Antimicrobial and Physicochemical Properties of Plasma Treated Bio-Coating Polypropylene Films Containing *satureja hortensis* Essential Oil

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ABSTRACT: *Carboxymethyl cellulose-coated polypropylene film containing Satureja hortensis Essential Oil (SEO) was developed based on the casting method as a novel composite bilayer film intended for food packaging. Polypropylene films were initially treated with an atmospheric plasma system to improve adhesion properties. The films were incorporated with 1-4% Satureja hortensis essential oil and were characterized for physical (thickness, moisture content, and water solubility), mechanical (Tensile Strength (TS, and elongation at break), optical, as well as Water Vapor*

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Permeability (WVP), and microstructure (SEM) properties. The antimicrobial activity of the films against five selected bacteria including S. aureus, B. cereus, E. coli, S. typhimurium, and P. aeruginosa was also examined with direct contact and vapor phase methods. Results showed that higher SEO incorporation dosage led to significantly lower solubility and permeability of bilayer film ($P<0.05$). Additionally, by increasing SEO concentration, more opaque and stretchable films with less resistance to breakage were obtained. Films incorporated with 2-4% SEO effectively inhibited all tested microorganisms both in direct contact and vapor phase. Results of the present study suggest that SEO incorporated PP/CMC films as a novel structure of biopolymer coatings on common plastics that can be potentially used as active packaging for food products.

KEYWORDS: *Satureja hortensis; Cold plasma; Film; Antimicrobial; Bio-coating*.

INTRODUCTION

Producing efficient packaging which could provide fresh, healthy, and high-quality food products have always been the aim of the food packaging industry [1]. Synthetic petroleum polymers are among the commonly used materials in the food packaging industry in which the most important concern is the waste-disposal problems associated with the environment and release of undesirable chemical additives into the packed food [2]. In recent years, biopolymer-based packaging materials have been substantially taken into consideration due to their biodegradability and recyclability [1].

Carboxymethyl cellulose (CMC) is one of the natural cellulose derivatives with β (1→4)-linked glucopyranose residues. CMC is a water-soluble anionic polysaccharide with widespread applications as an additive in foodstuff products. CMC has many remarkable properties including biodegradability, great film-forming, and thermal gelation properties, non-toxicity, and biocompatibility that make it an appropriate option in film formulations [3]. The principal limitation of CMC films is their poor mechanical and water vapor barrier properties due to its high water affinity.

Many studies have aimed to improve the general characteristic of biopolymer-based films, especially their mechanical and water vapor barrier properties [4], however, full satisfaction has not been achieved yet, and there are still some problems in applications of edible films in the food packaging industry [5].

A strategy to best overcome the overuse of synthetic polymers and to improve the properties of biopolymers in the packaging is to use edible films in combination with synthetic polymers, and therefore, creating a bilayer composite film composed of two components. In addition to better recyclability of the packaging [6], this technique has other advantages including lowering production costs, defeating migration problems, improving oxygen permeability resistance [7], and water vapor resistance [8]. Moreover, it provides an efficient carrier for different bioactive compounds in the packaging [9], especially for heat liable bioactive components which are prone to be lost during extrusion of plastic materials or may be incompatible with the polymer matrices. Therefore, biopolymers could act as a qualified carrier for the incorporation of these compounds into the active packaging [10].

Among different additives, essential oils are more preferred as a result of consumers' perception of the probable harms of chemical additives, and thus there is an increasing trend for evaluating different essential oils for use in the active packaging.

Satureja hortensis is an annual, medicinal plant belonging to the Lamiaceae family, which primarily grows in southern Europe. This herbaceous plant is used as a flavoring agent in a variety of foods. The main constituents of *Satureja hortensis* Essential Oil (SEO) are phenolic compounds with previously demonstrated antimicrobial and antioxidant activity [11, 12].

Polypropylene as a practical polymer in the packaging industry represents good traits such as low density, thermal stability, easy processing, and good chemical resistance in addition to its low cost and abundance [13].

In general, it is very difficult to coat non-polar plastic films with polar biopolymer solutions due to their

different natures. The non-polar surface of PP represents no open biding sites to be coated with biopolymers [14]. For improving adhesion properties of synthetic polymers, surface modification prior to the coating process is very essential, otherwise, final composites would have weak physical properties [15]. Different methods can be utilized to modify polymer surface including corona discharge, exposure to flame, chemical etching, and plasma treatment.

