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Physical, mechanical and barrier properties of corn starch films incorporated with plant essential oils

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Abstract

Corn starch-based films are inherently brittle and lack the necessary mechanical integrity for conventional packaging. However, the incorporation of additives can potentially improve the mechanical properties and processability of starch films. In this work two essential oils, *Zataria multiflora* Boiss (ZEO) or *Mentha pulegium* (MEO) at three levels (1%, 2% and 3% (v/v)), were incorporated into starch films using a solution casting method to improve the mechanical and water vapour permeability (WVP) properties and to impart antimicrobial activity. Increasing the

content of ZEO or MEO from 2% to 3% (v/v) increased values for elongation at break from 94.38% to 162.45% and from 53.34% to 107.71% respectively, but did not significantly change tensile strength values of the films. The WVP properties of the films decreased from 7.79 to 3.37 or 3.19 g mm m⁻² d⁻¹ kPa⁻¹ after 3% (v/v) ZEO or MEO incorporation respectively. The oxygen barrier properties were unaffected at the 1% and 2% (v/v) oil concentration used but oxygen transmission increased with 3% (v/v) for both formulations. The films' color became slightly yellow as the levels of ZEO or MEO were increased although transparency was maintained. Both films demonstrated antimicrobial activity with films containing ZEO more effective against *Escherichia coli* and *Staphylococcus aureus* than those containing MEO. These results suggest that ZEO and MEO have the potential to be directly incorporated into corn starch to prepare antimicrobial biodegradable films for various food packaging applications.

Keywords: Corn starch, Physical properties, Essential oil, Atomic-force microscopy, Antimicrobial properties

1. Introduction

There are increasing public concerns regarding the environmental pollution caused by excessive waste from packaging materials. Indeed food packaging is one of the main contributors to this waste and although some materials can be recovered and recycled, many synthetic polymer based materials end up in landfill or the environment. To address some of the problems arising from this plastic waste, considerable research has been undertaken to obtain environmentally friendly food packaging materials (Janjarasskul & Krochta, 2010). Edible and biodegradable natural-polymer films offer alternatives to conventional packaging due to their excellent biodegradability, biocompatibility and edibility, and the range of their potential applications (Salmieri & Lacroix, 2006). Moreover, these films may operate as carriers for a range of functional ingredients thus expanding their capabilities into active packaging.

A range of naturally derived materials including polysaccharides, proteins and lipids can be modified and processed in order to obtain packaging films and coatings. Among them, carbohydrate-based edible films, which have good film-forming ability due to their unique

colloidal properties, are particularly attractive. Starch is the most commonly used raw material in agriculture due to its relatively low cost, wide availability and ease of handling. Furthermore, it is known to be completely biodegradable in soil and water, which is of great advantage from an environmental point of view (Jiménez et al., 2012). Additionally, it is a promising candidate polymer for the development of biodegradable films because it can be readily cast into edible films. Nevertheless, technological applications of starch reveal several disadvantages as a result of its inherently strongly hydrophilic character (water sensitivity) and poor mechanical properties compared to conventional synthetic polymers. This makes it unsatisfactory for some applications such as packaging (Pelissari et al., 2009) although some of these problems can potentially be solved with the addition of plasticizers. Glycerol has often been used as a plasticizer since it is compatible with amylose and as such, it improves the mechanical properties of films by decreasing intermolecular forces and interfering with amylose packing. Conversely, plasticizers generally increase a film's hydrophilicity, which in turn promotes water vapor permeability but this may be improved by the incorporation of lipidic additives such as some essential oils (Pranoto et al., 2005). In particular, the addition of these oils allows improvements in food safety and shelf life by reducing or sometimes preventing the growth of pathogenic and spoilage microorganisms. Several studies have reported the use of starches from different sources to prepare films and coatings with different properties (Han et al., 2006; Shen et al., 2010; Kuorwel et al., 2013).

Despite recent achievements in food safety technologies, epidemiological studies have demonstrated that the number of diseases caused by food-borne pathogenic microorganisms has increased in recent years (de Moura et al., 2012). Therefore, one of the major challenges for food technologists is the design of active food packaging and in particular, antimicrobial packaging. This technology offers a promising alternative to conventional packaging since its application can improve food safety by inhibiting pathogenic bacteria while reducing the environmental impact of food and packaging waste (Cagri et al., 2004). Currently, consumers are more conscious about the potential health risks associated with the consumption of synthetic additives that may be present in food products for preservation and other uses. Indeed the shift towards the incorporation of antimicrobial agents in packaging rather than directly into food products has been a significant focus of active packaging research (Suppakul et al., 2003). More recently, research has focused on the incorporation of natural antimicrobial compounds such as plant extracts and

bacteriocins into biobased packaging materials as replacements for conventional plastic films (Ma et al., 2013).

Essential oils extracted from plants or spices are rich sources of biologically active compounds such as terpenoids and phenolic acids (Burt, 2004). The ability of plant essential oils to protect foods against pathogenic microorganisms has been reported by several researchers (Rojas-Graü et al., 2006; Kristo et al., 2008). Although many essential oils are classified as Generally Recognized as Safe (GRAS), high concentrations of essential oils are generally needed to achieve effective antimicrobial activity in direct food applications and as such, these concentrations may exceed organoleptically acceptable levels (Viuda-Martos et al., 2008). To avoid this problem, the addition of essential oils to edible films has the potential to inhibit the diffusion of the oils into food products by incorporation into the chemical structure of the film. Furthermore, compared with direct application, smaller quantities of essential oils can be applied to achieve a specific food shelf life, due to the gradual release of the oils onto the food surfaces or packaging headspace (Ponce et al., 2008).

Zataria multiflora Boiss (ZEO), or Avishan shirazi, and *Mentha pulegium* (MEO), or Pune, are aromatic and medicinal plants belonging to the Labiatae family. Plants in this family are rich in polyphenolic compounds and a large number of them are well known for their antioxidant, antibacterial and antifungal properties (Kamkar et al., 2010; Saei-Dehkordi, et al., 2010; Sajed et al., 2013). However, the antimicrobial properties of ZEO and MEO when incorporated into edible films are relatively unknown.

The use of essential oils has become very attractive for several applications in the food industry, particularly due to the successful results obtained so far. However, further studies are necessary on the potential of each essential oil. Therefore, the aim of this study was (1) to develop an antimicrobial film based on corn starch and two essential oils (ZEO and MEO); (2) to study the effect of the incorporation of ZEO and MEO on the antimicrobial properties of composite starch films; and (3) to evaluate some physical, mechanical, barrier, optical and morphological properties of these films to examine their potential applications as food-packaging material.

