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Characterization of soluble soybean polysaccharide film incorporated essential oil intended for food packaging

Davoud Salarbashi[a], Sima Tajik[b,1], Mehran Ghasemlou[c,*], Saeedeh Shojaee-Aliabadi[d], Mostafa Shahidi Noghabi[e], Ramin Khaksar[d]

[a] Department of Food Science and Technology, Sabzevar Branch, Islamic Azad University, Sabzevar, Iran
[b] Department of Food Science and Technology, Gorgan University of Agricultural Sciences and Natural Resources, Beheshti Avenue, Gorgan, Iran
[c] School of Packaging, Michigan State University, East Lansing, MI 48824, USA
[d] Department of Food Science and Technology, National Nutrition and Food Technology Research Institute, Faculty of Nutrition Sciences and Food Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[e] Department of Food Chemistry, Research Institute of Food Science and Technology, PO Box 91735-147, Mashhad-Quchan Highway, Mashhad, Iran

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ABSTRACT

This study examines the development of new bio-active polysaccharide-based bioplastics through casting and solvent-evaporation. Soluble soybean polysaccharide (SSPS) films incorporated with *Zataria multiflora Boiss* (ZEO) or *Mentha pulegium* (MEO) at various concentrations were prepared and characterized. The presence of ZEO and MEO improved polysaccharide interactions, reducing the films' water solubility and water vapor barrier properties, but did not markedly modify their moisture content or thickness. Differing amounts of ZEO or MEO had no significant effect on mechanical behavior, with the exception of 3% oil concentration, which decreased tensile strength and significantly increased elongation at break. DMTA curves revealed a single T_g, which may indicate the compatibility of essential oil and SSPS. The electron scanning micrograph for the composite film was homogeneous, without signs of phase separation between the components. These results suggest that ZEO and MEO can potentially be directly incorporated into SSPS to prepare active biodegradable films for food-packaging applications.

1. Introduction

The growing accumulation of synthetic plastic wastes together with the difficulty of recycling the majority of packaging has stimulated food and packaging industries to explore for new biodegradable packaging materials (Tharanathan, 2003). In recent years, there has been growing interest in edible films and coatings, which offer several advantages over synthetic materials, such as being biodegradable and environmentally friendly (Salmieri & Lacroix, 2006). However, the nature of edible packaging films, which is rigid and brittle, causes limitations in food applications. Therefore, to overcome film's brittleness and also to increase the workability and flexibility of these films, various types of plasticizers have been widely used, glycerol being one of the most preferred and most studied (Ghasemlou, Khodaiyan, Oromiehie, & Yarmand, 2011a).

Nowadays, one of the major challenges for food technologists is the design of active food packaging. This technology seems to be a promising alternative since application of this method can improve safety of foods by inhibiting pathogenic bacteria or controlling spoilage flora using minimum amounts of active compounds (Ma, Tang, Yang, & Yin, 2013). The natural antimicrobial agents frequently employed in active packaging include antimicrobial enzymes, bacteriocins, essential oils and phenolic compounds.

Essential oil compounds, which their inhibitory effect on the growth of various microorganisms are well documented and described extensively, are of great potential use in active coatings (Burt, 2004).

Although most of the essential oils are classified as Generally Recognized as Safe (GRAS) their use as food preservatives is often limited due to flavoring considerations since effective antimicrobial doses may exceed organoleptically acceptable levels (Viuda-Martos, Ruiz-Navajas, Fernández-López, & Pérez-Álvarez, 2008). To avoid this problem an alternative is the incorporation of them within edible films. Edible films can reduce the diffusion of antimicrobial compounds into the product since the essential oil forms part of the chemical structure of the film

* Corresponding author. Tel.: +1 517 355 9580; fax: +1 517 353 8999.
E-mail addresses: mehran.ghasemloo@gmail.com, ghasemlo@msu.edu (M. Ghasemlou).
[1] This author has equally contributed as first author in this research.

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and interacts with the polymer and the plasticizer (Ponce, Roura, del Valle, & Moreira, 2008). Furthermore, compared with direct application, smaller amounts of antimicrobial agents would be needed when edible films are used as carriers in order to achieve a specific food shelf life due to a gradual release on food surfaces. *Zataria multiflora Boiss* (ZEO) and *Mentha pulegium* (MEO), which are locally called Avishan shirazi and Pune, respectively, are aromatic and medicinal plants belonging to Labiatae family. They are rich in polyphenolic compounds and a large number of them are well known for their antioxidant, antibacterial and antifungal properties (Kamkar, Javan, Asadi, & Kamalinejad, 2010; Saei-Dehkordi, Tajik, Moradi, & Khalighi-Sigaroodi, 2010; Sajed et al., 2013).