Amidst these techniques, plasma treatment is more favorable due to several advantages including environmental safety, decreasing consumption of harmful chemicals, uniformity and reproducibility, energy-saving, and the ability to modify without altering the bulk properties [16,17].

To our best knowledge, although some studies have already been implemented regarding composite films constituted of a biopolymer and a synthetic polymer, or biodegradable films activated with different essential oils, there is just one report on the incorporation of essential oils in composite structured films by the coating method [18]. Furthermore, so far there has been no attempt for characterization of PP/CMC bilayer films containing *Satureja hortensis* Essential Oil (SEO) in the literature. Results of this study could be promising for potential applications of these bilayer films for diverse foodstuffs in the food packaging industry.

EXPERIMENTAL SECTION

Materials

Commercial carboxymethyl cellulose (CMC) was supplied by SINOCMC Co., LTD (Qingdao, China), Essential oil was purchased from Barij Essence Pharmaceutical Co. (Kashan, Iran). PP films with 20-μm thickness were catered by Pol-film Co. (Tehran, Iran). Tween 80, glycerol, Sodium Chloride, Calcium chloride, Magnesium Nitrate, Mueller–Hinton agar (MHA), and Mueller–Hinton Broth (MHB) were bought from Merck Co. (Darmstadt, Germany). All other reagents used were of analytical grade.

Bacterial strains

Staphylococcus aureus ATCC 25923; *Bacillus cereus* PTCC 1154, *Escherichia coli* ATCC 25922; *Pseudomonas aeruginosa* ATCC 27853; *Salmonella typhimurium* ATCC 14028 were supplied by the Iranian Research Organization for Science and Technology (Tehran, Iran). Stock cultures of the target bacteria were allowed to grow in a nutritious broth (Mueller–Hinton broth (MHB)) at 37°C for 24 h before testing.

Plasma treatment of Polypropylene films

Rolled synthetic polypropylene films were cut into rectangles of 20 cm x 30 cm, and were cleaned with alcohol-dipped cotton to remove any probable dust on the surface. After drying, PP films were treated with an atmospheric plasma system (roll to roll plasma system, Satiya Co. Iran). The plasma system consisted of a silicon-coated roller electrode connected to the high voltage and 4 stainless steel electrodes with 4 mm diameter connected to the earth. This system uses air as working gas to generate plasma. After applying the 14kV (the frequency of the power supply is fixed to 20 kHz with AC form) to the silicone coated electrode, the plasma will be generated. Polypropylene films were passed continually throw the plasma gap with 3.6 m/min speed which was constant during the experiment. Treated films were subjected to air for about 2 minutes to let the created free radicals on the film surface react with air molecules to form active functional groups which were subject to post-reactions. Plasma treatment could impart some level of oxidation followed by an increase in the surface energy level [19].

Preparation of the films

The bilayer films were made by the casting method. Initially, CMC films were prepared according to the method of *Shojaee-Aliabadi et al.* (2013) with some modifications [12]. CMC film solutions (1% w/v) containing glycerol as the plasticizer (50% v/w based on CMC) were made at 75-80°C under continuous magnetic stirring for 40 min. The Film-Forming Dispersion (FFD) was cooled to about 55°C to eliminate any air bubbles and was used as the control film.

For preparing antimicrobial films, 1 to 4% (v/v based on FFD) of *Satureja hortensis* Essential Oil (SEO) was added to the FFD following the addition of tween 80 as an emulsifier in quantities proportional to the SEO (0.1 to 0.4% v/v). For Homogenization, a rotor-stator homogenizer (IKA T25-Digital Ultra Turrax, Staufen, Germany) was utilized at 13,500 rpm for 4 min.

Finally, the FFDs were cast on the center of rectangular glass plates (20*30 cm^2) which were previously covered with plasma-treated polypropylene and then dried at 35°C for about 20 h. Dried films were peeled off the plates and

kept inside desiccators at 25°C and 53% Relative Humidity (RH) provided by saturated magnesium nitrate solution until evaluation.

Determination of physical properties of films

Thickness

Film thickness was determined using a manual digital micrometer (Mituto, Tokyo, Japan) to the nearest 0.001 mm. Measurement was carried out at ten different film locations and mean thickness value was used to calculate the permeability and mechanical properties of the film.

Moisture content

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.