2. Materials and methods

2.1. Materials and bacterial strains

Corn starch (12% moisture) was provided from Glucosan Industry (Ghazvin, Iran). Glycerol, sorbitol and Tween 80 were purchased from Merck, Germany. The ZEO and MEO were obtained from Barij Essence Pharmaceutical Co., Kashan, Iran, and stored in a dark container at 4 °C until used. Mueller–Hinton agar (MHA) and Mueller–Hinton Broth (MHB) were purchased from Merck Co., Darmstadt, Germany. All other chemicals used were of analytical grade or the highest grade available. *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 were provided by Iranian Research Organization for Science and Technology, Tehran, Iran. Stock cultures of the studied bacteria were grown in MHB at 30 °C for 24 h prior to testing.

2.2. Film preparation

Starch-based films were prepared by the method of Ghanbarzadeh, et al. (2011), with some modifications. Corn starch was dispersed in distilled water in order to obtain 5% (w/w) polysaccharide suspensions. To achieve complete dispersion, the mixture was stirred constantly for 40 min using a magnetic stirrer at 500 rpm on a hot plate. Preliminary evaluation was also performed to compare the effectiveness of using sorbitol or glycerol as a plasticizer. It was revealed that glycerol was significantly better than sorbitol with the latter producing wet films that were difficult to peel. Accordingly, the dispersion was mixed with glycerol as a plasticizer at a loading of 50% w/w of the polysaccharide. This suspension was transferred to a water bath at 90 °C for 10 min, and agitated by magnetic stirrer at 500 rpm. After cooling, about 70 mL of the sample was spread evenly over a Teflon casting plate (15 cm diameter) placed on a leveled surface. The films containing essential oils were obtained by adding ZEO and MEO to the dispersion 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) to assist dispersion. Samples were then homogenized at 20,000 rpm for 3 min in an Ultra-Turrax T-25 homogenizer (IKA T25 Digital Ultra-Turrax, Staufen, Germany). Following this process, the resulting dispersions were rested for several minutes to allow natural removal of most of the air bubbles incorporated during stirring. All films were allowed to dry for approximately 48 h at 30% RH and 20 °C. These optimum conditions were established after previous experiments to ensure that

homogeneous, flawless films were obtained. For example, at a higher relative humidities, films did not dry adequately. Dried films were peeled off the casting surface and stored inside desiccators at 25 ± 1 °C until evaluation. For each test, 5 different samples were prepared by taking 3 portions from each film at different positions (two at the edges and one at the centre) with the exception of the water vapour and oxygen permeability analysis, where the whole sample was used, and replicates of each type of film were evaluated.

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 0.0001 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 dry weight was obtained. Three replications of each film treatment were used to measure the moisture content. The film solubility was determined by a method adapted from Shojaee-Aliabadi et al. (2013). The film samples were cut into square pieces of 4.0 cm² and accurately weighed to record the dried film mass. The films were 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 remaining pieces of film were then filtered and dried in a hot air oven at 110 °C until a final constant weight was obtained. The percentage of solubility of the film was calculated according to the equation $WS (\%) = ((W_0 - W_F) / W_0) \times 100$, where W_0 is the initial weight of the film expressed as dry matter and W_F is the final weight of the desiccated undissolved film.

2.5. Film color measurement

For measuring the color of the films a Hunter Lab colorimeter (MiniScan XE Plus 45/0-L, USA) was used to determine the values of L , a , and b . The tests were performed in accordance with ASTM D2244 (ASTM, 2011) using a D65 illuminant with an opening of 14 mm and a 10° standard observer. The colorimeter was calibrated using a standard white plate ($L^* = 93.49$, $a^* =$

-0.25, $b^* = -0.09$). The color measurements were performed by placing the film specimens over colorimeter with at least three points of each sample selected randomly to measure the color parameters of the films. The following equation was used to calculate the index ΔE to estimate the color difference between samples:

$$\Delta E = \sqrt{\left(L^* - L\right)^2 + \left(a^* - a\right)^2 + \left(b^* - b\right)^2} \quad (1)$$

where L^* , a^* and b^* are the color values of the starch film samples and L , a and b are the color parameters of the white standard tile.

2.6. Water vapor permeability

The water vapor permeability (WVP) of the films was determined in accordance with the ASTM E96/E96M (ASTM, 2012) gravimetric method, taking into account the modification proposed by Hosseini et al. (2009). Films were prepared and sealed over the circular opening of 0.00287 m^2 of a permeation cell that was stored at $20 \text{ }^\circ\text{C}$ in a desiccator. The inside of the cell was completely filled with calcium anhydride ($\text{CaCl}_2 - 0\% \text{ RH}$), and the system was placed in a desiccator containing a saturated sodium chloride solution ($\text{NaCl} - 75\% \text{ RH}$). 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 day period to the nearest 0.0001 g and 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 ($\text{g H}_2\text{O/s}$) divided by the cell area (m^2). WVP value, expressed as $[\text{g mm m}^{-2} \text{ d}^{-1} \text{ kPa}^{-1}]$, was calculated according to Eq. (1):

$$\text{WVP} = \frac{w}{\theta} \times \frac{24 \times t}{A \times \Delta p} \quad (2)$$

where w = weight gain obtained from the straight line (g); θ = time during which w occurred (h); t = film thickness (mm); A = permeation area (cell top area, m^2); Δp = vapor pressure difference (kPa). The WVP was measured in triplicate for each type of film.

2.7. Oxygen permeability

The oxygen transmission rate was determined in accordance with ASTM D3985 (ASTM, 2010) using an Ox-Tran modular system (Mocon, Inc., Minneapolis, MN, USA) at 23 °C and 50 ± 1% RH. The samples were equilibrated at 50 ± 1% RH (23 ± 1 °C) for a period of 48 h before analysis. Film samples were placed on a stainless-steel mask with an open test area of 5 cm². On one side of the film 100% oxygen gas was flowing with nitrogen gas (98% N₂ and 2% H₂) on the other side. Nitrogen gas was conducted to the coulometric sensor and measurements were obtained when a steady state was reached. The relative humidity of both gases was controlled by a humidifier, and varied from 50 to 90%. The permeability analysis was not performed below RH 50% because the starch films began cracking at these lower RH levels. Oxygen permeability was calculated by dividing O₂ transmission rate by the difference in O₂ partial pressure between both sides of the film (101 kPa) and multiplying by the average thickness of the film (μm). Measurements were performed in duplicate and the units for OP were cm³ μm m⁻² d⁻¹ kPa⁻¹.

