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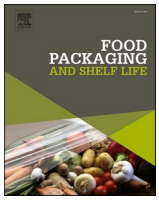

# Active packaging film of chitosan and *Santalum album* essential oil: Characterization and application as butter sachet to retard lipid oxidation

María Flórez, Patricia Cazón, Manuel Vázquez *

*Department of Analytical Chemistry, Faculty of Veterinary, University of Santiago de Compostela, 27002 Lugo, Spain*

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ABSTRACT

Chitosan with sandalwood (*Santalum album*) essential oil (SEO) using malic acid as solvent were evaluated as an active packaging film. It was applied as sachet to butter packaging. The effects of SEO concentration (0.5 %, 1 % and 2 % v/v) on chitosan film properties were studied by measuring the equilibrium moisture content, solubility, water vapor permeability (WVP), mechanical, optical, heat sealability, antioxidant properties, surface morphology and thermostability. SEO showed a significant effect on the film properties except for the puncture properties and the equilibrium moisture values. SEO promoted a significant decrease in tensile strength from 5.78 to 2.99 MPa, Young's Modulus (YM) from 35.74 to 6.81 MPa, WVP from $6.70{\cdot}10^{-11}$ to $3.34{\cdot}10^{-11}$ g/m·s·Pa, and sealability from 195.20 to 107.94 N/m. The antioxidant properties of the films were improved with the presence of SEO. The addition of SEO significantly improved the UV-barrier of the films. The color and transparency of the samples showed significant variations by the addition of SEO. The active packaging film was evaluated as butter sachet. After 3 months of butter storage, a significant decrease of the thiobarbituric acid reactive substances was observed, showing a 36 % decrease in the lipid oxidation compared to unpackaged samples. The films are completely water-soluble and can be easily removed from foodstuffs after use without generating solid wastes.

## 1. Introduction

Food packaging design is one of the most important phases in the food chain. A perfect food packaging material should protect food quality over time, being transportable, convenient to use, economical, and renewable or biodegradable (Priyadarshi & Rhim, 2020). Moreover, if the packaging has a positive effect on the food, as in the case of active packaging, its use becomes even more interesting. Active films are films deliberately enriched with active components. These components release or absorb chemicals in order to prolong the shelf life of the food while maintaining its sensory and quality characteristics (Yildirim et al., 2018). Natural and renewable materials are gaining interest due to the major environmental problems generated by the uncontrolled use of petroleum derivatives, but also because the consumer has undergone a trend towards eco-friendly habits.

A wide variety of polysaccharides are being studied for development as films intended for food use, such as cellulose (Sirviö et al., 2014), starch (Abreu et al., 2015), alginate (Parreidt et al., 2018) and chitosan (Priyadarshi et al., 2018). Chitosan is the second most abundant polysaccharide in the world. The exoskeleton of crustaceans is the main source to obtain chitin and then chitosan by deacetylation. Chitosan and its derivatives can inhibit the growth of a wide range of molds, yeasts and bacteria. Chitosan properties like solubility, film forming ability, viscosity, chelating ability, antimicrobial properties, among others, make it an interesting material to apply in multiple areas, including food packaging (Priyadarshi & Rhim, 2020). Chitosan is soluble in dilute organic acid which facilitates its application as a film or coating. The properties of the chitosan matrix are highly dependent on the type of solvent used (Rhim et al., 1998). The correct selection of the acid used as solvent will allow to adapt the properties of the matrix to its purpose. For example, the use of malic acid results in water-soluble films that can be easily removed from foodstuff (Cazón et al., 2021). Water soluble films can be applied in foods with high fat content and low moisture content, such as butter, margarine, lard, and oil.

On the other hand, several natural substances, as essential oils are often added to chitosan films to enhance their active properties. Essential oils are aromatic oily liquids obtained from various parts of plants, such as flowers, seeds, roots, wood, herbs, leaves or shoots (Burt,

* Corresponding author.
*E-mail address:* manuel.vazquez@usc.es (M. Vázquez).

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**Table 1**
Films composition of lecithin and sandalwood essential oil (SEO). Samples contains also chitosan 1 % (w/w) and malic acid 2 % (w/v).

| Film samples | Lecithin % (w/v) | SEO % (v/v) |
|---|---|---|
| SEO-chitosan_0 | 0 | 0 |
| SEO-chitosan_0.5 | 0.1 | 0.5 |
| SEO-chitosan_1 | 0.1 | 1.0 |
| SEO-chitosan_2 | 0.1 | 2.0 |

2004). Sandal is a type of wood from trees of the genus *Santalum*. Sandalwood essential oil (SEO) is clear to yellowish in hue and thick. Its main use is in the perfume industry, due to its aroma and fixative properties. However, it is also used in the food industry as a flavor ingredient. This essential oil is made up of two main components known as sesquiterpene alcohols: *cis*-α-santalol (7–60 %) and *cis*-β-santalol (7–33 %). These alcohols are responsible of the characteristic odor of sandalwood. Nevertheless, there are minor constituents that should be taken into account, such as sesquiterpene hydrocarbons (6 %), esters (2–4 %), phenols, lactones, terpenes, volatile compounds and fragrant substances (Burdock & Carabin, 2008; Subasinghe et al., 2013).

SEO has been approved as generally recognized as safe (GRAS) for use in food as a flavoring component by the Flavor and Extract Manufacturers Association (No. 3005). It also has been approved by the FDA as a natural flavoring substance that can be used in combination with other flavors. Council of Europe added sandalwood to the list of substances, spices, and seasonings (Burdock & Carabin, 2008).

The chitosan films enriched with essential oils have been tested in multiple foodstuffs. However, no studies have been carried out on water-soluble chitosan-based active films for extending the shelf-life of foods. The main advantage of these films is that they are easily disposable without environmental impact, but their soluble nature limits their applications in foods with high moisture content. Nevertheless, they can be an useful tool for preserving foods with high lipid content. This type of food tends to suffer easily from lipid oxidation, which causes off-flavors and degradation of colors and nutrients (Vieira et al., 2017).

To our knowledge, SEO-chitosan films have not been studied. Considering the potential application of SEO in food packaging and the suitability of chitosan as a matrix to carry active agents, the objectives of this study were to develop and characterize the physic-chemical properties of a novel SEO-chitosan film. The effect of SEO at different ratio on the mechanical, water vapor permeability (WVP), optical, equilibrium moisture content (%W), solubility, sealability (S), and antioxidant properties of chitosan films were evaluated. The surface morphology, compatibility, and thermostability of the film were also evaluated using scanning electron microscope (SEM), Fourier transform infrared spectroscopy (FT-IR) and simultaneous thermal analysis (TGA/DSC). Considering the water-soluble nature of chitosan film from malic acid solutions (Cazón et al., 2021), its antioxidant and lipid oxidation retarding capacity has been evaluated in high-fat foods, by selecting butter as a model food. Its application in butter has been tested by analysing the antioxidant and optical properties of the films as well as the thiobarbituric acid reactive substances (TBARS) of the butter samples after 90 days of storage.