Materials available for forming films and coating generally fall into the categories of polysaccharides, proteins and lipids. Among them, carbohydrate-based edible films, which have good film-forming ability due to their unique colloidal properties, are most attractive. They are capable of yielding tough and flexible transparent films due to the linear structure of their polymer backbone. Various polysaccharides have been used for the preparation of edible films including cress seed (Jouki, Khazaei, Ghasemlou, & HadiNezhad, 2013), psyllium seed gum (Ahmadi, Kalbasi-Ashtari, Oromiehie, Yarmand, & Jahandideh, 2012) and corn starch (Kuorwel, Cran, Sonneveld, Miltz, & Bigger, 2013a). However, over the past few years, there has been a renewed interest in other new resources for the production of edible and biodegradable films. Recently, a novel polysaccharide has been extracted from the cell-wall material of soybean cotyledon. This polysaccharide, soluble soybean polysaccharide (SSPS), has a pectin-like structure composed of a galacturonan backbone of homogalacturonan (α-1,4-galacturonan) and rhamnogalacturonan (repeating units being comprised of α-1,2-rhamunose and α-1,4-galacturonic acid) branched by β-1,4-galactan and α-1,3- or α-1,5-arabinan chains (Nakamura, Furuta, Maeda, Takao, & Nagamatsu, 2002). SSPS has been reported to provide health benefits to humans through lowering blood cholesterol, improving laxation and reducing the risk of diabetes (Shorey, Willis, Lo, & Steinke, 1985; Tsai, Vinik, Lasichak, & Lo, 1987). Apart from its nutritional value, it has various functions such as dispersion, stabilization, emulsification and adhesion (Asai et al., 1994). Previous studies have found that SSPS can be used as a functional ingredient in fortified foods. For example, Nakamura, Furuta, Kato, Maeda, and Nagamatsu (2003) used SSPS as a stabilizer in acid milk dispersions, and demonstrated that the stabilizing properties of SSPS are comparable to those of pectins. The new published paper in our research group showed that SSPS can produce biodegradable films with good appearance and satisfactory mechanical properties (Tajik et al., 2013). Although significant improvements in various properties of SSPS based films have been reported in that study, they are still not comparable to those of the synthetic plastic films. Therefore, the physical and barrier properties of these films need to be improved. Preliminary studies in our laboratory have shown that ZEO and MEO were more compatible essential oils with SSPS polymer matrix. It seems that their combination in SSPS films can be an appropriate way to produce antimicrobial films with improved barrier and mechanical properties. This study brought a novel approach by showing possibility of developing flexible active packaging film using SSPS as film-forming material. The aim of this study was to develop a composite edible film based on SSPS and two essential oils – ZEO and MEO – and to assess their influence on properties of the films. This was achieved by relating the information gathered from thermo-mechanical analyses, solubility measurements, moisture content determinations, water vapor permeability measurements, mechanical tests and structural properties analyzed through scanning electron microscopy (SEM) and atomic force microscopy (AFM).

2. Materials and methods

2.1. Materials

Soluble soybean polysaccharide (abbreviated as SSPS) was kindly donated by Fuji Oil Company (Osaka, Japan) for this study. According to our preliminary analysis, the composition of the product was: 4.7% crude protein, 4.9% moisture 5.3% ash and 81.6% total sugar. Glycerol and Tween 80 were purchased from Merck, Germany. *Z. multiflora Boiss* (ZEO) and *M. pulegium* (MEO) were obtained from Barij Essence Pharmaceutical Co., Kashan, Iran, and stored in dark container at 4 °C until used.

2.2. Preparation of films

The SSPS films were prepared as described in our previous study (Tajik et al., 2013). Briefly, an SSPS solution was prepared by dissolving 3 g SSPS in 100 ml distilled water to obtain 3% (w/v) film-forming solutions. To achieve a complete dispersion of the SSPS, the solution was stirred constantly for 40 min using a magnetic stirrer at 300 rpm on a hot plate. The solution was then mixed with plasticizer (50% w/w of the polysaccharide). Glycerol was added to the medium as a plasticizer, and stirring was continued for a further 15 min at 82 °C. Control films (without essential oils) were cast from this solution. Composite SSPS films were achieved according the procedure previously described by Shojaee-Aliabadi et al. (2013), who had worked with carageenan-based films. The emulsions were obtained by adding ZEO and MEO to the SSPS solution to reach final concentrations of 1, 2 and 3% v/v. Tween 80 was added as an emulsifier in quantities proportional to the essential oils (0.1, 0.2 and 0.3% v/v). The film solutions were homogenized at 20,000 rpm for 3 min in an Ultra-Turrax T-25 homogenizer (IKA T25 Digital Ultra-Turrax, Staufen, Germany). The film solutions were left for several minutes to naturally remove most of the air bubbles incorporated during stirring. All prepared film solutions, including the control and those with essential oil, were cast by pouring the mixture onto polystyrene Petri dishes (14 cm in diameter) placed on a leveled granite surface for approximately 18 h at room temperature and room relative humidity. Dried films were peeled off the casting surface and stored inside desiccators at 25 ± 1 °C and 53% RH until evaluation. Saturated magnesium nitrate solution was used to achieve the required relative humidity.

2.3. Film thickness measurement

Film thickness was determined using a hand-held digital micrometer (Mitutoyo No. 293-766, Tokyo, Japan) having a precision of 0.01 mm. Measurements were carried out at ten different film locations and the mean thickness value was used to calculate the permeability and mechanical properties of the films.

2.4. Moisture content and film solubility in water

The films' moisture content was determined by measuring the weight loss of films, upon drying in an oven at 110 °C until a constant weight was reached (dry sample weight). Three replications of each film treatment were used for calculating the moisture content. The film solubility was determined by a method adapted from Shojaee-Aliabadi et al. (2013). The film samples were cut to a square piece of 2.0 cm × 2.0 cm and accurately weighed to give the dried film (W_0). The films placed into test beakers with 100 ml distilled water. The samples were immersed and shaken under constant agitation at 180 rpm for 6 h at 25 °C. After that period, the remained pieces of film were then filtered and dried in a hot air oven at 110 °C until a final constant weight was obtained (W_1). The percentage of solubility of the film was calculated according to the equation WS

(%) = $((W_0 - W_1)/W_0) \times 100$, where W_0 is the initial weight of the film expressed as dry matter and W_1 is the weight of the desiccated undissolved film.

2.5. Moisture uptake

Moisture uptake was measured by following the method of Ghanbarzadeh and Almasi (2011) instead of the classical technique (immersion in water), because SSPS is very sensitive to liquid water and can partially dissolve after long time exposure to water. Prior to testing all specimens were first conditioned using anhydrous $CaSO_4$ at 0% RH for 48 h. The film samples were cut to a square piece of 2.0 cm × 2.0 cm and accurately weighed to give the dried film (W_i). After weighing, they were conditioned at 25 °C in a desiccator containing K_2SO_4 saturated solution to ensure a relative humidity of 97%. The samples were removed from the desiccator after 48 h and weighed (W_1). The moisture uptake values of the samples were calculated using the following equation:

$$\%\,Moisture\ uptake = \frac{W_1 - W_i}{W_i} \times 100 \quad (1)$$

where W_1 is the weight of the film sample after exposure to 97% RH for 48 h, and W_i is the initial weight of the sample. Each test consisted of triplicate measurements and expressed as the mean value.