2.8. 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 and elongation at break of the antimicrobial starch films were determined using a Testometric Machine M350-10CT (Testometric Co. Ltd., Rochdale, Lancs., England) according to ASTM standard method D882 (ASTM, 2012). 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 with a minimum of five replicates of each film tested.

2.9. Microbiological activity of the films

The disc-diffusion assay was used to examine the antimicrobial characteristics of the films. Composite films were cut into 6 mm diameter discs using a sterile punch and these were then placed on plates containing MHA which had been previously seeded with 100 μL of an overnight broth culture containing approximately 10⁸ CFU/ml of the test bacteria. There were a total of four disks on each plate which were incubated at 30 °C for 24 h. On each plate, one disk was the control essential oil-free film with the other three disks comprised three replicates of each

formulation. Following incubation, the whole zone area of growth was measured then subtracted from the film disc area and this difference in area was reported as the zone of inhibition (Seydim & Sarikus, 2006).

2.10. Scanning electron microscopy

The microstructure of films was observed by scanning electron microscopy (SEM) using an Oxford Instruments INCA Penta FET×X3 microscope. Before the analysis each film was fixed on a support using double side adhesive tape, placed horizontally with an angle of 90°. The 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.

2.11. Atomic force microscopy

Before testing, samples were preconditioned at 50% RH and at room temperature for at least 48 h. The AFM scan was performed using an Autoprobe CP Research instrument (Veeco Instruments) in tapping mode with a 125 × 125 μm scan size and a 6 μm vertical range. Measurements were taken from several areas of the film surface (50 × 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); and root-mean-square roughness (R_q : root-mean-square average of height deviations taken from the mean data plane).

2.12. 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 mean values of the measured film properties were compared using Duncan's multiple range tests and a probability value of $p < 0.05$ was considered significant.

3. Results and discussion

3.1. Film formulation optimization

Preliminary studies in our laboratory showed that a corn-starch concentration of less than 5% is not sufficient to obtain a strong supporting matrix and the resulting films are soft. A 5% corn starch concentration was selected as the optimum polysaccharide concentration in the film-forming solution. The composite films formed using ZEO and MEO were visually homogeneous without signs of phase separation between the components. There were no brittle areas or bubbles and they could be easily peeled from the casting plates. However, films formulated without essential oil were brittle, and it was not possible to evaluate their mechanical properties. The addition of essential oils to starch films may induce a plasticizing effect that can improve the mechanical integrity of resulting films. To determine the maximum concentration of ZEO and MEO that could be incorporated into the film matrix, increasing amounts (up to 5%) were added to the film-forming dispersion. It was found that films formulated with ZEO and MEO at concentrations lower than 1% v/v did not impart significant antimicrobial activity and those containing more than 3% resulted in samples with an unacceptably strong aroma.

3.2. Physical properties of films

Table 1 shows the effects of incorporating essential oils on the physical properties of starch-based films. The starch film thickness varied from 0.17 mm to 0.24 mm, and incorporation of different antimicrobial compounds did not significantly ($P > 0.05$) affect the resulting film thickness. The films prepared with either ZEO or MEO showed lower moisture content than the control film and these decreased significantly ($P < 0.05$) as essential oil concentration increased. This may be due to the incorporation of the hydrophobic essential oils which can affect the ability of the film to retain water.

Table 1. Physical properties of corn starch films incorporated with ZEO or MEO.

Film type	Essential oil conc. (% v/v)	Thickness (mm)	Moisture content (%)	Solubility in water (%)
Control	0	0.151 ± 0.004 b	21.95 ± 1.69 a	27.88 ± 2.01 a
ZEO	1	0.170 ± 0.010 ab	17.59 ± 2.11 b	24.03 ± 1.56 b
ZEO	2	0.195 ± 0.007 b	15.47 ± 2.89 bc	23.00 ± 1.51 c
ZEO	3	0.266 ± 0.010 a	14.04 ± 1.56 c	18.96 ± 1.54 d
MEO	1	0.182 ± 0.003 ab	17.02 ± 1.28 b	23.18 ± 1.65 c
MEO	2	0.212 ± 0.002 a	14.62 ± 0.93 c	19.36 ± 1.51 d
MEO	3	0.247 ± 0.005 a	13.21 ± 0.47 c	19.55 ± 0.78 d

Values are given as mean ± standard deviation. Different letters in the same column indicate significantly different ($p < 0.05$) when analyzed by Duncan's Multiple Range Test.

Water solubility of film forming materials is an important factor when choosing a film for specific applications. Indeed solubility is a desired property in many cases such as the encapsulation of foods. When water resistance and integrity are required for packaging foods with high moisture contents, solubility is disadvantageous. The water solubility of the prepared starch films with and without antimicrobial additives is shown in Table 1. Generally, the effects of additives on the solubility of films depend on the type of compounds and concentrations and their inherent hydrophilicity and hydrophobicity indices. It would be expected that hydrophilic compounds should increase a film's solubility, whereas hydrophobic compounds should decrease it (Kavoosi et al., 2013). In the present study, the solubility of the control and composite films followed the same trend as moisture content which is in accordance with the expectation that increasing addition of essential oils subsequently decreases the hydrophilicity of the film.

3.3. Optical properties

Table 2 shows the color parameters L , a , and b and the total color difference (ΔE) values for pure starch-based films and those containing essential oils. There were no differences observed between the a values of any film studied. Generally, starch films with ZEO showed higher L values but lower b values than those with MEO although films with MEO were darker in appearance. There was no significant difference among the L and b values of composite films containing ZEO with exception of films containing 3% (v/v) essential oil, which had a significantly ($P < 0.05$) lower L value and higher b value. There were no obvious differences ($P < 0.05$) in L values when the MEO concentrations increased from 1 to 3% (v/v) whereas the b

values significantly changed over the same concentration range ($P < 0.05$). The ΔE value showed a similar trend to changes in L values. This result is consistent with our previous findings (Shojaee-Aliabadi et al., 2013) where emulsified carrageenan films became more opaque with the incorporation of essential oils. This effect is probably due to the increase in diffuse reflectance provoked by light scattering in the lipid droplets which lowers both the light-scattering intensity and the film's whiteness index. In this case, the starch composite films became slightly yellow but they remained transparent. In the present study, all the starch films either with ZEO or MEO, even at highest concentration of essential oil, were not significantly colored and their transparency was suitable for food packaging.