Film solubility in water

The water solubility was measured according to the method of *Ojagh et al.* with some modifications [20]. The film samples were cut to a square piece of 2.0 cm ×2.0 cm, and their initial dry weight was determined after drying at 110 °C to constant weight (W_0). The samples were immersed and shaken under constant agitation in 50 mL of distilled water for 1 h at 25°C. The solution was then filtered, and the undissolved remaining film was dried in a hot air oven at 110◦C until a final constant weight was obtained (W_1).

The water solubility (%) of the film was calculated according to the following equation:

$$\%WS = \left(\frac{W_o - w_1}{W_o}\right) \times 100$$

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.

Mechanical properties

Mechanical properties, including tensile strength (MPa) and elongation at break (%) of the film samples, were determined according to ASTM standard method D882 using a Testometric Machine M350-10CT (Testometric Co., Ltd., Rochdale, Lancs., England) at 25 °C [21]. Film specimens were cut into rectangular strips (1.5 cm × 10 cm), and conditioned at 25 °C and 53% RH in desiccators containing Mg $(NO_3)_2$ saturated solutions for 48 h prior to testing. The initial grip separation and cross-head speed were set to 50 mm and 50 mm/min, respectively. Moreover, 50 N load cell was employed. Tensile strength values were calculated by dividing the maximum stress by the cross-sectional area of the specimen and Elongation values were expressed as percent units, with the ratio of extended length to the initial length (distance between the grips). At least three replicates of each film were tested.

Water Vapor Permeability (WVP)

WVP of the film samples was determined at 25 °C and 75% RH gradient according to the ASTM E96 gravimetric method [22]. Circular test cups with an opening surface area of 0.00287 m^2 were applied and sealed by the test films. Prior to sealing, the cups were filled with anhydrous calcium chloride ($CaCl_2$– 0% RH). The cups were then placed in a desiccator containing a saturated sodium chloride solution (Merck, Darmstadt, Germany) with 75% RH. The difference in RH contributes to a driving force of 1753.55 Pa, expressed as water vapor partial pressure. Weight gain of the cell was recorded and plotted as a function of time (with an accuracy of 0.0001 g). The Water Vapor Transmission Rate (WVTR) was calculated from the slope of the weight gain vs. time plot divided by the cell area (m_2). WVTR was multiplied by the thickness of the film and divided by the pressure difference between the inside and outside of the test cup to obtain the WVP. All tests were performed in triplicate.

Optical properties

The lightness (L), redness (a), and yellowness (b) color system was used to assess the color of films by a colorimeter (Minolta CR 300 Series, Minolta Camera Co., Ltd., Osaka, Japan). The measurements were performed on a standard white plate (L* = 99.94, a* = −0.52 and b* = 1.43). All measurements were carried out in triplicates. The total color difference (ΔE) and Whiteness Index (WI) were calculated according to the following equations:

$$\Delta E = \sqrt{\left(L^* - L\right)^2 + \left(a^* - a\right)^2 + \left(b^* - b\right)^2}$$

$$WI = 100 - \sqrt{(100 - L)^2 + a^2 + b^2}$$

Where *L**, *a**, and *b** are the color parameter values of the standard background and *L*, *a*, and *b* are the color parameter values of the test films.

The opacity of the film specimens was determined by measuring the absorbance at 600 nm using a spectrophotometer (Shimadzu UV-Vis 1601, Japan) according to the method of *Gómez-Estaca et al.* [23]. An empty test cell was used as the reference. The opacity was calculated using the following equation:

$$Op = \frac{Abs600}{X}$$

Where Abs600 is a value of absorbance at 600 nm and x is the film thickness (mm).

Scanning Electron Microscopy (SEM)

The microstructure of the cross-sections of bilayer films was examined by scanning electron microscopy (VEGA, TESCAN, Czech Republic). Before the analysis, each film was fixed on support using double-sided adhesive tape. Then samples were gold coated with K450X sputter coater (Emitech**,** England) under 10^{-1} pa vacuum. All the images were captured using an accelerating voltage of 25 kV.

Evaluation of the antimicrobial activity of films

Disc diffusion method

The agar diffusion assay was used to qualitatively examine the antimicrobial characteristics of the films. PP/CMC films were cut into 6 mm diameter discs using a sterile punch and placed on plates containing MHA which had been previously seeded with 100 µL of an overnight broth culture containing approximately 10^8 CFU/mL of the target bacteria. The plates were then incubated at 30 °C for 24 h. Following incubation, the whole zone area was measured by calculating the diameter of the growth inhibition zones and subtracted from the film disc area. This difference in the area was reported as the zone of inhibition [20]. Each film formulation was evaluated in three replicates.