2.6. Water vapor permeability (WVP)

WVP of the films was determined by using the ASTM E96/E96M (ASTM, 2012a, 2012b) gravimetric method, taking into account the modification proposed by Hosseini, Razavi, and Mousavi (2009). Films were cut, adjusted and sealed over a circular opening of 0.00287 m^2 in a permeation cell that was stored at 20 °C in a desiccator. The inside of the cell was completely filled with calcium anhydride ($CaCl_2$ – 0% RH), and the system was placed in a desiccator containing a saturated sodium chloride solution (NaCl – 75% RH). The RH inside the cell was always lower than outside, and water-vapor transport was determined from the weight gain of the permeation cell at a steady state of transfer. Eight weight measurements were made at 12 h intervals over a 7 days period to the nearest 0.0001 g. Changes in the weight of the cell were recorded and plotted as a function of time. The slope of each line was calculated by linear regression (Microsoft® Office Excel 2007) and the water vapor transmission rate (WVTR) was calculated from the slope (g H_2O/s) divided by the cell area (m^2). All values for WVTR were corrected for air–gap distance between the calcium chloride and the film surface according to the equations of Gennadios, Weller, and Testin (1993). After the permeation tests, the film thickness was measured, and water-vapor permeability (WVP) (g Pa^{-1} s^{-1} m^{-1}) was calculated as:

$$WVP = \frac{\Delta m}{A\Delta t}\frac{X}{\Delta p} \quad (2)$$

where $\Delta m/\Delta t$ is the weight of moisture gain per unit of time (g/s), X is the average film thickness (mm), A is the area of the exposed film surface (m^2), and Δp is the water vapor pressure difference between the two sides of the film (Pa). WVP was measured for three replicated samples for each type of film.

2.7. Mechanical properties

Film specimens were cut into rectangular strips, 1 cm wide and 10 cm long, after conditioning in 50% relative humidity for >70 h. Tensile strength (TS) and elongation at break (EB) of the SSPS films were determined using a Testometric Machine M350-10CT (Testometric Co. Ltd., Rochdale, Lancs., England) according to ASTM standard method D882 (ASTM, 2012a, 2012b). The initial grip separation and cross-head speed were set to 50 mm and 50 mm/min, respectively. A microcomputer was used to record the stress–strain curves. Tensile properties including TS and EB were calculated as outlined in ASTM D882 (2012a). At least five replicates of each film were tested.

2.8. Dynamic mechanical thermal analysis (DMTA)

DMTA is a thermoanalytical technique used to study and characterize materials. It is most useful for observing the viscoelastic nature of polymers. The glass-transition temperatures (T_g) of SSPS films were determined using a dynamic mechanical thermal analyzer (DMTA, Triton Technology, UK), working with liquid nitrogen and film grip clamps that allowed possible uniaxial traction tests. All the necessary thermal and mechanical calibrations of the instrument were performed before the experiments according to its operation manual. Film samples were clamped in the instrument with the initial grip separation of 5.5 mm. The films were subjected to a sinusoidal strain on top of a static deformation. The test was conducted at a constant frequency of 1 Hz and a strain of 0.02% over a temperature range of −50–150 °C, at a heating ramp rate of 5 °C/min. To prevent water loss during the experiment, the exposed surface of each sample was partially wrapped with aluminum foil, leaving the bottom and top in direct contact with the clamp holders (Ghasemlou, Khodaiyan, & Oromiehie, 2011b). The storage modulus (E') and loss tangent (tan δ) of each film sample were obtained as a function of temperature. The transition temperature was determined at the point of inflection of the curve of loss tangent (tan δ) as a function of temperature. All the measurements were conducted at least in duplicate.

2.9. Scanning electron microscopy (SEM)

The microstructure of films was observed using scanning electron microscopy (Oxford Instruments INCA Penta FET × X3). Before the analysis each film was fixed on a support using double side adhesive tape, placed horizontally with an angle of 90°. Then samples were gold coated with a sputter coater (BALTEC AG, Balzers, Liechtenstein) under 15 mA for 60 s. All samples were examined using an accelerating voltage of 20.0 kV.

2.10. Atomic force microscopy (AFM)

Before testing, samples were preconditioned at 50% RH and at room temperature for at least 48 h. The AFM was carried out by an Autoprobe CP Research instrument (Veeco Instruments) in contact mode with a 125 μm × 125 μm scan size and a 6 μm vertical range. In our measurements, we used silicon probes (Veeco probes), with a spring constant of 5 N m^{-1}. The resulting data were transformed into a 3D image. Measurements were taken from several areas of the film surface (50 μm × 50 μm). According to method ASME B46.1 (ASME, 2009), the following two statistical parameters related with sample roughness were calculated: average roughness (R_a: average of the absolute value of the height deviations from a mean surface), root-mean-square roughness (R_q: root-mean-square average of height deviations taken from the mean data plane). A minimum of three replicates were considered to obtain these parameters.

2.11. Statistical analysis

The data were presented as the mean ± standard deviation of each treatment. The experiments were factorial with a completely randomized design using analysis of variance (ANOVA) in the SAS program (Version 9.1; Statistical Analysis System Institute Inc., Cary, NC, USA). Differences between the films' properties mean

values were compared using Duncan's multiple range tests. A probability value of $p < 0.05$ was considered significant.

3. Results and discussion

3.1. Film formulation

Preliminary studies in our laboratory showed that a SSPS concentration of less than 3% is not sufficient to obtain a strong supporting matrix, and the resulting films are soft. A 3% SSPS concentration was selected as the suitable polysaccharide concentration in the film-forming solution. The composite films formed using ZEO and MEO were visually homogeneous with no brittle areas or bubbles, and could be easily peeled from the casting plates. To determine the maximum concentration of ZEO or MEO that could be incorporated into the SSPS matrix, increasing amounts (up to 5%) were added to the film-forming dispersion. As expected, a stronger aroma was observed as the concentration of essential oil went above 3%. Moreover, regarding the optical properties of films, it was observed that the color intensified and the transparency decreased as the concentration of both essential oils increased (data not published yet). It is important to note that no structural gradation was found, which confirms both the good dispersion of the essential oils and the lack of significant creaming during drying.