## 2. Experimental

### 2.1. Materials

Chitosan ($M_w$ 100000–300000 and CAS number 9012–76–4) was purchased from Acros organics (Geel, Belgium) and DL-malic acid extra pure (CAS number 6915–15–7) was provided by Scharlau Microbiology (Barcelona, Spain). Soy lecithin supplied by Ynsadiet (Madrid, Spain) and pure sandalwood essential oil supplied by Pranarôm (Ghislenghien, Belgium) were used to prepare the chitosan film-forming solution. The food packaging properties of the developed films were tested on butter purchased from Mantequería Arias (Madrid, Spain).

### 2.2. Preparation of films

Chitosan films were made by dissolving 1 % (w/w) chitosan in an aqueous solution of 2 % (w/v) malic acid (Table 1). The acid concentration was adjusted until the chitosan was completely dissolved, yielding a clear solution. The stock solution was kept stirred overnight at room temperature. As a natural emulsifier, soy lecithin (0.1 % w/v) was added to the solution. Chitosan-soy lecithin solution was homogenized at 15,000 rpm for 5 min (Ultra Turrax®, IKA, Staufen, Germany). The foam formed in the mixture was removed with the aid of an ultrasonic bath for 15 min. Subsequently, the SEO was added until a final concentration ranged 0–2% (v/v), and homogenized at 15,000 rpm for 5 min following by a degassed step in an ultrasonic bath for 15 min.

From the resulting film-forming solution, 40 ml were poured in a Petri dishes of 210 mm diameter and dried for 48 h at room temperature. The films were cut to a specific size and stored for 5 days at determined conditions depending on the test. The thickness (mm) was measured at five random locations using a Thickness Meter ET115S (Etari GmbH, Stuttgart, Germany).

### 2.3. Scanning electron microscope (SEM)

SEM pictures were used to examine and characterize the morphology of SEO-chitosan films made with malic acid as solvent. High-vacuum microscope (JEOL JSM-6360LV, Jeol Ltd, Tokyo, Japan) was used to photograph dried and gold-coated samples at an accelerating voltage of 20 kV. Using conductive double-sided carbon tape, samples were adhered to slides.

### 2.4. Fourier transform infrared spectroscopy (FT-IR)

FT-IR ABB Bomen 102 (ABB Ltd, Zurich Switzerland) fitted with a Universal Attenuated Total Reflectance (UATR) accessory (SPECAC Golden Gate) with diamond crystal was used to examine the presence of certain chemical groups and crosslinking in the films. The selected samples were conditioned for 48 h at 65 ± 2 % relative humidity and 21 ± 1 ºC. FTIR spectra in the range (400–4000) $cm^{-1}$ were recorded at a spectral resolution of 4 $cm^{-1}$.

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

Water solubility of the samples was measured using the gravimetric method of immersion in a known volume of distilled water, as described elsewhere (Cazón et al., 2019). The equilibrium moisture content (%W) was estimated using the gravimetric method by comparing the weights of conditioned samples at 33 % relative humidity (% RH) and dried samples (Cazón et al., 2019). Water vapor permeability (WVP) was measured following the ASTM Standard Test Method E96 (https://www.astm.org/Standards/E96.htm). The WVP of the samples was determined at 30 ºC and considering 50 % RH, as an intermediate value between the two levels which define the RH gradient utilized in the WVP determination. The test was run at least 4 h, enough time to reach a dynamic equilibrium in the vapor flux. Each test was carried out by triplicate.

### 2.6. Mechanical properties analysis

Tensile and puncture tests were performed on the films with a texturometer (TA-XT plus, Stable Micro System, UK) and the accessories recommended for each test. Tensile strength (TS, MPa), percentage of elongation at break (%E, %) and Young's Modulus (YM, MPa) were measured by the tensile test. The test was performed using the ASTM standard method D-882 (https://www.astm.org/d0882–18.html), with the initial grips spacing set to 40 mm and the load rate at 1 mm/s. The

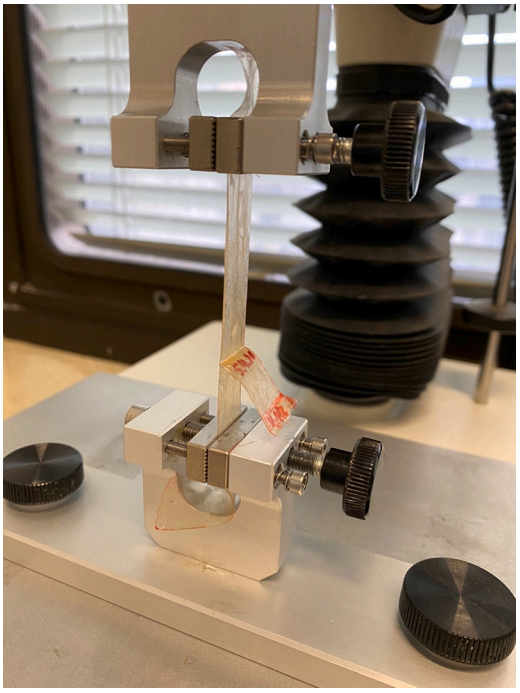

**Fig. 1.** SEO-chitosan film running sealability test. SEO is sandalwood essential oil.

samples were cut to 15 × 100 mm and conditioned for 5 days at room temperature and 57 % RH before being clamped between the grips of the texturometer. Seven replicates were tested for each batch (Cazón et al., 2018).

In the puncture test, a film holder (Reference HDP/FSR, Stable Micro System, UK) was used with the texturometer to hold the film sample. Sample squares for each batch were cut to 30 × 30 mm and conditioned at 57 % RH for at least 5 days before analysis. A cylindrical probe (d = 3 mm) with a speed of 1 mm/s was used and the force was recorded until rupture. The burst strength (BS) and distance to burst (DB) were computed using the force (g) against strain (mm) curves.