Disc volatilization method

Antimicrobial activities of the films in the vapor phase were examined according to the method of *Lopez et al.* [24]. The test method is similar to the disc diffusion method, however, the discs are mounted on the inside surface of the upper lid without direct contact with the culture surface. The plates were sealed using parafilm to avoid the escape of essential oil vapor prior to incubation at 30 °C for 24 h. The diameters of the zone area were measured and the whole zone area was reported as "zone of inhibition". The tests were carried out in triplicate for each test film.

Statistical analysis

The statistical analysis of the data was performed using SPSS statistical software version 21 (SPSS Inc., Chicago, IL). Analysis of variance (ANOVA) followed by Duncan's multiple range test was used to determine any significant differences among the treatments at a 95% confidence level.

RESULTS AND DISCUSSION

Physical properties of films

Table 1 shows the effect of SEO incorporation on the physical properties of PP/CMC films. The thickness of films varied from 0.048 to 0.125 mm, significantly increasing as the SEO percentage increased ($p<0.05$). The bilayer films containing SEO represented lower moisture content than the control film, however, incorporation of essential oils less than 3% did not significantly affect it ($p > 0.05$).

The water solubility of the film is particularly important for their specific application in the food packaging indicating their integrity in high-moisture conditions. More solubility of the film is linked to lower water resistance. The solubility of the control film was 89.16% indicating lower solubility compared to what Dashipour reported (100%) which could not be detectable [25]. This can be attributed to PP layer of bilayer film and good attachment of CMC coating to it which decreases water solubility.

The result of this study showed that the addition of SEO into the film formulation remarkably decreases film solubility ($P<0.05$) indicating that the SEO decreased the hydrophilicity of the films. Another reason for this reduction could be an increase in interaction between the hydroxyl groups of CMC chains and SEO components, contributing to a decrease in the availability of hydroxyl groups, and thus reducing CMC–water interactions needed for the solubility. Because of lack of information in the literature about the solubility and moisture content of biopolymer coated plastic films, results are compared to the edible films and are in accordance with GhasemLou

Table 1: Physical, WVP and mechanical properties of PP/CMC films formulated with different concentrations of SEO [a]

Film	Thickness (mm)	Moisture content (%)	Water Solubility (%)	WVP ($g\ s^{-1}\ m^{-1}\ Pa^{-1} \times 10^{-12}$)	Tensile Strength (MPa)	Elongation (%)
control	0.048±0.001^{e}	17.31±0.36^{a}	89.16±0.50^{a}	0.847±0.032^{a}	61.82±4.08^{a}	20.31±2.55^{d}
SEO1%	0.064±0.005^{d}	17.09±0.24^{ab}	84.15±0.29^{b}	0.673±0.040^{b}	17.03±0.82^{b}	19.00±4.36^{d}
SEO2%	0.097±0.002^{c}	16.87±0.89^{ab}	79.55±0.55^{c}	0.287±0.022^{c}	15.52±5.52^{bc}	26.17±2.09^{c}
SEO3%	0.112±0.0005^{b}	16.15±0.15^{b}	74.40±0.45^{d}	0.171±0.028^{d}	14.84±0.34^{bc}	34.26±1.62^{b}
SEO4%	0.125±0.003^{a}	14.58±0.51^{c}	70.54±0.95^{e}	0.160±0.006^{d}	10.32±0.02^{c}	49.63±0.66^{a}

[a] *Data reported are average values ± standard deviations. Values within each column with different letters are significantly different in each section ($P < 0.05$)*

findings who observed that solubility and moisture content of corn starch films incorporated with plant essential oils, both decreases as essential oil concentration increased [26].

Mechanical properties

In order to predict the mechanical properties of the bilayer films, tensile strength (TS) and elongation at break (EB) of the films, as two important features representing the strength and flexibility of films, were characterized. Table 1 shows the effect of essential oil concentration on the mechanical properties of the PP/CMC films.

The results revealed that SEO incorporation in PP/CMC films significantly influenced the mechanical resistance and stretching properties of the bilayer films ($P<0.05$).

