as ionic strength, degree of dissociation and counter ion structure. Besides, different interactions are represented by the spatial configuration of the chitosan molecules during film formation [12–14]. Consequently, the effect of the oil and how it interacted with the polymer chains, depended on the solvent used, which determined the interactions between polymer chains and the conformation of the chitosan network.

The phospholipid compositions of the soy lecithin could contribute to the negative charge of the chitosan solution. The inclusion of phospholipids into chitosan solution could contribute to formation the surface-active amphiphilic molecules. The anionic charge may interact with the positive charge of protonated chitosan in acid solution and modify the chitosan network [16]. The plasticizing effect of oils also depends on the presence or absence of emulsifying agents which allow homogeneous dispersion and avoid the formation of large droplets. Ch-films enriched with citronella essential oil, cedarwood oil [27], *Zataria multiflora* Boiss essential oil [34] showed a plasticizing effect up to 10% of oil concentration. [27] reported that at higher essential oil concentrations, the mechanical values dropped by an excessive substitution of polymer-polymer bonds by polymer-essential oil bonds, giving weak structures with lower deformation capacity.

Ch-films enriched with TTEO or bergamot essential oil films without emulsifying agent showed a significant decrease in tensile strength, deformation at break and elastic modulus. The authors justify the general decrease in all mechanical properties due to the no homogeneous

Table 4
Optical properties of the chitosan-based films.

Film samples	UV-C %T	UV-B %T	UV-A %T	Transparency	Opacity
CH_M1	11.45 ± 2.85	29.06 ± 5.69	51.34 ± 3.63	51.88	3.01
CH_M2	9.86 ± 1.97	26.72 ± 4.02	49.28 ± 3.29	48.80	3.29
CH_M3	7.73 ± 1.54	23.43 ± 3.29	48.14 ± 2.46	52.07	3.06
CH_M4	4.28 ± 2.99	18.15 ± 6.12	43.85 ± 6.72	49.03	3.48
CH_L1	13.75 ± 1.80·10 ⁻²	27.13 ± 6.23	48.27 ± 4.90	49.35	2.79
CH_L2	9.99 ± 2.23·10 ⁻²	17.34 ± 11.79	41.70 ± 13.67	46.49	2.83
CH_L3	6.30 ± 7.07·10 ⁻⁴	14.55 ± 5.80	38.89 ± 6.23	43.14	2.72
CH_L4	5.53 ± 0.46	21.12 ± 6.24	41.69 ± 6.33	51.10	3.62

CH - chitosan; M - malic acid; L - lactic acid.

UV-C (200–280 nm).

UV-B (280–315 nm).

UV-A (315–400 nm).

%T - percentage of transmittance.

Values are expressed as mean ± standard deviation (SD).

Different letters in the same column indicate significant differences ($p < 0.05$).

Experiments are defined in Table 1.

arrangement of the lipid phase in the polymeric matrix [8,35].

The values obtained by the puncture test showed a significant effect of TTEO addition on values of BS in Ch-films with malic acid (Table 2). Addition of TTEO decreased the values BS. There were no such clear results of BS in Ch-films with lactic acid. The deformation suffered by the samples and how the polymer chains must restructure under the pressure of forces with different directions is different from the restructuring and deformation under horizontal forces. For this reason, the same interactions within the matrix can show different resistance and deformation behaviour under tensile or puncture forces [36].

3.8. Antioxidant properties

Table 3 shows the antioxidant activity of all Ch-films determined by two methods: ABTS^{•+} and DPPH[•], which allow to assay the capacity of scavenge free radicals. As it was reported in previous works, all pure Ch-films (CH_M1, CH_M2, CH_L1 and CH_L2) showed antioxidant activity in both methods, regardless of the presence of an emulsifier [9,34].

The antioxidant activity of the CH_M1 and CH_L1 samples represented 1.31 and 3.3% inhibition by ABTS^{•+} method, respectively. This activity can be attributed to the capacity of chitosan amino groups to react with free radicals to form ammonium groups (NH₃⁺). The scavenging activity of these samples might also be provided by the antioxidant properties of the malic and lactic acid [37,38]. The solvent used to obtain the film-forming solution has a direct effect on the chitosan protonation and the matrix conformation, affecting the antioxidant properties of pure and enriched films.

After adding TTEO, the antioxidant activity of films was substantially increased. The degree of antioxidant capacity of supplemented films was proportional to the amount of TTEO added (0.5 and 1%). The well-known antioxidant properties of TTEO are attributed to terpenic compounds which substantial amount confirmed in our study (Table S1). They can remain in the chitosan matrix and provided the improved antioxidant properties to the film. The obtained results are in accordance with the increasing scavenging activities observed in Ch-films with *Eucalyptus globulus* essential oil [9].