### 2.7. Seal strength property

Film samples were cut into 15 × 100 mm. Two film were placed together and an area of 10 × 10 mm at the edge of the films was heat-sealed using a vacuum heat-sealer (Model PH7 Food Machinery, Madrid, Spain) and a sealing time of 2.5 s. Seal strength of the heat-sealed films was determined according to the ASTM standard method F88/F88M-21 (https://www.astm.org/f0088_f0088m-21.html) using a texturometer (TA-XT plus, Stable Micro System, UK). Each tail of the sealed film was clamped to the opposing grips and the seal remains unsupported while the test is being conducted (technique A: unsupported) (Fig. 1). The distance between the grips was set to 50 mm. A 5 kg load cell and a test speed of 30 mm/min were used. The seal strength (N/m) was given as the highest force required to cause seal failure. Seal strength was calculated by the following Eq. 1 (Prateepchanachai et al., 2019):

$$\text{Seal strength (N/m)} = \text{peak force/film width} \quad (1)$$

### 2.8. Antioxidant properties of films

Two procedures were used to determine the antioxidant capacity of the pure SEO and the SEO-chitosan films: 1,1-diphenyl-2-picrylhydrazyl radicals ($DPPH^{\bullet}$) and the 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) free radical scavenging assay. The aliquots were obtained by the methanolic extracts of the films, using 1 g of sample in 24 ml of methanol and left overnight in darkness.

UV-Vis spectrophotometer V-670 (Jasco Inc, Japan) at 515 nm was used to examine the radical scavenging on $DPPH^{\bullet}$, and at 734 nm to analyze the $ABTS^{\bullet+}$ radical cation scavenging activity following the method described elsewhere (Antoniewska et al., 2018; Rutkowska et al., 2020). The results were expressed as % $DPPH^{\bullet}$ and % $ABTS^{\bullet+}$ scavenging activity.

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

Spectrophotometer V-670 (Jasco Inc, Japan) was used to measure the UV-Vis spectra of the film samples in the UV-Vis light ranges (190–800 nm) and optical properties of the samples were determined as described elsewhere (Cazón et al., 2019).

TGA/DSC (Mettler Toledo, Switzerland) thermogravimetry and differential scanning calorimetry equipment were used to analyze the thermal stability properties of the films. The samples were placed in hermetic aluminum pans, and the test was carried out at a heating rate of 10 ºC/min from 50 to 400 ºC, in a nitrogen atmosphere (50 ml/min).

### 2.10. Storage stability of butter packed in SEO-chitosan sachets

Rectangular samples of butter (6 g) were packed in chitosan sachets with different SEO concentration (0–2 %). Unpackaged butter samples were considered as control sample. The samples were stored at 5 °C for 3 months. The storage time was selected based on previous studies which indicate that there are significant changes in the quality of butter after 3 months in the refrigerator (Krause et al., 2008; Okturk et al., 2001).

The $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ parameters were analysed in unpacked and packed butter samples after 3 months of storage. The procedure followed was as aforementioned and the analysis was by triplicated.

The TBARS values in the butter samples were determined using a spectrophotometric method (Zeb & Ullah, 2016). Butter samples (1 g) was mixed with 5 ml of acetic acid glacial. Then, the solution was added to 0.5 µL butylhydroxytoluene (BHT). Extraction was performed with funnel and filter paper. Aliquot of 1 ml of extract was mixed with 1 ml of thiobarbituric acid solution. The mixture was incubated at 95 ºC for 1 h, then the absorbance was measured at 532 nm. The results were expressed as mg malondialdehyde (MDA)/kg butter.

Samples of packaged butter with the films were analyzed for color (L*, a*, and b* values) after 3 months of refrigerated storage at 5ºC using a colorimeter ColorStriker (Mathai, Hannover, Germany). Two measures were carried out for control, each treatment and replicate.

### 2.11. Statistical analysis

The results obtained were statistically analyzed employing Microsoft Excel® software by one-way analysis of variance (ANOVA). The Tukey Post Hoc test was used to analyze differences between pairs of values based on confidence intervals. The least significance difference was $p < 0.05$.

## 3. Results and discussion

The formulations of the experiments are shown in Table 1. The film-forming solution was prepared at room temperature, which resulted in a low-energy procedure. For complete dissolution of chitosan, a malic acid concentration of 2 % was required. During the incorporation of soy lecithin and SEO, some foam was formed under stirring. An ultrasonic bath was applied to remove the foam, resulting in a bubble-free solution with a milky appearance. The films were peeled off the Petri dishes. They were easy to handle. It was detected a certain adhesion characteristic that could be of interest for their application as food packaging.