The tensile Strength (TS) of the control film was 61.82 MPa which is twice more than the TS of MC and HPMC coated PP films which showed a tensile strength in the range of 30-35 MPa [14]. This could be attributed to the molecular characteristics of the coating matrix and the difference in the amount of applied plasticizer, which was 35% and 15% for MC and HPMC, respectively compared to 50% in the present study. In addition, TS of the control PP/CMC film was remarkably higher than what Shobita et al (31.3 MPa) and Dashipour et al (17.75 MPa) reported for monolayer CMC film [25, 27] indicating that the presence of the PP layer as the base film has successfully increased the maximal force per original cross-sectional area that the film could tolerate before breaking. Furthermore, it can be due to interactions of plasma-induced polar groups of PP film and the hydroxyl group of CMC and also higher roughness of plasma-treated PP layer resulting in better adhesion of PP to CMC [18].

Bilayer films having SEO showed significantly lower tensile strength ($P<0.05$) than the control film. Furthermore, the addition of SEO in the film matrix led to a significant decrease ($p<0.05$) in TS of the bilayer films from 17.03 to 10.32 MPa as oil concentration increased from 1 to 4%. The mechanical properties of bilayer films are primarily affected by the base film (here; PP film) rather than the coating layer [28]. For CMC-coated PP films, however, the tensile property was affected by oil concentration as an antimicrobial agent.

Particularly, by increasing the percentage of essential oil, negative impacts could be exerted on tensile properties, contributing to the reduction of mechanical resistance in PP/CMC films. The primary reason for this trend could be the slight replacement of strong intermolecular interactions by weaker polymer-oil interactions resulting in the weakness of the film network and, thus decreasing TS [12]. On the other hand, the presence of hydrophobic SEO components such as carvacrol and thymol as main compounds, increased hydrophobicity of CMC coating which in turn reduced plasma-induced surface hydrophilicity of PP film, probably leading to a more difficult coating process compared to a similar work having *Satureja hortensis* extract [29]. However, even at the concentration of 4%, the bilayer films retained their integrity. The result of TS coincides with the results reported for CMC–PVOH–clove oil films [27].

As Table 1 shows, although 1% incorporation of SEO caused no significant difference ($P > 0.05$) in the stretch ability of the films, in higher percentages there was a meaningful difference in the films' elongation in comparison to the control film ($P < 0.05$), significantly increasing as oil concentration increased ($P < 0.05$).

It seems that the described changes in the FFD interactions induced a plasticizing effect which resulted in more flexibility of the films (high elongation values at break). These results are consistent with our previous observation for CMC coated PP films containing *Zataria multiflora* essential oil which showed an increase in elongation from 22.86 to 44.24 as the oil concentration increased from 1 to 4% [18]. Moreover, another reason for the better flexibility of essential oil incorporated films could be due to good adherence of CMC to the PP implying that more stretch ability of the CMC layer caused the more stretch ability of PP layer as the base film. Good attachment of two layers to each other is due to the created roughness, as well as the induced polar groups on PP surface followed by plasma treatment [18]. This is while in monolayer edible films controversial results are reported regarding the effect of essential oil on elongation. Some authors indicated the same result following the addition of essential oil [30] and some authors indicated reverse results [25].

Water vapor permeability

One of the most required functions of the packaging for increasing the shelf life of the food product is to prevent or minimize moisture transfer between the food and its surrounding environment, therefore, water vapor permeability should be subsided to the lowest possible degree [31]. The water vapor permeability values of PP/CMC films with different essential oil percentages are shown in Table 1. WVP of the control film (0.847 g/s m Pa $\times 10^{-12}$) was considerably lower than the WVP of a single CMC or PP film was measured in our previous work [18], and was significantly improved ($P < 0.05$) by incorporating different amounts of SEO (1-4%). The increased resistance to water vapor in the bilayer films is due to the presence of a hydrophobic layer (PP) which limited the transfer of water vapors. Moreover, by increasing SEO concentration from 1 to 4%, WVP decreased from 0.673 to 0.160 g/s m Pa $\times 10^{-12}$. This reduction in permeability was meaningful from 1 to 3% ($P < 0.05$), however, for 4% SEO no significant difference was observed in comparison with 3% incorporation ($P > 0.05$). It could be attributed to the induced tortuosity followed by the addition of essential oil as a hydrophobic dispersed phase making the diffusion of water vapor more difficult through the film matrix [32]. For edible films, so many studies have already proved a reduction in WVP as the oil concentration increased [33-35]. Moreover, Tihminlioglu reported a remarkable improvement in water vapor barrier properties of corn–zein-coated polypropylene films [8]. Therefore, the combination of water vapor resistant PP film with CMC incorporated with more hydrophobic SEO coating resulted in films with high WV barrier properties.