Irrespectively of the kind of organic acid in formulation, the DPPH[•] scavenging rates of TTEO supplemented films were about 6.5–12-fold higher than that of pure films (Table 3). However, such spectacular

Table 5
Colour parameters of the chitosan-based films.

Film samples	L*	a*	b*
CH_M1	91.41 ± 0.18	-0.58 ± 0.13	4.12 ± 0.68
CH_M2	90.10 ± 0.77	-0.41 ± 0.02	4.09 ± 0.34
CH_M3	91.63 ± 0.04	-0.46 ± 0.10	5.38 ± 0.43
CH_M4	90.15 ± 2.29	-0.34 ± 0.16	6.51 ± 1.52
CH_L1	91.85 ± 0.35	-0.93 ± 0.16	5.73 ± 1.12
CH_L2	91.49 ± 3.59	-1.11 ± 0.33	5.64 ± 2.38
CH_L3	91.03 ± 0.17	-1.27 ± 0.27	6.84 ± 1.87
CH_L4	90.16 ± 1.17	-0.80 ± 0.04	7.30 ± 1.70

CH - chitosan; M - malic acid; L - lactic acid.

L*, lightness: black = 0 and white = 100; a*, green = -a* and red = +a*; b*, blue = -b* and yellow = +b*.

Values are expressed as mean ± standard deviation (SD).

Experiments are defined in Table 1.

effect in increasing of antioxidant activity after adding TTEO, assayed by scavenging of ABTS^{•+} free radicals, was stated only in the case of films with malic acid. It is necessary to note, that enriched with TTEO Ch-films contained malic acid were distinguished by 6.5–7.5 higher DPPH[•] scavenging capacity than that contained lactic acid. It can be explained by proved synergic effect of malic acid as promoter agents for antioxidant activity of terpenes [39].

3.9. Evaluation of the optical properties

Optical properties of the Ch-films were evaluated through the transmittance properties in the UV region. The colour, transparency and opacity values were calculated from the transmittance values in the Vis region. These parameters provide useful information regarding the potential applications of these films to protect food against UV-light, keeping transparency high and opacity low. Formulation of polysaccharide films with UV blocking properties may be an effective tool to prevent negative effect on flavour quality, light-sensitive nutrients and loss of vitamins. The direct UV light on food might lead to the prompting of a series of reactions that are initiated by the release of singlet oxygen, causing discoloration, nutrient losses, off-flavours and photooxidation of lipids and vitamins [40,41].

The average percentage of transmittance (%T) in the UV-C (200–280 nm), UV-B (280–315 nm) and UV-A (315–400 nm) regions of the studied Ch-films are shown in Table 4. The UV profile spectra of the films are shown in Fig. 4.

Ch-films from malic acid solutions showed %T values ranged 11.45–4.28%, 29.06–18.15% and 51.34–43.85% in the UVC, UVB and UVA regions, respectively. Similar trend was observed for Ch-films using lactic acid as solvent, with average values ranged between 13.75 and 5.53%, 27.13–21.12% and 48.27–41.69%, respectively. Pure chitosan samples showed similar data and spectra that those previously reported [10,42]. Data revealed that the active compounds of TTEO prompted a slightly increase of the absorbance values, enhancing the UV-blocking properties of the formulated films. No significant differences were found as function of the solvent used to prepare the film-forming solution.

These results were remarkable in the UV-C region, obtaining transmittance values below 6%. The extra protection of antioxidant agents in Ch-films against oxidative processes induced by UV light were also observed by the addition of mint and pomegranate peel extract [43], clove essential oil [44] ellagic acid [45], ginger, rosemary, sage, tea tree and thyme essential oils [46] or α -tocopherol among others samples [47]. On the other hand, [46] reported lower blocking capacity of the essential oils than theirs hydro-alcoholic extracts. Besides the UV-blocking properties, the developed films should also be pretty transparent to achieve the approval of the consumers. The calculated transparency values (Table 4) remained in the range 52.07–48.80 and 51.10–43.14 for Ch-films from malic acid and lactic acid solutions,