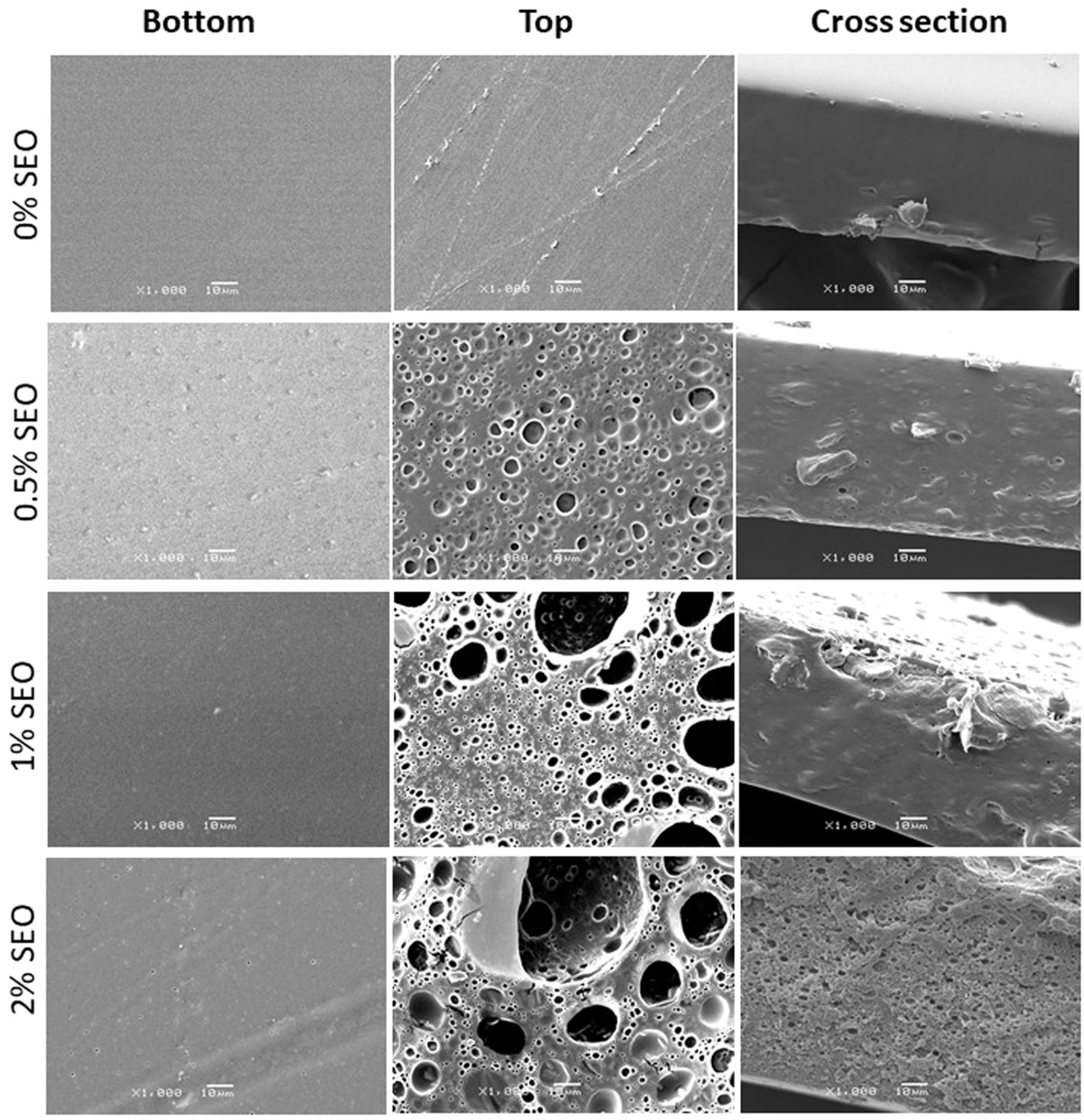


**Fig. 2.** Scanning electron microscopy images of the top, bottom and cross section of the SEO-chitosan films using malic acid as solvent. SEO is sandalwood essential oil.

### *3.1. Scanning electron microscopy (SEM)*

The SEM images of the bottom, top and cross section are shown in Fig. 2. Pure chitosan films showed a smooth and flat surface. Nevertheless, a discernible change in the chitosan film microstructure was detected when the concentration of SEO in the films increased. The bottom SEM images, which correspond to the side in direct contact with the Petri dish, suggested that the addition of soy lecithin allowed a homogeneous distribution of the oil in the films. No irregularities or breaks were detected on the films that could indicate that the ultrasonic bath was effective to remove any bubbles (Cazón et al., 2021).

However, the top SEM images indicated that when SEO was incorporated into the chitosan film, the uniformity of the film's morphology was compromised. The top surface suggested that there were certain areas of heterogeneity. This is attributed to oil droplets trapped in the polysaccharide network. When the SEO concentration reached 2 %, the formation of larger oil droplets was observed, this could be due to a higher frequency of collisions between oil droplets, resulting in a probable coalescence (Peng & Li, 2014). These oil droplets were not exactly spherical, as is usual in other emulsions such as oil/water. This could be related to the traction forces generated by the chitosan network when the solvent evaporates (Hafsa et al., 2016).

On the other hand, the cross-section images confirm the presence of an oil phase in the film, which also causes the film to cut less cleanly compared to pure chitosan film. This was also noticeable when handling the films, an increase in adhesiveness could be observed as the oil concentration increased. Similar behavior has been described for chitosan films with *Citrus limonia* essential oil (Gonçalves De Oliveira Filho

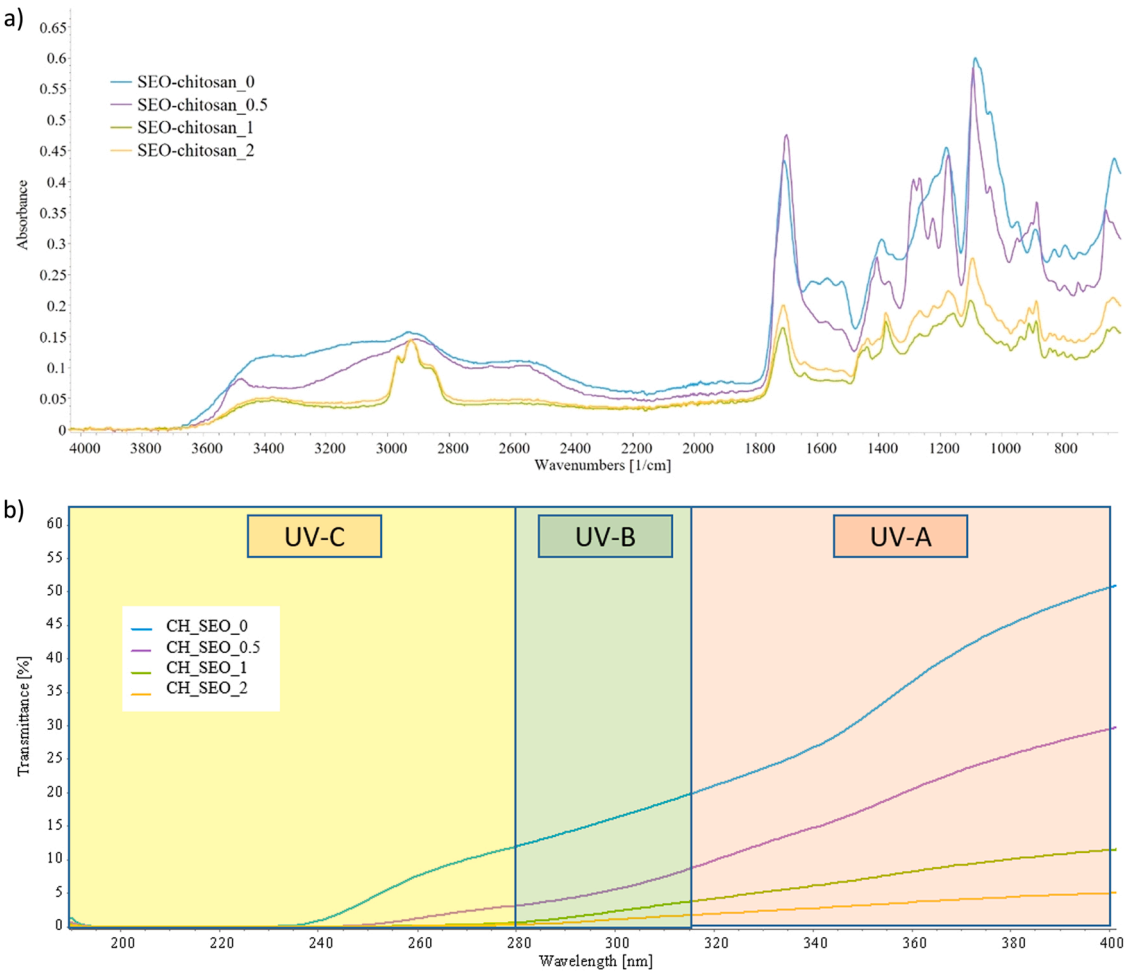


**Fig. 3.** a) FT-IR spectra of SEO-chitosan films using malic acid as solvent, b) UV–VIS spectra profile of SEO-chitosan films using malic acid as solvent. SEO is sandalwood essential oil.