3.2. Physical properties

Table 1 shows the effects of incorporating essential oils on the physical properties of SSPS-based films.

The film thickness varied from 0.073 mm to 0.083 mm, and incorporation of different essential oils did not significantly ($p > 0.05$) affect it. Aguirre, Borneo, and León (2013) reported that the incorporation of oregano essential oil had no effect on the thickness of triticale protein films. Rubilar et al. (2013) did not find any difference in the thickness of chitosan film incorporating various extract of carvacrol and grape seed extract. SSPS films without any essential oil had significantly higher ($p < 0.05$) moisture content than did SSPS films incorporating either ZEO or MEO. The films containing ZEO displayed lower moisture content than those formulated with MEO, indicating that the ZEO decreased the hygroscopicity of the films much more than did the MEO. These findings were in accordance with those reported by Zinoviadou, Koutsoumanis, and Biliaderis (2009), who also showed that adding oregano oil at 0.5, 1 and 1.5% did not markedly affect the water content of whey protein isolate films.

Water solubility of film-forming materials is an important factor when choosing a film for specific applications. Solubility is a desired property in many cases, such as the encapsulation of foods. The water solubility of the prepared SSPS films with and without ZEO and MEO is shown in Table 1. It would be expected that hydrophilic compounds should increase a films' solubility, whereas hydrophobic compounds should decrease it (Kavoosi, Dadfar, & Mohammadi Purfard, 2013). In the present study, the solubility of the control and emulsified films followed the same trend as moisture content, which is in accordance with the expectation that increasing the concentration of essential oils decreases the films' hydrophilicity. Lower solubility of ZEO-containing films compared to MEO-containing ones may be explained by an increase in interaction between the hydroxyl groups of SSPS chains and ZEO components, leading to a decrease in the availability of hydroxyl groups, and thus reducing polysaccharide–water interactions. This in turn would result in a decrease in the films' solubility. ZEO addition at all three levels did not markedly affect the water uptake of SSPS films. However, lower moisture uptake was observed for the films containing 3% ZEO compared to the control films. Nor did increasing MEO content have a significant effect ($p > 0.05$) on the films' moisture uptake.

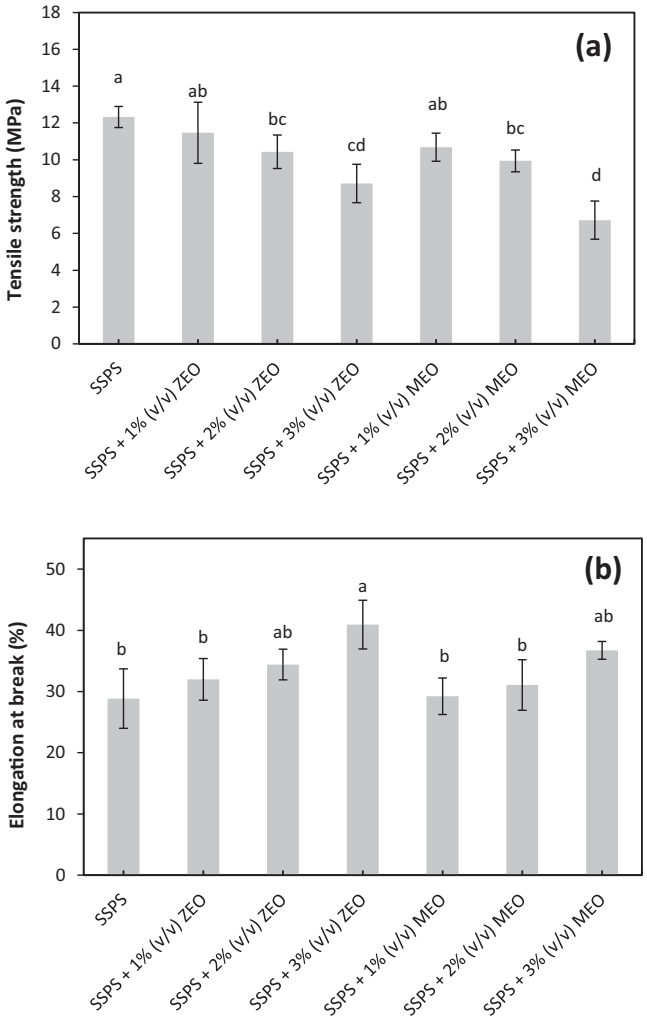

Fig. 1. Tensile strength (a) and elongation at break (b) of SSPS films formulated with ZEO or MEO. Different letters indicate a statistically significant difference ($p < 0.05$).

3.3. Water vapor properties

One of the main functions of food packaging is to avoid or minimize moisture transfer between the food and the surrounding atmosphere. Water vapor permeability (WVP) should therefore be as low as possible to optimize the food package environment and potentially increase the shelf-life of the food product (Hosseini, Rezaei, Zandi, & Ghavi, 2013). The WVP of SSPS films incorporated with ZEO or MEO are shown in Fig. 1.

In the present study, the WVP of the SSPS composite films was not significantly ($p > 0.05$) affected by the incorporation of 1–2% of ZEO or MEO compared to control films. However, a significant decrease ($p < 0.05$) was observed when 3% ZEO was added ($1.17\ g\ s^{-1}\ m^{-1}\ Pa^{-1} \times 10^{-10}$), much lower than that for films emulsified with MEO. The lower WVP of the SSPS films with either ZEO or MEO could be explained by the action of two opposite effects: the high contact degree between SSPS and oil, which makes the SSPS chain aggregation forces weaker and thus contributes to a decrease in the cohesion forces of the polymer network (as deduced from the analysis of mechanical properties); and the overall increase in the hydrophobic character of the matrix by the oil incorporation. At the low oil concentrations, the effect of the overall increase in

Table 1
Physical properties of SSPS films incorporated with various concentrations of ZEO and MEO[a,b].