Table 2. Hunter color parameters (L , a , b), and total color differences (ΔE) of corn starch films incorporated with ZEO or MEO.

Film type	Essential oil conc. (% v/v)	L	a	b	ΔE
Control	0	78.09 ± 1.32 ab	-1.67 ± 0.11 a	14.70 ± 1.11 b	21.44 ± 0.19 c
ZEO	1	77.02 ± 1.97 abc	-1.68 ± 0.01 a	14.23 ± 1.98 b	21.93 ± 1.83 c
ZEO	2	79.86 ± 1.75 a	-1.66 ± 0.02 a	14.99 ± 1.56 b	20.43 ± 1.21 c
ZEO	3	74.02 ± 2.63 dc	-1.69 ± 0.01 a	19.20 ± 0.50 a	27.48 ± 1.87 ab
MEO	1	76.67 ± 2.06 abc	-1.66 ± 0.02 a	14.14 ± 2.83 b	22.14 ± 1.67 c
MEO	2	74.69 ± 1.05 bdc	-1.68 ± 0.06 a	16.47 ± 1.52 b	25.10 ± 1.68 b
MEO	3	73.06 ± 1.41 dc	-1.67 ± 0.05 a	19.73 ± 0.94 a	28.50 ± 1.57 a

Values are given as mean ± standard deviation. Different letters in the same column indicate significantly different ($P < 0.05$) when analyzed by Duncan's Multiple Range Test.

3.4. Barrier properties

3.4.1. Water vapor permeability

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 should therefore be as low as possible in order to optimize the food package environment and potentially increase the shelf-life of the food product (Hosseini et al., 2013).

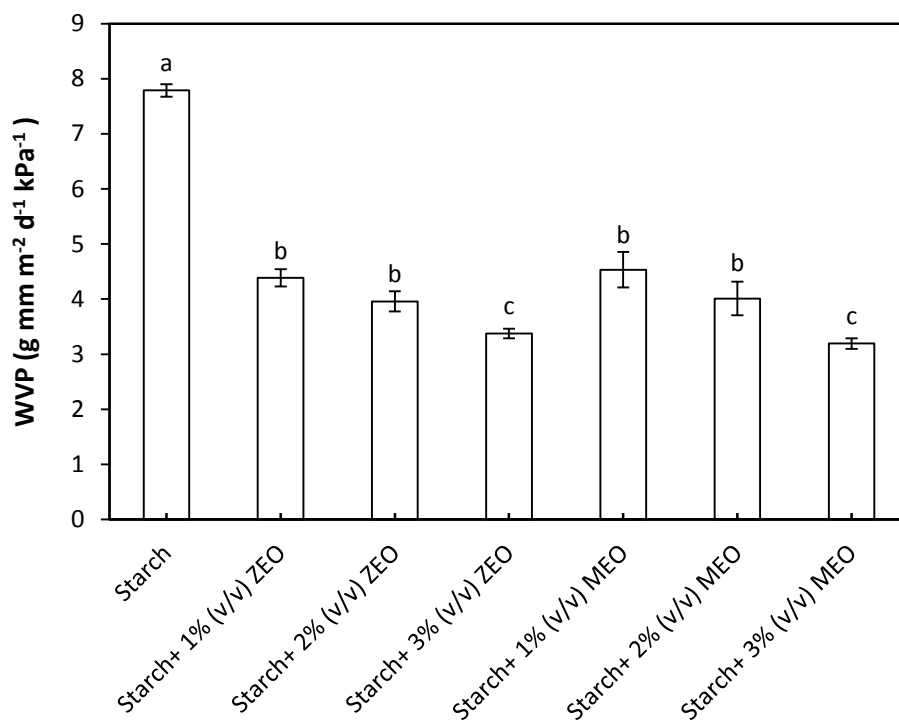


Fig 1. Water vapor permeability of corn starch films formulated with ZEO or MEO. Different letters indicate a statistically significant difference ($P < 0.05$).

Figure 1 shows the WVP values for the film samples and the results suggest there was a significant difference ($P < 0.05$) in the observed WVP values between the films containing ZEO or MEO and control films. The addition of ZEO or MEO improved barrier properties of starch films, decreasing WVP up to 50% relative to the control sample. The lower WVP of the starch films with either ZEO or MEO may be due to the hydrogen and covalent interactions between the starch network and these polyphenolic compounds. These interactions may limit the availability of hydrogen groups to form hydrophilic bonds with water, and subsequently lead to a decrease in the film's affinity for water (Shen et al. 2010). Moradi et al. (2012) reported a significant decrease in WVP of chitosan films formulated with ZEO and grape-seed extract and in another study, Shojaee-Aliabadi et al. (2013) reported that incorporation of *Satureja hortensis* essential oil into carrageenan films also resulted in films with reduced WVP rates. The results obtained in the present study are more promising than those reported by Han et al. (2006) who prepared biodegradable films based on pea starch and beeswax and reported relatively high WVP

values between 6.56 and 7.78 g mm m⁻² d⁻¹ kPa⁻¹. Kechichian et al., (2010) reported WVP values ranging between 4.65 and 9.01 g mm m⁻² d⁻¹ kPa⁻¹ for cassava starch incorporated with natural antimicrobial additives. However, even when essential oils were added, the WVP obtained in this work, as well as those reported by the authors mentioned above, were high compared to those of high barrier synthetic polymers: 0.11 g mm m⁻² d⁻¹ kPa⁻¹ for PVC, 0.08 g mm m⁻² d⁻¹ kPa⁻¹ for LDPE, and 0.02 g mm m⁻² d⁻¹ kPa⁻¹ for HDPE (Salame, 1986). Nevertheless, the WVP of corn-starch films incorporating essential oils were similar to or better than that of cellophane (7.3 g mm m⁻² d⁻¹ kPa⁻¹) and there is indeed some scope for use in some food packaging applications.