**Table 2**
Physical and mechanical properties of chitosan films. Film composition is described in Table 1.

| Film samples | %W<br>% | WVP<br>g/m·s·Pa | TS<br>MPa | %E<br>% |
|---|---|---|---|---|
| SEO-chitosan_0 | 0.78 ± 0.13 | $6.70{\cdot}10^{-11} \pm 7.09{\cdot}10^{-12\ a}$ | 5.78 ± 2.62[a] | 27.68 ± 2.91[a] |
| SEO-chitosan_0.5 | 0.60 ± 0.06 | $4.52{\cdot}10^{-11} \pm 5.22{\cdot}10^{-12\ b}$ | 11.40 ± 2.70[b] | 48.86 ± 6.38[b] |
| SEO-chitosan _1 | 0.68 ± 0.11 | $4.26{\cdot}10^{-11} \pm 1.56{\cdot}10^{-12\ bc}$ | 2.20 ± 0.43[c] | 74.01 ± 10.9[c] |
| SEO-chitosan _2 | 0.78 ± 0.20 | $3.34{\cdot}10^{-11} \pm 8.28{\cdot}10^{-12c}$ | 2.99 ± 0.43[c] | 94.53 ± 9.98[d] |
| **Film samples** | **YM** | **BS** | **DB** | **Sealability (S)** |
| | MPa | g | mm | N/m |
| SEO-chitosan_0 | 35.74 ± 12.07[a] | 3209.49 ± 1222.02 | 7.18 ± 0.78 | 195.20 ± 33.74[a] |
| SEO-chitosan_0.5 | 68.43 ± 12.89[b] | 3147.06 ± 404.13 | 6.34 ± 0.67 | 80.88 ± 6.49[bc] |
| SEO-chitosan_1 | 7.84 ± 0.74[c] | 2680.35 ± 543.72 | 6.36 ± 0.89 | 79.09 ± 1.72[b] |
| SEO-chitosan_2 | 6.81 ± 0.80[c] | 2874.04 ± 380.04 | 6.19 ± 0.34 | 107.94 ± 8.45[c] |

SEO – sandalwood essential oil; %W - equilibrium moisture content; WVP - water vapor permeability; TS - tensile strength; %E - percentage of elongation to break; YM - Young's modulus; BS - burst strength (puncture properties); DB - distance to burst (puncture properties);
Values are expressed as mean ± standard deviation. Different letters in the same column indicate significant differences ($p < 0.05$).

et al., 2020) or *Eucalyptus globulus* essential oil (Hafsa et al., 2016).

### 3.2. Fourier transform infrared spectroscopy (FT-IR)

Fig. 3a shown the FT-IR spectra in the range between 4000 and 800 $cm^{-1}$ of SEO-chitosan films. This analysis was done with the aim of identifying possible molecular interactions between the functional groups of chitosan, malic acid, soy lecithin and SEO in the film structure. The FT-IR spectra of pure chitosan samples showed all the characteristic bands. The stretching vibrations of -OH and -NH are represented by the absorption bands at 3500–3400 $cm^{-1}$. The stretching vibrations of the C-H bond in the -$CH_2$ group give the absorption peaks at 2928 $cm^{-1}$ (Kalaycıoğlu et al., 2017). Whereas the amide-I vibrations of stretching C═O are represented by the absorption at 1700 $cm^{-1}$, the amide-II vibrations of stretching $NH_2$ are represented by the absorption at 1554 $cm^{-1}$ (Priyadarshi et al., 2018). The vibrations of the C-C y C-O stretches and the bending of C-H bonds in polysaccharide structure can explain the spectrum absorption between 1200 and 800 $cm^{-1}$ (Liu et al.,

**Table 3**
Antioxidant properties of the developed chitosan-based films and SEO. Film composition is described in Table 1.

| Samples | DPPH• (%) | ABTS•+ (%) |
|---|---|---|
| SEO | 78.23 ± 4.87 | 15.65 ± 0.86 |
| SEO-chitosan_0.5 | 7.34 ± 1.64[a] | 2.54 ± 0.27[a] |
| SEO-chitosan_1 | 12.74 ± 3.38[a] | 8.75 ± 0.10[b] |
| SEO-chitosan_2 | 23.69 ± 6.03[b] | 19.22 ± 0.57[c] |

SEO - sandalwood essential oil
Values are expressed as mean ± standard deviation.
Different letters in the same column indicate significant differences ($p < 0.05$).

2013). An important change was found with a new peak at 1745 $cm^{-1}$, indicating that with increasing SEO concentration there was an increase in the interaction between CH and the essential oil. The C═O stretching vibrations in the ester structures contained in fatty acids were responsible for this band, which is the distinctive band of oils (Priyadarshi et al., 2018). As a result of the probable interactions between chitosan, oil and soy lecithin, the intensity of the amide-II peak of chitosan in the blend film was reduced.

It can be determined that the addition of SEO to the chitosan films exerts modifications on the spectra of the chitosan films. All these changes could be related to a possible intermolecular interaction and molecular compatibility between the functional groups of the essential oil and the hydroxyl and amino groups of the chitosan matrix. Similar results were observed when various types of fats (Akyuz et al., 2018), citronella and cedarwood essential oil (Shen & Kamdem, 2015) or turmeric extract (Kalaycıoğlu et al., 2017) were added to chitosan films.

### 3.3. Evaluation of the water solubility, equilibrium moisture content and water vapor permeability (WVP)

To determine the water solubility of the films, the soluble matter of the samples was analysed. After a few seconds of immersion in distilled water, the films lost the whole structure and is completely water soluble. This is because malic acid is not a volatile solvent, therefore it remained in the matrix and improved film solubility. In addition, it was observed that the presence of SEO in the matrix did not decrease the solubility of the films. This is an interesting because the film can be easily removed from foodstuffs avoiding solid wastes.

The equilibrium moisture content (%W) of SEO-chitosan films ranged between 0.60 % and 0.78 % as shown in Table 2. The addition of SEO to the chitosan films showed no significant differences ($p > 0.05$) in equilibrium moisture content.

The WVP values calculated for chitosan films were ranged between $3.34{\cdot}10^{-11} - 6.70{\cdot}10^{-11}$ g/m·s·Pa, as shown Table 2. The addition of SEO showed a significant difference on WVP values ($p < 0.05$) as compared with pure chitosan films. The presence of hydrophobic compounds of the essential oil promoted the decrease of WVP, reaching a reduction of almost 50 % at a 2% SEO concentration. The presence of the oil droplets observed in the SEM images (Fig. 2) created a tortuous path that made it difficult for the water molecules to permeate through the matrix. Moreover, the increased number of hydrogen bonds between the functional groups of the chitosan and the SEO may decrease the availability of hydrophilic groups to interact with water molecules, resulting in a more water-resistant layer (Shen & Kamdem, 2015).