Film type	Essential oil concentration (% v/v)	Thickness (mm)	Moisture content (% d.b.)	Solubility in water (%)	Water uptake (%)
Control	0	0.073 ± 0.005[b]	32.05 ± 0.21[a]	90.12 ± 1.89[a]	22.22 ± 0.11[b]
ZEO	1	0.074 ± 0.005[b]	23.49 ± 0.19[bc]	69.64 ± 3.72[ab]	25.31 ± 0.57[b]
ZEO	2	0.076 ± 0.003[ab]	21.22 ± 1.67[bc]	67.71 ± 2.51[ab]	19.57 ± 3.10[bc]
ZEO	3	0.077 ± 0.002[ab]	18.92 ± 0.53[c]	63.97 ± 2.64[c]	13.86 ± 2.15[c]
MEO	1	0.075 ± 0.006[ab]	26.65 ± 2.58[b]	79.00 ± 2.01[b]	27.02 ± 2.07[ab]
MEO	2	0.081 ± 0.002[ab]	28.51 ± 2.01[b]	78.45 ± 2.89[b]	28.51 ± 2.01[ab]
MEO	3	0.083 ± 0.005[a]	27.02 ± 2.07[b]	75.69 ± 3.16[b]	32.65 ± 2.58[a]

Values reported are means and standard deviation. Superscript letters indicate the significant difference of data within the same column at $p < 0.05$.

the hydrophobic nature of the matrix could predominate over the effect of the loss of matrix cohesion, limiting water-vapor transport. In contrast, for high oil content, the two effects seem balanced (Bonilla, Atarés, Vargas, & Chiralt, 2012). The effectiveness of ZEO in reducing WVP can be partially explained by the more hydrophobic nature of the main components of this essential oil (thymol, carvacrol and γ-terpinene), which are less polar than those of MEO (pulegone, menthone and piperitone) (Kamkar et al., 2010; Saei-Dehkordi et al., 2010). The same results were found by Moradi et al. (2012) for ZEO incorporation into chitosan films, and by Shojaee-Aliabadi et al. (2013) for incorporation of *Satureja hortensis* essential oil into carrageenan films. This result also confirmed the outcome of earlier research by Aliheidari, Fazaeli, Ahmadi, Ghasemlou, and Emam-Djomeh (2013), who had reported that incorporation of *Matricaria recutita* essential oil in casein-based films decreased its WVP. However, some studies, the incorporation of essential oils has not shown improvements in WVP (Zinoviadou et al., 2009). The opposite behavior was found in the study of Atarés, De Jesús, Talens, and Chiralt (2010), who suggested that it cannot be assumed that the WVP of films is reduced simply by adding a hydrophobic component to the formulation.

3.4. Mechanical properties

Mechanical properties of films were characterized by measuring the tensile strength (TS) and elongation at break (EB), which are key indicators of a film's strength and flexibility. The effect of different essential oil types and concentrations on the mechanical properties of film samples is presented in Fig. 2.

The results showed that both ZEO and MEO significantly affected ($p < 0.05$) the mechanical resistance and extensibility of the SSPS-based films. The incorporation of ZEO or MEO into the SSPS films caused a reduction in TS: it did not significantly change when essential oil concentration was increased from 1 to 2%, but significantly changed when concentration was increased from 2 to 3%. This effect was the most pronounced in films containing 3% MEO, which displayed the lowest TS values (6.72 MPa). This can be attributed to the complex structures formed between the lipids and the SSPS

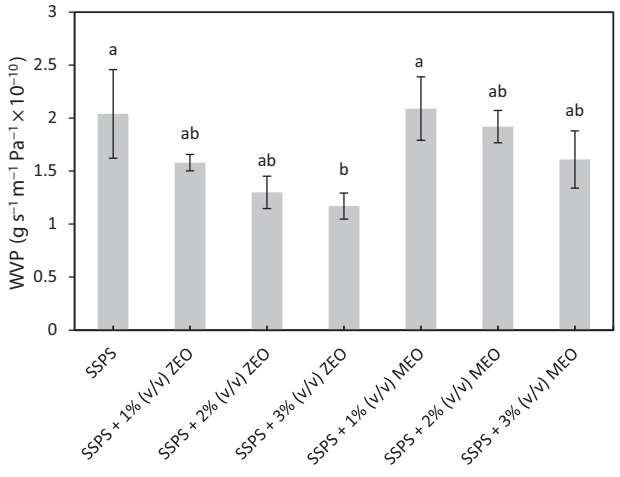

Fig. 2. Water vapor permeability of SSPS films formulated with ZEO or MEO. Different letters indicate a statistically significant difference ($p < 0.05$).