3.4.2. Oxygen permeability

Figure 2 shows the changes in oxygen permeability (OP) of composite corn-starch films incorporated with ZEO and MEO. The OP value of the pure starch film was 12.11 cm³ μm m⁻² d⁻¹ kPa⁻¹, indicating that this film is a relatively good oxygen barrier. The OP values measured in this study were significantly higher than those obtained by Araujo-Farro et al., (2010), who reported a value of 4.34 cm³ μm m⁻² d⁻¹ kPa⁻¹ for quinoa-starch film. These differences may be due to the use of a different type of starch, various modes of sample preparation or a difference in residual water content. The starch films containing ZEO or MEO in the present study showed no significant differences (P > 0.05) at concentrations of 1% and 2% (v/v) although a general upward trend is suggested by the data when comparing films containing essential oils. These lower levels might not have been at a sufficiently high concentration to induce structural changes in the starch structure that would otherwise increase the molecular mobility of oxygen within the film structure. However, the addition of 3% (v/v) of either essential oil to the films significantly increased the OP values (P < 0.05). This may be due to swelling of the starch polymer matrix in the presence of volatile oils at these higher concentrations (Ahvenainen, 2003). Han et al. (2006) reported similar results in pea-starch films when beeswax concentration was increased to more than 40%. These authors suggested that oxygen may penetrate through beeswax/starch interfaces between the beeswax particles, providing oxygen-penetration channels when higher concentrations of beeswax are added to the films. Conversely, increased amounts of beeswax in the pea-starch films imparts more beeswax on the surface, and consequently decreases the surface energy of starch films. This lower surface

energy with higher hydrophobicity of the film surface may accelerate the absorption of oxygen from the atmosphere and increase the solubility of diffusion.

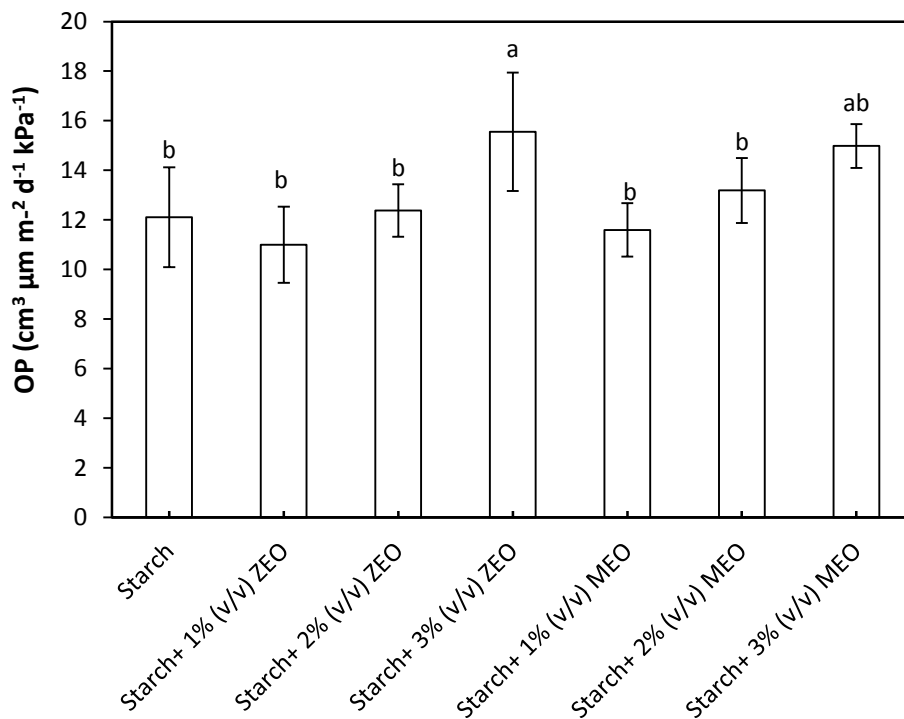


Fig 2. Oxygen permeability of corn starch films formulated with ZEO or MEO. Different letters indicate a statistically significant difference ($P < 0.05$).

3.5. 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 Figure 3.

Increasing the content of MEO into the starch films caused a reduction in TS which significantly changed ($P < 0.05$) when the essential oil concentration was increased from 1% to 2% (v/v), but did not significantly change ($P > 0.05$) when the concentration was increased from 2% to 3% (v/v). In contrast, the EB values of the film improved ($P < 0.05$) when the content of ZEO or MEO increased from 1% to 3% (v/v). This can be attributed to the complex structures formed

between the lipids and the starch polymers which reduces the cohesion of the starch network forces subsequently decreasing the films resistance to breakage (Jiménez et al., 2013). Zivanovic et al., (2005) observed a decrease in TS and an increase in EB for chitosan films combined with essential oils which is consistent with the present results. Maizura et al., (2007) reported a decrease in the TS of the films prepared from starch-alginate film to which different concentrations of lemongrass oil had been added. They reported that this was due to the effect of the lipid on the starch chains and that the phase rich in polysaccharide had higher TS than the lipid phase. This result confirms the outcome of the work by Pelissari et al. (2009) who had reported that incorporation of oregano oil in starch films increases its stretchability, however the results obtained by Bourtoom and Chinnan (2009) are different from those presented here. Decreases in TS and increases in EB are common results of essential-oil incorporation, and have been broadly discussed in research into other biopolymer films (Atarés et al., 2010; Benavides et al., 2012).

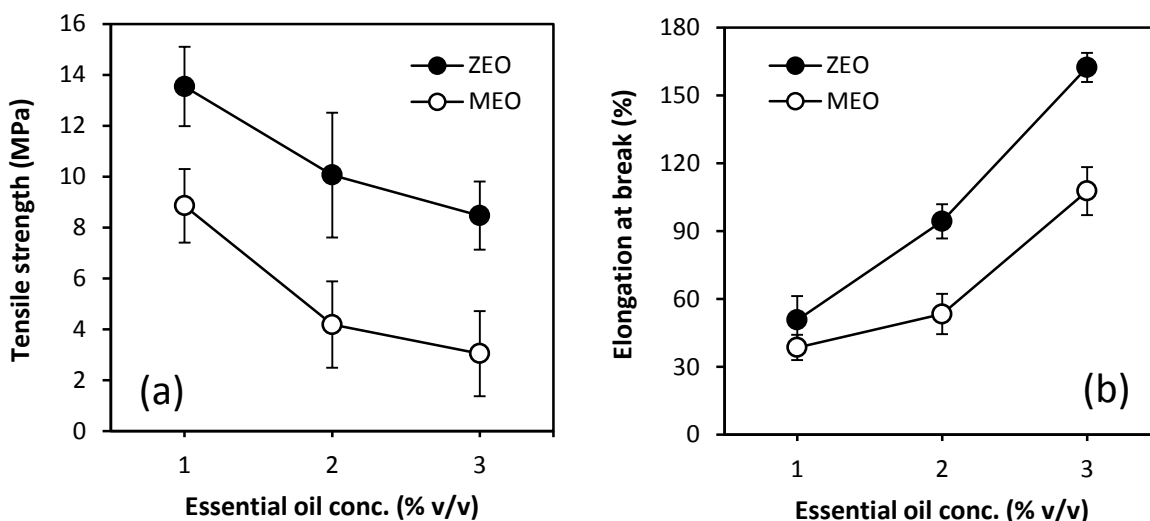


Fig. 3. Tensile strength (a) and elongation at break (b) of the corn starch films formulated with ZEO or MEO.