The observed effect of SEO addition on chitosan films in the present study is in agreement with the results obtained by Shen and Kamdem (2015), who found that adding 10 % (w/w) citronella and cedarwood essential oil into chitosan films resulted in a considerable reduction in the WVP of the samples. In a similar way, Yuan et al. (2016) observed a significant decrease in the WVP of chitosan films with cinnamon essential oil.

It should be noted that certain factors such as chitosan properties, film composition, WVP method and measurement conditions or film thickness, are important for the subsequent WVP results (Rhim et al., 1998).

### 3.4. Evaluation of the mechanical properties

Table 2 shows the results of mechanical properties obtained from the tensile and puncture tests. The tensile test for SEO-chitosan films showed values in the range 2.20–11.40 MPa for TS, 27.68–94.53% for E% and 6.81–68.43 MPa for YM. The addition of SEO to chitosan film had a significant effect ($p < 0.05$) on the three tensile properties analysed.

The values of TS and YM decreased while the %E values increased by the addition of SEO, indicating a decrease in the resistance to break and an increase in the deformation capacity by the addition of SEO. The changes in the mechanical behavior could be explained by the structural arrangement of the lipid phase in the chitosan matrix, as shown the SEM images (Fig. 2). The structural arrangements resulted in a discontinuous and open matrix with unequal physical interactions (Flórez et al., 2022). Besides, the incorporation of essential oil into chitosan films could disrupt polymer-polymer interactions, leading to weaker essential oil-polymer interactions, resulting in a plasticizing effect of the essential oil (Hosseini et al., 2015). The new interactions gave a less stiff structure due to the reduction of the cohesion of the polymer network forces, decreasing the TS and YM values and increasing the %E. Same trend in the mechanical properties was observed when *Origanum vulgare* (Hosseini et al., 2015) or *Melaleuca alternifolia* (Cazón et al., 2021) essential oil were added to chitosan films.

The puncture test results revealed a statistically non-significant effect of SEO concentration on BS and DB values, despite a slightly decrease of the values was observed (Table 2). The samples' deformation and how the polymer chains must arrange under deformation forces in several directions differ from the restructuring and deformation produce

**Table 4**
Optical properties and color parameters of the films. Film composition is described in Table 1.

| Film samples | UV-C %T | UV-B %T | UV-A %T | Transparency | Opacity |
|---|---|---|---|---|---|
| SEO-chitosan_0 | 6.01 ± 0.78 | 16.53 ± 1.28 | 35.88 ± 1.84 | 34.90 | 3.87 |
| SEO-chitosan_0.5 | 1.59 ± 0.01 | 6.54 ± 0.04 | 19.73 ± 0.08 | 37.40 | 8.38 |
| SEO-chitosan_1 | 0.33 ± 0.00 | 2.45 ± 0.01 | 7.87 ± 0.03 | 32.22 | 19.39 |
| SEO-chitosan_2 | 0.16 ± 0.18 | 1.14 ± 0.95 | 3.50 ± 2.09 | 18.01 | 22.13 |
| **Samples** | **L*** | **a*** | **b*** | | |
| SEO-chitosan_0 | 84.73 ± 3.81[a] | -0.48 ± 0.27[a] | 6.18 ± 0.45[a] | | |
| SEO-chitosan_0.5 | 66.62 ± 8.17[b] | 1.67 ± 1.01[b] | 15.1 ± 4.45[b] | | |
| SEO-chitosan_1 | 59.87 ± 11.14[a] | 1.21 ± 0.31[b] | 8.40 ± 1.27[a] | | |
| SEO-chitosan_2 | 33.88 ± 6.52[a] | 1.62 ± 0.71[b] | 8.15 ± 1.50[a] | | |

SEO - sandalwood essential oil; UV-C (200–280 nm); UV-B (280–315 nm); UV-A (315–400 nm); %T - percentage of transmittance; 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.
Different letters in the same column indicate significant differences ($p < 0.05$).

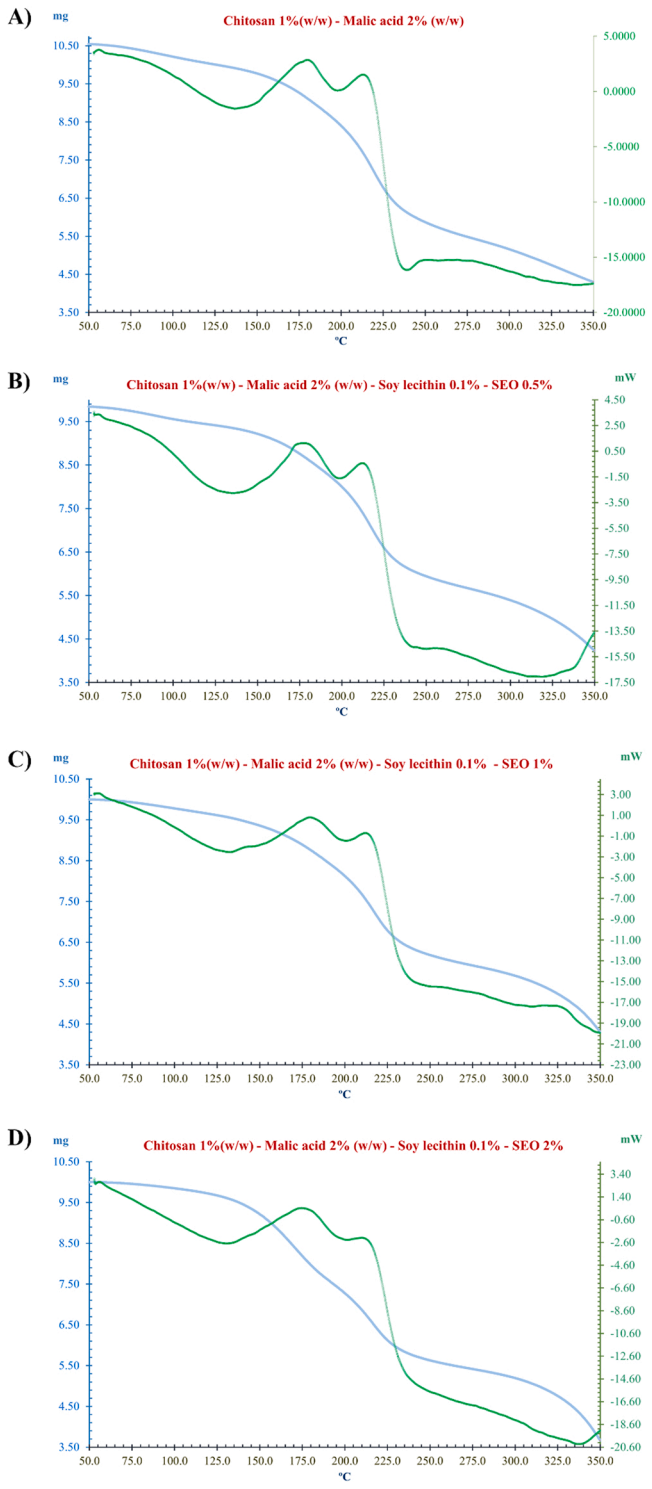


**Fig. 4.** Thermogravimetry and differential scanning calorimetry of SEO-chitosan films using malic acid as solvent. SEO is sandalwood essential oil.