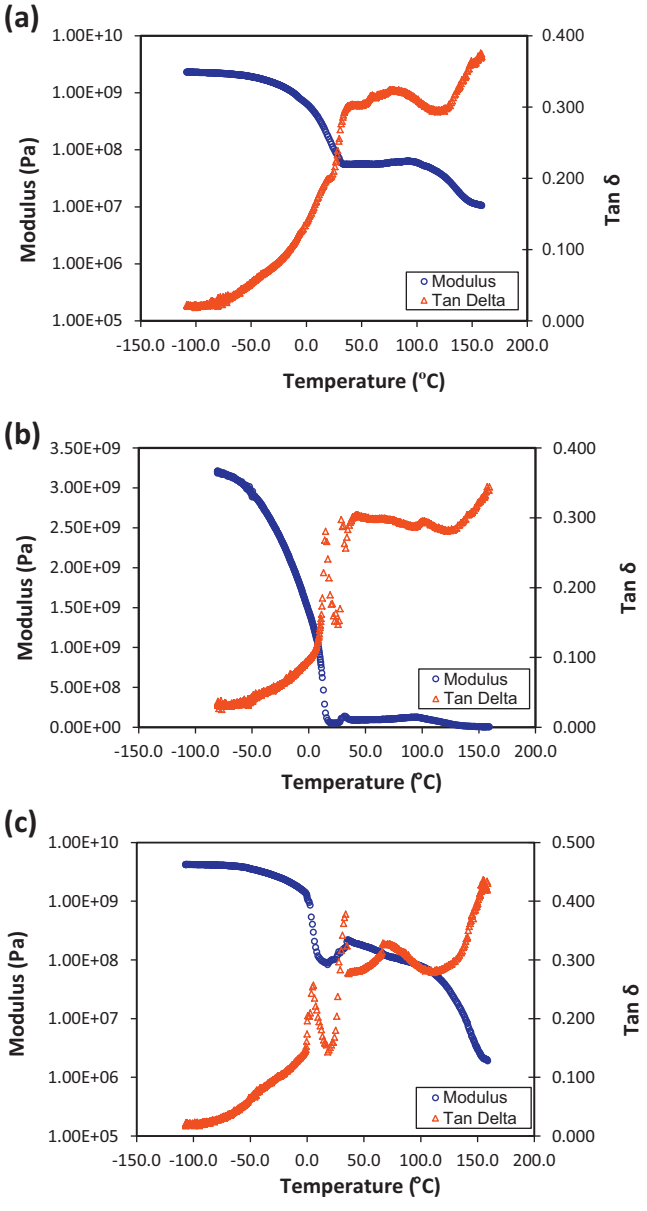

Fig. 3. DMTA plots ($\log E'$, $\tan\delta$) for glycerol-plasticized SSPS films containing different concentrations of ZEO and MEO; (a) SSPS, (b) SSPS/ZEO and (c) SSPS/MEO.

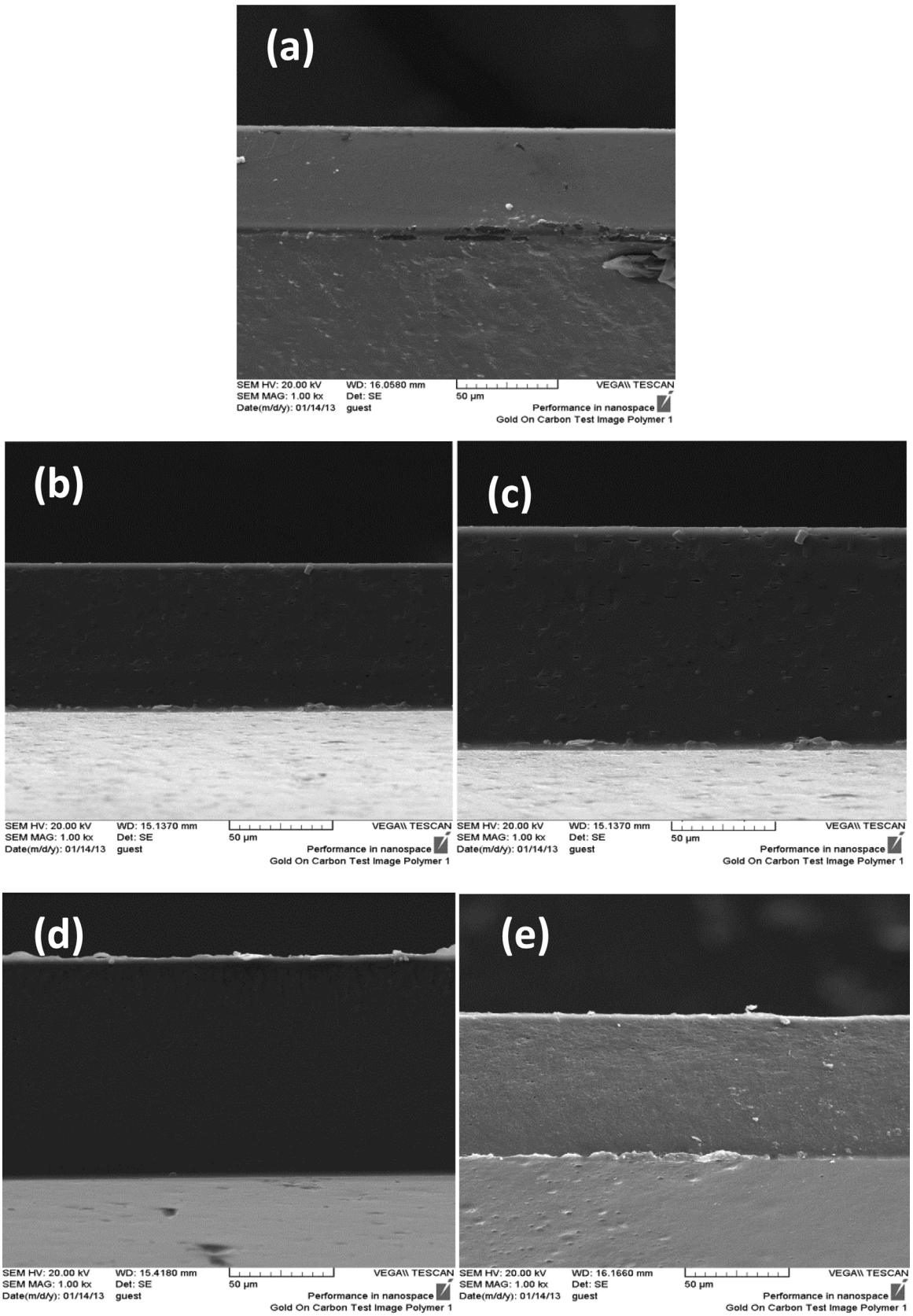

Fig. 4. Scanning electron micrographs of cross-section of different SSPS films, (a) SSPS, (b) SSPS/1% ZEO, (c) SSPS/3% ZEO, (d) SSPS/1% MEO, and (e) SSPS/3% MEO viewed at a magnification of 1000×.