3.6. Antimicrobial activity

Photographs of the inhibitory effect of films incorporated with 3% (v/v) of either ZEO or MEO against two tested microorganisms in comparison with the control are shown in Figure 4. In each case, no inhibition is observed for the control film as expected. Higher inhibition zones were

observed when ZEO was incorporated into the film and the inhibition for both essential oils was more significant against *S. aureus*.

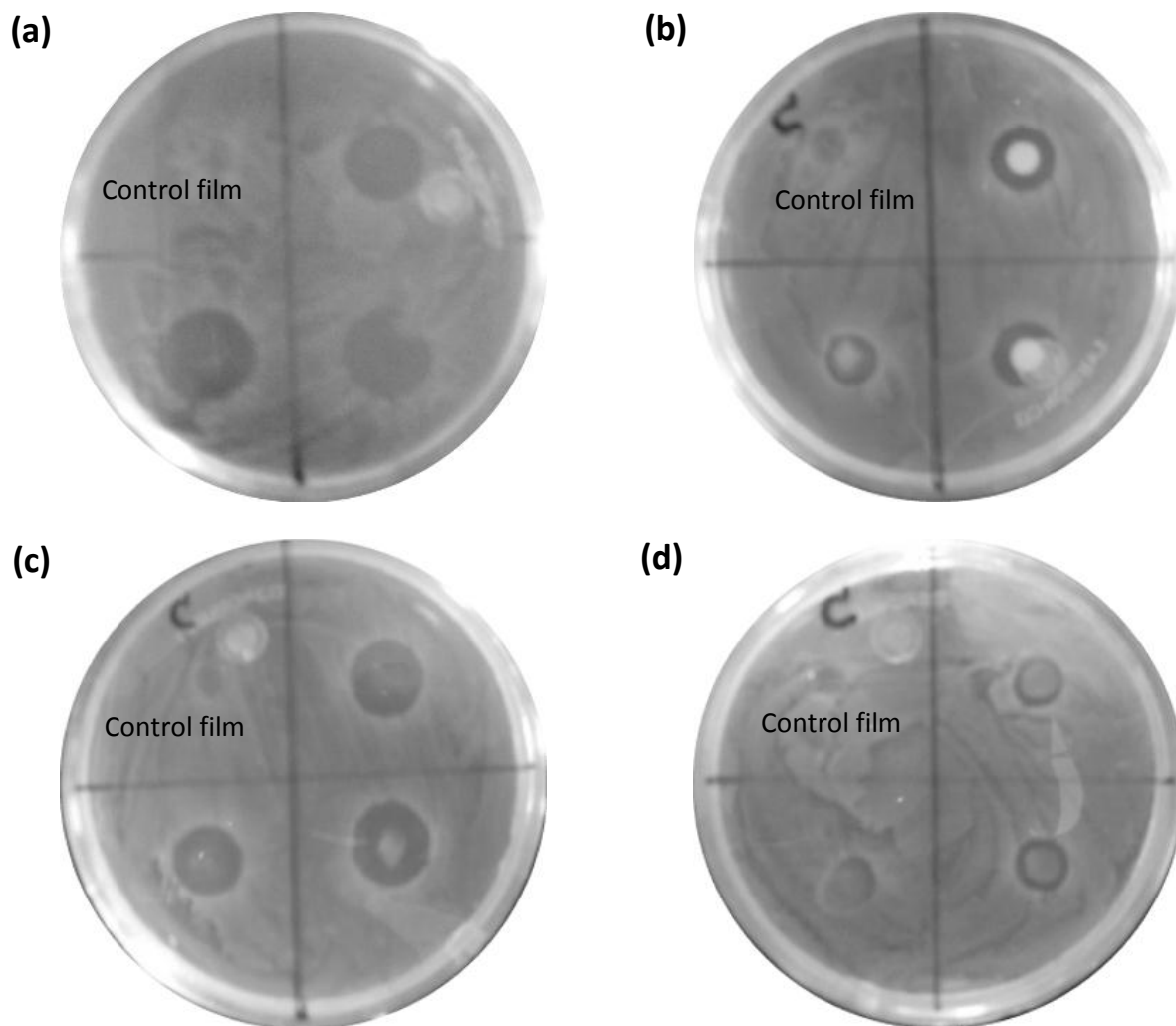


Fig. 4. Images of inhibitory zones of corn starch films incorporated with (a) 3% (v/v) ZEO, (b) 3% (v/v) MEO against *S. aureus*, and (c) 3% (v/v) ZEO and (d) 3% (v/v) MEO against *E. coli* O157:H7.

Figure 5 shows the calculated inhibitory effect of starch films against the two selected bacteria. The results showed that all composite films containing ZEO or MEO inhibited the growth of the two tested bacteria. Even the lowest concentration of MEO (1%) resulted in significant inhibition with areas of 37.23 mm² for *E. coli* O157:H7 and 98.76 mm² for *S. aureus*. As the concentration of essential oil increased, the zone of inhibition also increased significantly ($P < 0.05$) at all levels.