The result of this study is in contradiction with *Hosseini et al.* concluding that application of thyme, clove, and cinnamon essential oils in the film matrix result in more water vapor permeability of chitosan-based films [36]. He suggested that physical factors (loose and sponge-like texture of incorporated films) had a dominant effect on WVTR. There are also other studies concluding that the presence of essential oil causes an interruption in the film network which is more influential that created hydrophobic nature, making the films more susceptible to water-molecule diffusion [37]. Additionally, *Maizura et al.* in favor of increasing WVP has justified that the addition of essential oil into a film matrix makes the polymeric structure more flexible leading to increased water absorption [30], however, the oil percentage that they used did not exceed 0.5% which is too lower compared to our study. In fact, increased hydrophobicity in our examined film is the most probable reason for this opposition.

Optical properties of films

The optical properties of bilayer films could directly influence the general perception of consumers from the packaging, therefore optimizing the formulation of films from this perspective is also important. For analyzing the optical properties of SEO-incorporated PP/CMC films, Hunter Lab color values (L, a, b), the total color difference (ΔE), and opacity values were examined (Table 2).

Oil incorporated films were more opaque with a lower whiteness index (WI) compared to the control film which was free from SEO ($P < 0.05$). In addition, SEO containing films represented markedly lower L and a-values versus showing significantly higher b-values ($P < 0.05$). This indicates that the presence of essential oil in the films caused a very slight yellowish appearance in comparison with the control film, however, in case of adding SEO up to 4% (examined in the present study) to the PP/CMC films, the color difference (ΔE) was almost steady and could not be detected with the naked eye.

Table 2: Effect of different concentrations of SEO on optical properties of PP/CMC films [a]

Film	L	A	B	ΔE	WI	Op
Control	99.830±0.036[a]	-1.28±0.26[a]	3.50±0.73[b]	2.21±0.74[b]	96.26±0.75[a]	1.87±0.34[c]
SEO1%	99.506±0.030[b]	-3.49±0.30[b]	9.96±0.90[a]	9.04±0.95[a]	89.43±0.95[b]	3.15±0.18[b]
SEO2%	99.433±0.153[b]	-4.06±1.10[b]	11.78±3.44[a]	10.95±3.61[a]	87.52±3.61[b]	3.41±0.30[ab]
SEO3%	99.420±0.020[b]	-4.21±0.04[b]	11.96±0.34[a]	11.17±0.33[a]	87.30±0.33[b]	3.56±0.27[ab]
SEO4%	99.396±0.025[b]	-4.33±0.05[b]	12.43±0.46[a]	11.65±0.41[a]	86.81±0.41[b]	3.88±0.13[a]

[a] Data reported are average values ± standard deviations. Values within each column with different letters are significantly different in each section (P < 0.05)

Surprisingly, no significant difference was recognized between different SEO concentrations in all the optical properties (P > 0.05) except for opacity which displayed a significant increase in films containing 4% SEO in comparison with 1% (P < 0.05).

The increased opacity in this study is in consistent with the observation of Rhim et al. who reported an increase in haze upon the incorporation of fatty acids in the edible film [38].

In general, transparency and haze of edible or synthetic films are primarily linked to morphological properties rather than chemical structure. Opacity is also affected by the morphological inhomogeneity of the coating, for instance when the coating thickness varies across the film [39]. According to this fact, lipid components as a reason for creating irregularities at the surface level (Fig. 1), directly influence the opacity. In particular, essential oils enhance the scattering phenomenon of the light passing through the film and thus making the films more haze and opaque.

Microstructure

Examining the microstructural order of different components in bilayer films helps to better interpret water-vapor transmission mechanisms and mechanical properties. Fig. 1 shows cross-sectional images of the PP/CMC film without SEO or with 1 and 4% (v/v) incorporation. SEO-free bilayer film had a smooth surface with no bubbles, but the addition of oil contributed to discontinuities (irregularities) in the film's structure due to the entrapment of oil micro droplets in the continuous polysaccharide network. Emulsified bilayer films represented a thicker appearance, depending on the amount of oil added to CMC matrix. Pictures well represent the carboxymethyl cellulose layer coated on a polypropylene surface.