by a single horizontal force. Hence, under tensile or puncture deformation forces, the same matrix can exhibit different resistance and deformation behavior (Cazón et al., 2021).

In the manufacture of films, heat sealability of the material and its durability is important. In addition, if these films are intended for food use, they should be strong enough to keep the product sealed and not release their contents during storage and handling. The SEO-chitosan samples showed a sealability values ranged from 79.09 to 195.20 N/m (Table 2). The addition of SEO to the chitosan films had a significant effect ($p < 0.05$) on the sealability of the films, resulting in a decrease in sealability values as the SEO concentration increased. It was noted that the film without SEO showed the highest values of sealability (195.20 N/m). This could be explained by the different active components of sandalwood essential oil, which could exert a modification on the network of the film. Results are in the range obtained for films of chicken protein/fish skin gelatin mixtures containing gallic acid or tannic acid where values of 64.83–113.5 N/m in the sealability of the films were observed (Nilsuwan et al., 2021).

### 3.5. Evaluation of antioxidant properties

Antioxidant activity of pure SEO and SEO-chitosan films was evaluated by two methods: $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ (Table 3). Pure sandalwood results showed values of 78.23 % for $DPPH^{\bullet}$ and 15.65 % for $ABTS^{\bullet+}$ verifying the antioxidant capacity of the essential oil and its suitability for application in food films. The results obtained from the film extracts showed values from 7.34 % to 23.69 % $DPPH^{\bullet}$ and the scavenging $ABTS^{\bullet+}$ activity ranged from 2.54 to 19.22 %. Both scavenging activities increased significantly ($p < 0.05$) with increasing oil concentration. After the addition of SEO (from 0.5 % to 2 %), the antioxidant capacity of the films increased significantly. Sandalwood essential oil contains a great variety of sesquiterpenoid alcohols called santalols (Demole et al., 1976). α-santalol is the main bioactive principle of the oil, therefore most of the antioxidant activities are attributed to it (Misra & Dey, 2013). This behavior can be attributed to the presence of these active components from the essential oil, which can exert their antioxidant activity by several possible mechanisms: free-radical scavenging activity, hydrogen donors, transition-metal-chelating activity, and/or singlet-oxygen-quenching capacity (Liyana-Pathirana & Shahidi, 2006).

### 3.6. Evaluation of the optical properties

The UV-barrier properties of the SEO-chitosan films were determined from the transmittance values in the UV region. The color and opacity of the films were determined from the transmittance values in the visible region.

Table 4 shows the average percentage of transmittance (%T) of the examined SEO-chitosan films in the UV-C (200–280 nm), UV-B (280–315 nm) and UV-A (315–400 nm) regions. Fig. 3b shows the UV profile spectra of the films. SEO-chitosan films showed %T values ranged 6.01–0.16 %, 16.53–1.14 % and 35.88–3.50 % in the UV-C, UV-B and UV-A regions, respectively. The results concluded that the addition of SEO to the chitosan films exerted a significant decrease in the transmittance values and thus improving the UV blocking capacity of the films.

The best results were obtained on films with 2% SEO, where the transmittance percentage was below 5 % in the 200–400 nm wavelength region, which reaffirmed the good UV-barrier properties of the film. These results showed that chitosan films containing SEO may retard UV-induced lipid oxidation.

Other important factors when assessing optical properties are transparency and opacity, as these have a direct impact on the visual appearance of the product to be coated. Table 4 shows the values of transparency ranged 18.01–37.40, while opacity values ranged 22.13–3.87. As can be seen, the addition of SEO promoted the decrease in the transparency values and an increase in the opacity of the films. A decrease in light transmission with the addition of SEO, with a consequent increase in opacity and a decrease in transparency, was also previously observed by the addition of essential oils in edible films (Tongnuanchan et al., 2012).

Table 4 shows the CIE coordinates of the SEO-chitosan films. Lightness (L*) varied from 33.88 to 84.73. The addition of SEO had a significant effect ($p < 0.05$) compared to pure chitosan films. Increasing SEO concentration, a significant decrease in L* values was shown, indicating a tendency of the film to darken (Zhang et al., 2018).


**Fig. 5.** Butter samples sealed in SEO-chitosan films stored at 4ºC for 90 days. SEO is sandalwood essential oil.

**Table 5**
Antioxidant and optical properties of chitosan films applied on butter after 3 months of storage. TBARS of butter after 3 months of storage. Film composition is described in Table 1.

| Samples | DPPH• (%) | ABTS•+ (%) | TBARS mg MDA / kg butter |
|---|---|---|---|
| Butter w/o film | – | – | 616.90 ± 9.58[a] |
| Butter with SEO-chitosan_0 | 10.70 ± 2.46[a] | 6.68 ± 0.31[a] | 417.91 ± 4.43[b] |
| Butter with SEO-chitosan_0.5 | 6.68 ± 1.65[a] | 11.42 ± 0.72[b] | 414.66 ± 5.60[b] |
| Butter with SEO-chitosan_1 | 12.11 ± 3.40[a] | 11.87 ± 0.38[b] | 410.77 ± 8.27[b] |
| Butter with SEO-chitosan_2 | 23.14 ± 6.07[b] | 19.34 ± 0.12[c] | 393.25 ± 6.22[c] |
| **Samples** | **L*** | **a*** | **b*** |
| Butter w/o film | 83.74 ± 0.29 | -1.03 ± 0.07[bc] | 36.26 ± 0.64 |
| Butter with SEO-chitosan_0 | 80.28 ± 2.22 | 1.54 ± 1.01[a] | 38.81 ± 4.73 |
| Butter with SEO-chitosan_0.5 | 81.83 ± 0.99 | 1.09 ± 0.55[ac] | 37.14 ± 2.71 |
| Butter with SEO-chitosan_1 | 78.17 ± 2.19 | 1.96 ± 0.72[a] | 45.17 ± 2.24 |
| Butter with SEO-chitosan_2 | 78.47 ± 1.51 | 1.13 ± 0.39[a] | 44.01 ± 1.49 |

SEO - sandalwood essential oil; TBARS – Thiobarbituric reactive substances
Values are expressed as mean ± standard deviation (SD).
Different letters in the same column indicate significant differences ($p < 0.05$).
Values of L* and b* showed no significant differences.