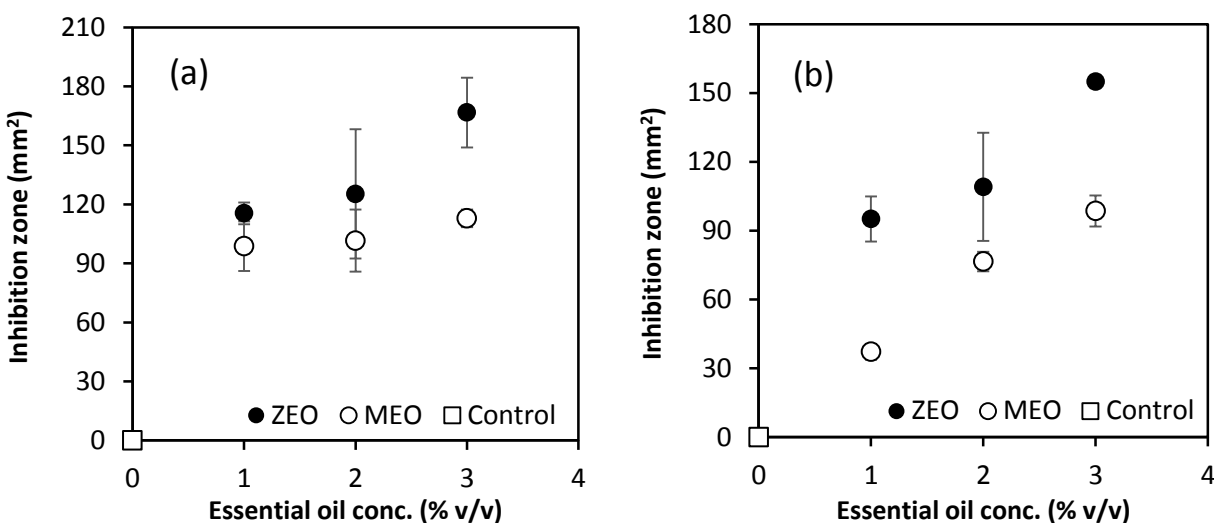


Fig 5. Antimicrobial activities of different concentrations of ZEO or MEO incorporated in corn starch films against *S. aureus* (a) and *E. coli* (b).

Regardless of essential-oil type or concentration, Gram-negative *E. coli O157:H7* was more resistant ($P < 0.05$) than Gram-positive *S. aureus*. This result may be due to the presence of an additional external membrane surrounding the cell wall in Gram-negative bacteria which can restrict diffusion of hydrophobic compounds through this lipopolysaccharide layer (Burt, 2004). Conversely, Gram-positive bacteria have a thick peptidoglycan layer that may function as a preventive barrier against some essential oil compounds (Burt, 2004). These results are in agreement with those of Kuorwel et al. (2011), who reported that thermoplastic starch films containing carvacrol, linalool and thymol effectively inhibited the growth of *S. aureus* on the surface of Cheddar cheese. The antimicrobial activity of films incorporated with ZEO and MEO has been attributed to their main constituents which includes a range of phenolic monoterpenes. Recently, Saei-Dehkordi et al. (2010) reported the presence and role of thymol, carvacrol, gterpinene, thymol methyl ether and carvacrol methyl ether as the main antimicrobial compounds of ZEO. Shakeri et al. (2011) reported that the addition of ZEO to whey protein-based films generally inhibited the growth of *E. coli*, *Salmonella enteritidis*, *S. aureus* and *B. cereus*. In another study, Broumand et al., (2011) analysed antimicrobial sodium caseinate films prepared with different concentrations of ZEO. These authors concluded that films containing

ZEO exhibited a large inhibitory effect on *S. aureus* but a somewhat reduced effect on *Salmonella typhimurium* and *E. coli* O157:H7. Additionally, Mahboubi and Haghi (2008) analyzed the components of MEO and found piperitone, piperitenone, terpineol and pulegone to be the major components and they linked the antimicrobial activity of this essential oil with these main constituents. In another study, Sivropoulou et al., (1995) reported a powerful inhibitory effect of MEO applied directly to *E. coli*, *L. monocytogenes*, *S. typhimurium* and *S. aureus*.

3.7. Film microstructure

Micrographs of the cross-section of starch based films with and without essential oil are presented in Figure 6. Films incorporated with ZEO had relatively smoother surfaces compared control starch films. However, many holes which appeared to be uniform in size were observed on these films and this is probably due to the presence of essential oils in the film formulation which may result from the volatility of the essential oil compounds. Although both the control and MEO-containing films presented similar surface characteristics, the incorporation of MEO at higher concentrations induced notable changes in the films' surface microstructure with a coarser microstructure was obtained in films incorporated with this agent. Generally, ZEO appears to be more finely distributed in the polymer matrix compared to MEO and this may be responsible for its greater antimicrobial activity and improved physico-mechanical properties.

3.8. Surface morphology

Atomic force microscopy (AFM) is a powerful tool for studying surfaces, and has been used to provide qualitative and quantitative information regarding the topography of biopolymers at the nanometer scale that are often inaccessible by any other experimental techniques. It has been used previously in our group to study the morphological properties of edible films made with kefiran (Ghasemlou et al., 2011). AFM allow the determination of roughness parameters which can be calculated for the surfaces of films. In addition, AFM allows a histogram to be plotted of the relative height of every pixel recorded during the scan and typical images of AFM scans of control and antimicrobial film is shown in Figure 7.

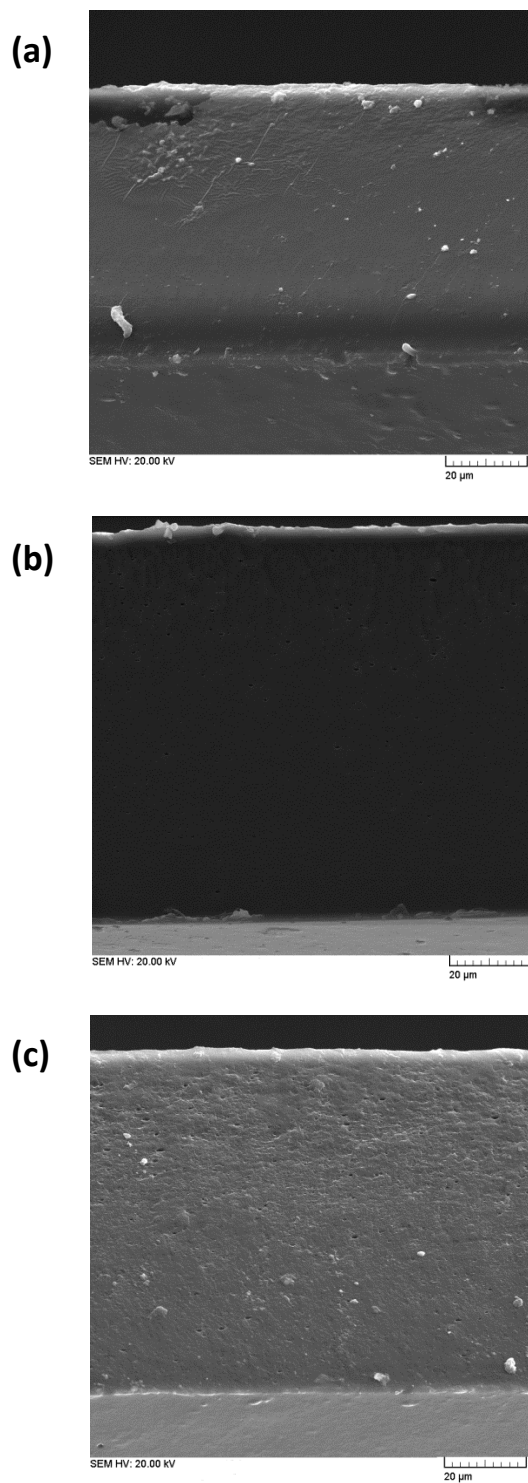


Fig 6. Scanning electron microscopic images of cross-section of corn starch films: (a) control, (b) corn starch with 2% (v/v) ZEO and (c) corn starch with 2% (v/v) MEO.