Even though from a macroscopic viewpoint SEO-containing films, either at low or high incorporation levels, displayed smooth and continuous surface characteristics, notable differences were identified in the surface microstructure of the bilayer films depending on the amounts of added SEO as revealed by SEM micrographs.

It seems that SEO distribution (at lower concentrations) in the polymer matrix occurred to an acceptable level since SEO particles can hardly be found in the continuous matrix.

Generally, bilayer films containing SEO at 1% concentration, although having a coarser surface compared to the control film, represented a more uniform structure in comparison to films that incorporated the highest concentration of SEO. This could be attributed to the fact that the higher lipid content facilitates the flocculation and coalescence rate [40].

As previously explained, our study proved that the addition of essential oil to the film formulation did not interrupt the film network even at high concentrations, and that is why we did not observe the increase in WVP of the bilayer films (Fig. 1).

Antimicrobial activity

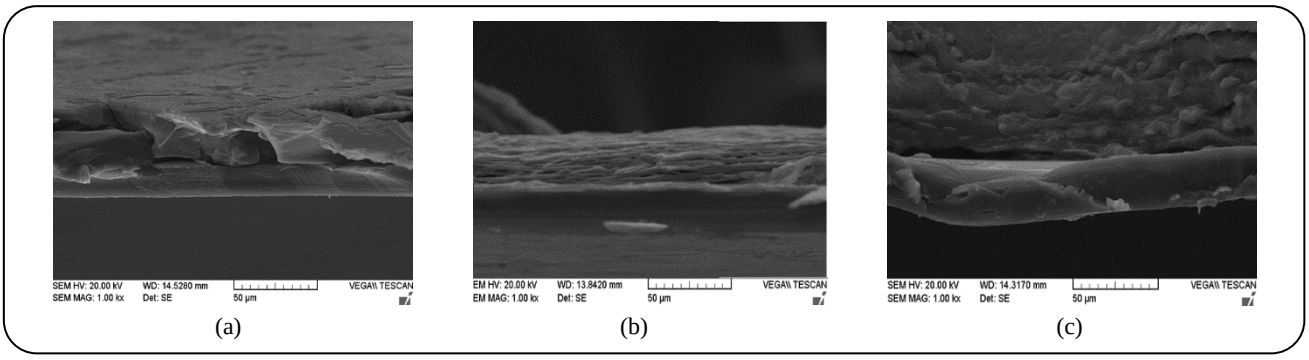
Antimicrobial activities of the SEO containing PP/CMC films in direct contact and vapor phase against five selected bacteria were tested and are shown in Table 3. An SEO-free PP/CMC film was used as a control for each of the tested bacteria and showed no inhibition against any of them which implies that the PP/CMC films don't have any potential antimicrobial activity.

In the direct contact method, films incorporated with 1% SEO showed a very inconsiderable antimicrobial activity against *S. aureus*, E. coli, and *B. cereus*, and, no inhibitory effect on either *S. typhimurium* or *P. aeruginosa* species. This is while in the vapor-phase test, films containing 1% SEO were quite ineffective against all target microorganisms.

Table 3: Antimicrobial activities of different concentrations of SEO incorporated in PP/CMC composite films in direct contact and in vapor phase [a]

Film	Inhibition zone (mm^2)				
	S. aureus	*B. cereus*	*E.coli*	*S. typhimurium*	*P. aeruginosa*
Direct contact					
control	0.00^{e}	0.00^{d}	0.00^{e}	0.00^{d}	0.00^{d}
SEO1%	28.91 ± 0.40^{d}	10.62 ± 0.61^{d}	23.92 ± 0.83^{d}	0.00^{d}	0.00^{d}
SEO2%	107.65 ± 2.37^{c}	95.78 ± 2.80^{c}	71.34 ± 3.28^{c}	43.52 ± 2.74^{c}	28.14 ± 0.69^{c}
SEO3%	264.63 ± 9.98^{b}	178.40 ± 12.13^{b}	154.76 ± 6.82^{b}	114.80 ± 3.81^{b}	86.37 ± 1.14^{b}
SEO4%	327.56 ± 3.65^{a}	277.02 ± 13.38^{a}	241.22 ± 4.06^{a}	235.86 ± 7.44^{a}	216.14 ± 5.35^{a}
Gas-phase					
control	0.00^{d}	0.00^{d}	0.00^{d}	0.00^{d}	0.00^{d}
SEO1%	0.00^{d}	0.00^{d}	0.00^{d}	0.00^{d}	0.00^{d}
SEO2%	87.39 ± 3.31^{c}	52.26 ± 3.44^{c}	48.04 ± 2.15^{c}	23.57 ± 2.30^{c}	11.26 ± 1.98^{c}
SEO3%	251.64 ± 4.57^{b}	107.58 ± 3.40^{b}	135.55 ± 3.97^{b}	91.22 ± 2.03^{b}	56.52 ± 2.04^{b}
SEO4%	274.58 ± 8.71^{a}	236.49 ± 3.87^{a}	193.11 ± 1.85^{a}	122.67 ± 2.30^{a}	77.17 ± 1.37^{a}