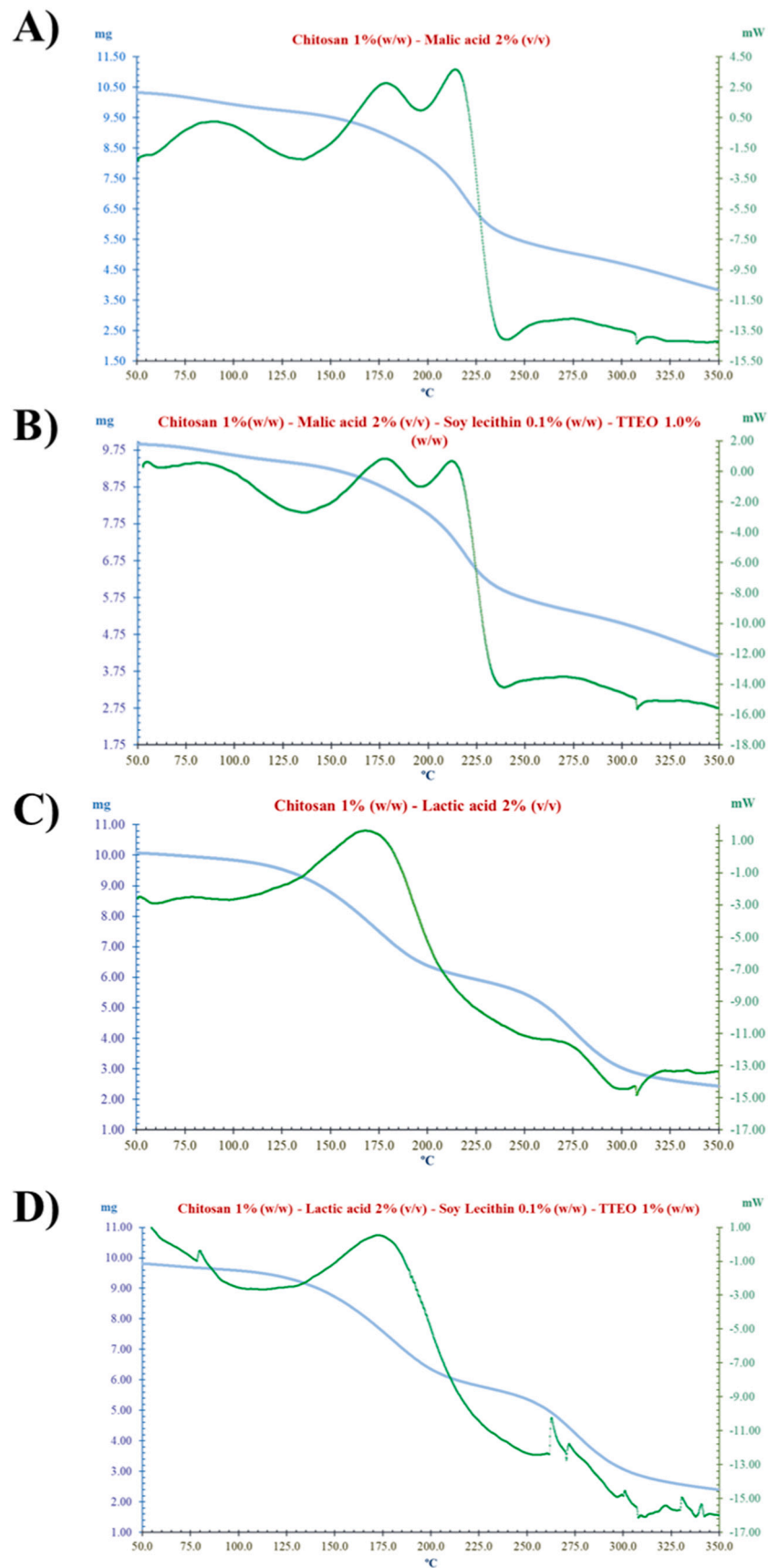


Fig. 5. Thermogravimetry and differential scanning calorimetry of the selected chitosan - tea tree essential oil films.

respectively. The opacity values ranged 3.01–3.48 and between 2.79 and 3.62, respectively. Overall, a clear trend of the TTEO effect on the transparency of the films was not observed, obtaining values in a similar range. However, at higher TTEO concentration, the opacity suffered a slight increase, but still maintaining very low values.