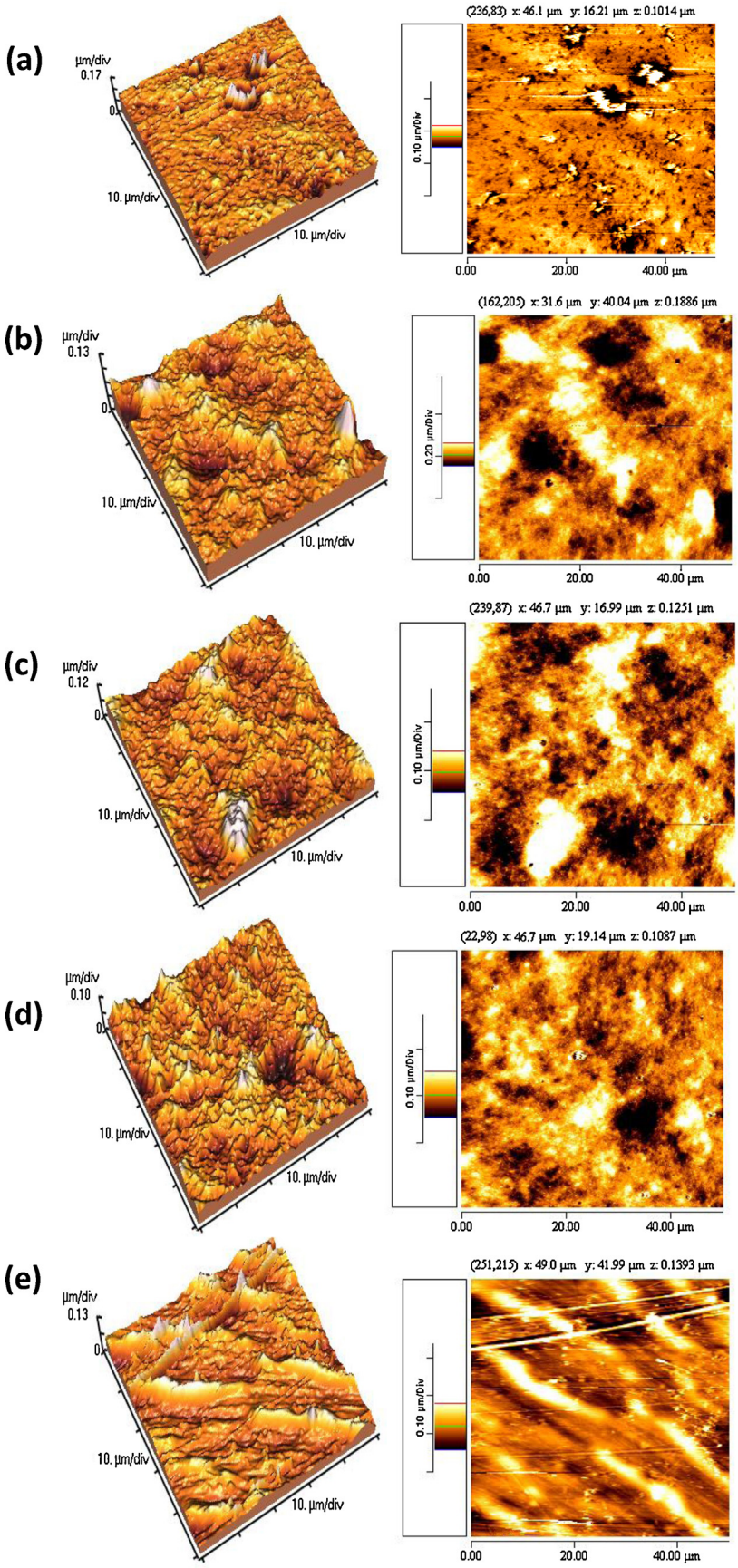

Fig. 5. AFM topographic images (50 μm × 50 μm) of the films, (a) SSPS, (b) SSPS/1% ZEO, (c) SSPS/3% ZEO, (d) SSPS/1% MEO, and (e) SSPS/3% MEO.

Table 2
Roughness parameters obtained from atomic force microscopy images. (three images were analyzed in each case).[a]

Film type	Essential oil concentration (% v/v)	Roughness parameters	
		R_a (nm)	R_q (nm)
Control	0	22.68 ± 1.41c	24.49 ± 3.31c
ZEO	1	35.11 ± 2.19b	24.95 ± 0.99c
ZEO	3	37.99 ± 2.49b	27.90 ± 1.93c
MEO	1	39.94 ± 0.64b	35.32 ± 3.54b
MEO	3	66.98 ± 4.65a	45.11 ± 5.37a

[a] Reported values correspond to the mean ± standard deviation. Values within each column followed by different letters indicate significant differences ($p < 0.05$).

chains, which reduce the cohesion of the polymer network forces, thus decreasing the films' resistance to breakage (Jiménez, Fabra, Talens, & Chiralt, 2013). No differences were observed for EB after adding ZEO or MEO up to a concentration of 2% except in the case of SSPS film incorporating 3% ZEO, which had a significantly higher EB value. Similar changes in mechanical properties were observed by Siripatrawan and Harte (2010) when green tea extract was incorporated into chitosan films; they reported that the TS and EB of chitosan films did not change when green tea extract concentration increased from 0% to 5%, but significantly increased when concentration increased from 5% to 20%.

3.5. Dynamic mechanical thermal analysis

Dynamic mechanical thermal analysis (DMTA) is one of the most effective means of characterizing the molecular chain segment motion, intermolecular interaction and miscibility of polymer blends. The thermo-mechanical behavior of SSPS films containing ZEO and MEO was studied using DMTA. Representative DMTA traces ($\log E'$ and $\tan\delta$) as a function of temperature are shown in Fig. 3.