[a] Data reported are average values ± standard deviations. Values within each column with different letters are significantly different in each section ($P < 0.05$)



(a) (b) (c)

Fig. 1: SEM micrographs of the cross-sections of the bilayer films: (a) control, (b) SEO1, and (c) SEO4.

By increasing SEO concentration, the antibacterial effect of PP/CMC films showed a remarkable increase proportional to the SEO concentration ($P < 0.05$) against all studied species in either direct contact or vapor-phase methods. Therefore, in both methods, the highest inhibitory property was obtained from 4%-SEO containing films as proved by the greatest zone of inhibition ($P < 0.05$). It was previously showed that the inhibitory effects of essential oils on the microorganism are proportional to the increasing doses [41-43] and the highest antimicrobial activity in essential oils has been attributed to the phenolic content [42,44-49].

Among all the tested microorganisms, *P. aeruginosa* was the most renitent species against SEO antimicrobial activity (inhibition zone 216.14 mm^2) while *S. aureus* with an inhibition zone of 327.56 mm^2 was the most affected bacteria. Similar results were reported for κ-carrageenan films containing *Satureja hortensis* essential oil [12] and origanum sanctum essential oil [41] Different antimicrobial activity with previous reports could be ascribed to variation in environmental growth conditions and bacterial species [42,45].

The size of the inhibition zone generally increased in the following order:

P. aeruginosa < S. *typhimurium* < E.coli < *B. cereus* < *S. aureus.* However, in some cases, there are counterexamples. For instance, films containing 3% SEO caused more inhibition for E.coli than *B. cereus*.

In general, gram-negative bacteria are more resistant species than gram-positive bacteria [43] due to the presence of a rather impermeable outer membrane in the former's species. The antimicrobial activity of SEO essential oil had been previously discussed in the literature and attributed to the high content of carvacrol, γ-terpinene, and p-cymene [50].

It is suggested that the hydrophobic nature of essential oil's constituents, particularly carvacrol in SEO, enable them to penetrate cell membranes and mitochondria contributing to disorganization and increased permeability of the cytoplasmic membrane [51].

Our observation showed that in the direct contact SEO components were more influential for inhibiting the growth of the tested bacteria compared to the vapor phase, however; in general, the antimicrobial effect was in approximately parallel trends. Lopez et al. pointed out similar results for basil and rosemary oil indicating that inhibitory effects in direct contact were more than the vapor phase [24].

This finding indicates that the diffusion of volatile components in the agar media was considerably more than in the air space. The reason might be due to the identical nature (hydrophilic) of agar media and CMC layer of the composite films which were in contact with the agar surface leading to water absorption by CMC matrix, and subsequently extending its structure and more release of essential oils into the agar.

CONCLUSIONS

The study was aimed to develop antimicrobial PP/CMC films and investigate the effects of adding different amounts of SEO (1-4%) on the general characteristics of the bilayer films. CMC as a typical biopolymer could be successfully coated on plasma-treated PP film as confirmed by the excellent visual quality. The incorporation of SEO in the films significantly influenced the characteristics of the resulting coated films compared to the control film in many properties. Our results indicated that the final SEO containing PP/CMC films displayed better water vapor barrier and lower solubility compared to the control film, however they were less resistant to fracture. The microstructure of the films showed that incorporation of SEO into the film, although making the structure less compact in comparison with the control, was not that influential to make the PP/CMC films more permeable which is a promising outcome relieving the concerns about the addition of essential oil to these films. The developed film presented good inhibition against the tested bacteria in either direct contact or vapor phase method, however, we suggest using this film in headspace-containing packaging to further decrease the solubility problems. More studies are needed to practically evaluate the effectiveness of the developed films for different food products.

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