However, the b* and a* values increased significantly from 6.18 to 15.1 and from − 0.48–1.67 when the oil was at a concentration of 0.5%. The increase in a* and b* values explains that the samples have a tendency towards redness and yellowness compared to the pure chitosan film. The same trend in the results was observed when *Melaleuca alternifolia* essential oil was added to chitosan films (Cazón et al., 2021).

### 3.7. Evaluation of the thermal properties (TGA/DSC)

The SEO-chitosan films were evaluated by TGA/DSC for study the effects of sandalwood essential oil on the thermal physical property. Fig. 4 shows the thermograms of chitosan films, pure or enriched with SEO up to 2 % (v/v).

Thermogravimetric curves indicated similar thermal behaviors with three steps. There are slight differences related to the composition of the samples. The weight loss between 50 and 150 ºC is due to the loss of water linked to the internal structure of the film (Chu et al., 2019). In comparison, weight loss was slower in pure chitosan films in agreement with the data obtained for %W. It must be taken into account that some of the water present in the film may evaporate before the TGA/DSC analysis is carried out and the temperature of 50 ºC is reached (Cazón et al., 2021). The second weight loss step is due to thermal degradation of the chitosan (Chen et al., 2008; Lewandowska, 2009). Finally, in the third step there is a weight loss probably due to the degradation of by-product generated by chitosan during the thermal degradation process.

Endothermic peaks were found between 140 and 240 ºC. These peaks correspond to chemisorbed water via hydrogen bonds, and the degradation of the chitosan amino group and the malic acid (Bonilla et al., 2014). At 225 ºC, the total weight losses of the samples increased with the SEO concentration, being 38.01 %, 33.68 %, 35 % and 40 %. According to that, the films incorporated with SEO up to 1.5 % showed a decreased weight loss compared to the control film, indicating greater thermal stability. However, when the SEO addition is up to 2 % there was a slight decrease in this property. This could be explained due to the presence of high concentrations of SEO in the films leads to an increase in the discontinuity of the film matrix. This generated a decrease in the compatibility of the multiphase system, which causes the films to lose more weight and therefore have lower heat resistance (Noshirvani et al., 2017). Similar results were obtained by Xu et al. (2019) when cinnamon and clove essential oil were added to gum arabic-chitosan films.

The results obtained suggest that the interactions produced in the matrix between chitosan and SEO (from 0.5 % to 1 %) increases the thermal stability of the films by reducing their weight. On the other hand, these films were stable until they reached 120ºC. This is an interesting finding, because it means that as the developed SEO-chitosan films could have applicability in pasteurization treatments.

### 3.8. Storage stability of butter packed in SEO-chitosan sachets

The antioxidant properties of the chitosan films as sachets for butter were tested after 3 months at 5 ºC refrigerated storage. Fig. 5 shows the butter samples sealed in SEO-chitosan films at day 90 of storage at 5 ºC. Table 5 shows DPPH• and ABTS•+ values in the films used for packaging butter after 90 days. Results revealed a significant increase ($p < 0.05$) of the DPPH• values from 10.70 % to 23.14 % for chitosan films with 1 % and 2 % SEO, respectively. ABTS•+ values ranged from 6.68 % to 19.34 % with a significant increase by the addition of any concentration of SEO compared with the chitosan films without SEO. DPPH• and ABTS•+ results suggested that the active films successfully kept the antioxidant properties after 3 months of storage.

The TBARS value was used to indicate that the second stage of lipid auto-oxidation is occurring. This assay detects malonaldehyde (MDA), an unfolded product of endoperoxides resulting from the oxidation of

unsaturated fatty acids (Han Lyn et al., 2021). Table 5 shows TBARS values of the unpackaged (control) and packaged butter after 3 months of refrigerated storage. The TBARS values for control sample was 616.90 mg MDA/kg butter and for packaged butter ranged from 417.91 to 393.25 mg MDA/kg butter. TBARS values showed a significant effect of packaged samples ($p < 0.05$) compared to the control. After 3 months of butter storage, a 36 % decrease in the lipid oxidation was observed due to the SEO-chitosan films. This behavior can be explained by the radical scavenging properties of the films, as supported by the results of the antioxidant assays (Han Lyn et al., 2021). On the other hand, butter samples packaged with SEO-chitosan films decreased lipid oxidation compared to butter packed with control films without oil. Therefore, the presence of sandalwood essential oil further decreases the oxidation of the butter. Same trend was observed in chitosan/graphene oxide bio-composite films that stored palm-oil based margarine (Han Lyn et al., 2021).

### 3.9. Optical properties of butter wrapping films

Table 5 shows the CIE coordinates of the unwrapped and wrapped butter samples with SEO-chitosan films. In this case, the lightness (L*) varied from 80.28 to 78.47 and the b* values were from 38.81 to 44.01. For both values, no significant difference was observed ($p > 0.05$). However, the a* (redness parameter) values varied from 38.81 to 44.01, producing a significant effect ($p < 0.05$) compared to butter without film. Results suggested that the brightness of the films and the yellowish of the samples were not affected by the presence of the film that wraps around the butter, only the redness color of the films was slightly modified. Hence, the films had sufficient transparency to maintain the visual appearance of the butter and the consumer could observe it without significant alterations.

## 4. Conclusions

The incorporation of SEO into chitosan matrix was successfully performed to obtain antioxidant films. The films are completely water-soluble and can be easily removed from foodstuffs after use without generate solid wastes. The films can be heat sealed and used as sachets. This makes them a good option for use in the food packaging industry partially replacing plastic containers. Regarding the optical properties, the color and transparency of the films applied on butter allowed to see the real color of the butter. The concentration of essential oil present in the film did not influence the visual appearance of the wrapped product under study. Considering the TBARS results, it can be stated that the application of SEO on chitosan films successfully delayed butter oxidation.

## CRediT authorship contribution statement

Maria Flórez: Writing- Original draft preparation. **Patricia Cazón**: Conceptualization, Supervision, Writing- Reviewing and Editing. **Manuel Vázquez**: Methodology, Writing- Reviewing and Editing, Project administration.

## 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.

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