The temperature at which the glass transition occurs depends on the free volume of polymer. At very low temperatures, the molecules are tightly compressed and there is negligible free volume. However, as temperature gradually increases, the free volume of the crystalline part of the polymer expands to permit localized movement in the main-chain molecules. This phenomenon is called γ relaxation. Continuing increase in temperature provides more free volume for the molecules, and this causes rotational and vibration movements in the side chains and end groups of polymer; this phenomenon is called β relaxation. Continuing heating causes movements in the main chain of the amorphous domains of the polymers (Ahmadi et al., 2012). This phenomenon happens at the glass transition temperature point (T_g) of polymers, mainly peak of $\tan\delta$, which is called α relaxation. When control SSPS films were scanned by DMA, two relaxations (β and α) were found with increasing temperature (Fig. 3). The higher-temperature peak was attributed to the glass transition (T_g) temperature, while the low-temperature peak corresponded to the β-relaxation, which is due to segmental motions in the non-crystalline phase. Addition of essential oil, either ZEO or MEO, in concentrations of up to 2 v/v% did not produce any remarkable change in the thermo-mechanical properties of SSPS films. It just seemed to very slightly extend the temperature interval over which the rubbery-like plateau appeared. However, essential oil content of 2 v/v%, was seen to modify the rheological behavior of the film. Moreover, ZEO addition generally induced an enhancement in modulus values as compared with those shown by the essential oil-free or MEO-containing films at the lowest temperature interval studied. The $\tan\delta$ curves provide information about molecular damping and mobility, which in turn is related to the impact strength of the material. It is obvious from the $\tan\delta$ curves, shown in Fig. 3, those films containing either ZEO or MEO exhibit almost similar values for glass transition temperature (T_g) and $\tan\delta$ peak height values. In these cases, a one-step drop in modulus, as well as a single peak in $\tan\delta$, was observed in their DMTA traces; separate transition was not detectable. If two different polymers or a blend of polymer and additives are immiscible, the mixture will show two α-relaxations corresponding to the two pure phases.

3.6. Film structure

A microstructural study of the structural arrangement of the different components in dried film matrices contributes to a better knowledge of water-vapor transmission mechanisms and mechanical properties (Fabra, Pérez-Masiá, Talens, & Chiralt, 2011). Differences in film structure caused by the addition of either ZEO or MEO were investigated by SEM. The micrographs of film surfaces showed a relatively smooth and continuous surface, and no differences due to the inclusion of essential oils were detected (data not shown).

Fig. 4 shows cross-sectional micrographs of various SSPS-based films, which show notable differences as a function of the film composition. While a smooth and continuous microstructure was observed for the pure SSPS film, emulsified films showed discontinuities, although to a lesser extent, due to the presence of the lipid dispersed phase in the polymer matrix. ZEO seems to be more finely distributed in the polymer matrix, giving rise to a more homogenous network where particles can hardly be detected in the continuous matrix.

Even though macroscopically both control and MEO-containing films presented similar surface characteristics, incorporation of MEO at higher concentrations brought out notable changes in the films' surface microstructure as revealed by SEM micrographs. In Fig. 4e small white spots were observed, which could be attributed to small particles produced during the film fracture before observation that deposited on the cross-section of the film. Generally, in the films containing MEO, at lower concentration, oil droplets were intimately integrated within the film matrix and were less evident on the film surface. However, a coarser microstructure was observed in the films that incorporated the highest concentration of MEO, due to the fact that the higher lipid content favors the flocculation rate (Sánchez-González, Vargas, González-Martínez, Chiralt, & Cháfer, 2009).

3.7. Film morphology

Fig. 5 shows typical surface topographies for the films; the corresponding results of roughness parameters – the average roughness (R_a) and root-mean-square roughness (R_q) – are shown in Table 2.

The oil-free film had a smooth surface, with R_a and R_q values of 22.68 and 22.49, respectively and showed the lowest values in Table 2. According to the AFM images, emulsified films containing 3% ZEO were rougher than those with 1%. However, a comparison of roughness parameters of these films did not show a significant difference ($p > 0.05$) between them. The surface irregularities of the ZEO-containing films were less accentuated than those of films prepared with MEO, thus producing a smoother film surface. At all

essential-oil concentrations tested, a trend toward higher roughness values was obtained in films with MEO, with lower roughness values for ZEO (Table 2). For films containing 3% MEO, a notable increase in the surface roughness was observed. The AFM images of either ZEO or MEO containing films revealed the presence of some large objects which could possibly be attributed to the presence of micro- or even nano-scale insoluble particles of SSPS. These particles may be present in the suspension which would appear completely dissolved until observed under very high magnification. This phenomenon was also seen when the microstructure of corn starch films was observed by SEM (Kuorwel, Cran, Sonneveld, Miltz, & Bigger, 2013b). In this study, the authors observed relatively small particles in both a commercial starch blend film and in films prepared directly from soluble corn starch powder and attributed these to insoluble starch particles.

4. Conclusion

This is the second report that demonstrates the ability of SSPS to form biodegradable film. The process developed here produced transparent films with good mechanical and barrier properties. SSPS-essential oil composite films with improved WVP were developed by emulsification. The incorporation of either ZEO or MEO did not cause major changes in the films' moisture-content values. Although emulsified films were less water soluble and less resistant to fracture, these changes did not negatively affect the handling or manipulation of the films. DMTA curves revealed a single T_g, which may be an indication of the compatibility of essential oil and SSPS. AFM images indicated that the surface roughness of the films was affected by the addition of either ZEO or MEO; as expected, the maximum surface roughness occurred at the ratio of 3% in the composite emulsified film. Based on the microstructures observed in SEM analysis, the micrographs for all films were homogeneous, without signs of phase separation between the components. Due to the lower WVP value of films produced in this study (in comparison with other polysaccharide-based films), SSPS films could be a potential alternative for synthetic packaging, and for the storage of low- or intermediate-moisture foods such as nuts. Overall, this study suggests that SSPS films incorporated with ZEO or MEO show a strong potential to be used as active films. Nevertheless, further studies are required before using such films as an active packaging for real food products.

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