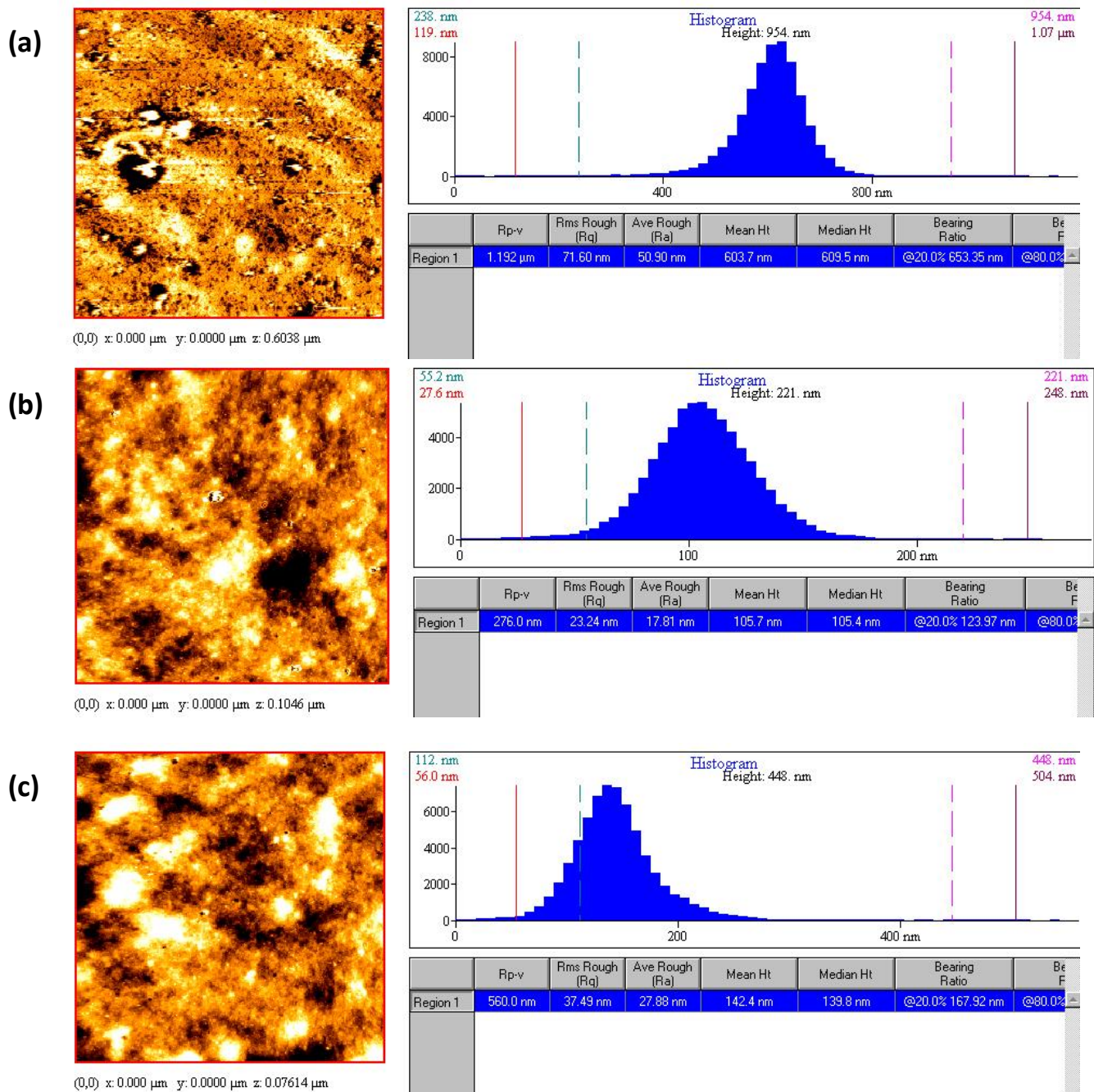


Fig. 7. 2D AFM topographic images of corn starch films: (a) control, (b) corn starch with 2% (v/v) ZEO and (c) corn starch with 2% (v/v) MEO as well as their relative pixel height histograms and roughness parameters. [Color figures can be viewed in the online version, which is available at sciencedirect.com]

The peak heights were determined to be at 954 nm for the control films, 221 nm for films incorporating ZEO and 448 nm for those incorporating MEO. According to these images, increasing the essential oils in the film dispersion favored modification of the surface to a smoother morphology. The films without essential oils were the roughest of all the films studied. The presence of either essential oil appears to result in reduced surface roughness which is probably due to the liquid state of the oils. This state contributes to the extension of creamed droplets on the film surface after drying, filling the irregularities of the film matrix surface and making it smoother. However, with an increase in ZEO or MEO content in the formulation, an increase in roughness parameters was observed. At all essential-oil concentrations tested, a trend towards higher roughness values was obtained in films with MEO with lower values for ZEO (data not shown). The AFM images revealed that ZEO remained homogeneously integrated with the film matrix during drying resulting in relatively regular surfaces. In contrast, MEO undergoes lipid and creaming aggregation during the drying step, thus causing irregularities on the film surfaces.

4. Conclusion

Antimicrobial starch films containing ZEO or MEO showed significantly different physico-mechanical properties between various composite films. An increase in oil content resulted in significantly lower tensile break strength but a concurrent increase in elongation at break. Although composite films were less water soluble, were more resistant to fracture, and were slightly colored, these changes did not negatively affect the handling or manipulation of the films. Incorporation of ZEO and MEO also improved the moisture barrier properties of the film with WVP more significantly reduced by the incorporation of ZEO. In addition, the results demonstrated that the films containing ZEO or MEO were effective in inhibiting the growth of *E. coli* and *S. aureus*. Again this effect was more pronounced when ZEO was added although inhibition was still observed when MEO was used. Overall, this study suggests that starch 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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