The CIE coordinates values (Table 5) obtained for lightness (L^*) varied from 90.10 to 91.63 for Ch-films with malic acid and from 90.16 to 91.85 for lactic acid. The addition of TTEO up to 1% (w/w) did not have a statistically significant effect ($p < 0.05$). The L^* results were close to 100 (white) and all films can be considered bright, in accordance with the transparency results obtained for all samples. Pure and TTEO Ch-films showed a light yellowish tint, with b^* values ranged from 4.09 to 6.51 using malic acid and from 5.73 to 7.30 using lactic acid. The yellowish colour is an intrinsic properties of chitosan [29]. As observed in the L^* values, the TTEO concentrations did not show a significant effect on the a^* and b^* parameters. Similar trend was observed in Ch-films enriched with gallic acid [48] and wheat starch-chitosan film enriched with basil essential oil, thyme essential oil, citric acid and α -tocopherol [49]. The CIE Lab values of the analysed samples were in agreement with previous works [29,46].

3.10. Evaluation of the thermal properties (TGA/DSC)

The effects of TTEO and the solvent on the thermal physical property of Ch-films were evaluated by TGA/DSC. The thermogram of pure chitosan samples from malic and lactic acid and enriched samples with TTEO 1.0% (v/v) are shown in Fig. 5.

The endothermic peak ranged 50–110 °C is associated to the loss of adsorbed water [50,51]. The volatilization of the water supposed a loss of weight for each sample. TTEO up to 1% (v/v) produced a slightly decrease of the loss weight until 110 °C due to its lipidic profile. However, these values were far from the values obtained in the analysis of the moisture content. Part of the water may be evaporated before carrying out the DSC-TGA analysis and until the analysis starting temperature was reached (50 °C). Besides, the period exposed at the temperature might affect the results.

As illustrated in Fig. 5a and b, the thermogram of samples CH_M1 and CH_M4 related to Ch-films obtained with malic acid as solvent, exhibited two endothermic peaks between 140 and 240 °C. These bands correspond to the chemisorbed water through hydrogen bonds, the degradation of the amino group of the chitosan and the degradation of the malic acid [5,10,47]. In this region, the weight loss of the pure chitosan samples CH_M1 was 37.82% while Ch-films with TTEO (CH_M4) was 33.90%.

The band observed between 240 and 350 °C was related with the dehydration, depolymerization and pyrolytic decomposition of the chitosan [47]. At 350 °C the total weight losses of the samples CH_M1 and CH_M4 were 62.08% and 57.31%, respectively.

The thermograms of the samples CH_L1 (pure chitosan) and CH_L4 (chitosan and 1% TTEO) produced from lactic acid in Fig. 5c and d, showed a second single broader peak from 125 to 200 °C. In this case, the weight losses at 200 °C were 36.26% and 34.73% for samples CH_L1 and CH_L4, respectively. The slightly decrease of the weight loss of samples with TTEO was observed, but to a lesser extent as well. At 240 °C, the weight losses were 43.09% for sample CH_L1 and 42.80% for sample CH_L4. At 350 °C, the total weight losses of the samples CH_L1 and CH_L4 were 75.55% and 75.21%, respectively.

Results showed higher weight loss values in lactic acid samples than those observed in samples formulated with malic acid at the same temperature. In accordance with the results obtained in the previous analysed properties, due to the greater number of interactions in the films made from malic than lactic acid, a greater thermal stability was also observed in films with malic acid.

Besides, results suggested that the new interactions between the chitosan polymer and the TTEO compounds could promote the thermal stability of the samples, diminishing slightly the weight loss of the

samples. These interactions were most effective in films with malic acid.

The results suggested that TTEO-chitosan films are stable at temperature below 120 °C. The developed films could be suitable to apply on foods under heat treatments, such as pasteurization for chitosan-lactic acid samples and even more intense heat treatments in the case of chitosan-malic acid samples [52].

4. Conclusions

The production of the chitosan film-forming solution using other solvents than acetic acid makes possible to obtain films with completely different functional properties which could be better adapted to specific purposes.

Malic acid or lactic acid as solvents for chitosan gave films with extraordinary mechanical properties (high mechanical deformation) that are instantly soluble in water. This quality makes it possible to apply this polymer as film or coating in foods such as fruits and vegetables, which once they are desired to be consumed can be easily removed by washing with water.

The addition of TTEO, an essential oil with well-known antimicrobial and antioxidant properties, allowed to improve the deformation properties of the film and giving films with powerful antioxidant capacity.

Thermal properties indicated the suitability of the TTEO-chitosan films for certain heat food treatments.

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Patricia Cazón: Investigation, Writing- Original draft preparation.
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Jaroslawa Rutkowska: Methodology, Writing- Reviewing and Editing.
Manuel Vazquez: Conceptualization, Methodology, Writing- Reviewing and Editing.

Declaration of competing interest

The authors have no conflict of interest to declare.

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