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Development of antifungal films based on low-density polyethylene and thyme oil for avocado packaging

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ABSTRACT: Trilayer low-density polyethylene (LDPE) films were prepared by incorporating varying concentrations of thyme oil, as the antifungal active additive for avocado packaging. A comprehensive thermal, structural, mechanical, and functional characterization of the prepared films was carried out. Thermal stability of the film reduced with the addition of thyme oil in higher concentration, whereas the degree of crystallinity increased upto 2.5 wt % thyme oil loading. The elastic modulus and elongation at break of the films decreased in presence of thyme oil. However, the incorporation of thyme oil did not change the water vapor transmission characteristics of the original film. The antifungal activity of the films was tested against *Colletotrichum gloeosporioides* causal organism of "anthracnose" postharvest disease in avocados. The results indicated that the films have great potential as antifungal packaging materials for avocado fruits. © 2015 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2016**, *133*, 43045.

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INTRODUCTION

Avocados (*Persea americana Mill.*) are highly nutritious (being rich in vitamins A, B, C, minerals, potassium, phosphorus, magnesium, iron, and antioxidants) and popular subtropical fruits of high economic importance to South Africa.[1] South Africa is the second largest exporter of avocados to Europe after Israel and approximately 64% of South African produced avocados are exported yearly.[2] However, the fruit is a highly perishable commodity as it is easily susceptible to postharvest diseases and losses. The incidence of postharvest decay in avocado depends on the cultivar, maturity level, ripening stage, storage and transportation, and retailer shelf conditions. However, fruit decay due to anthracnose (*Colletotrichum gloeosporioides*)[3] is mainly responsible for high percentage of losses during postharvest storage affecting the shelf-life and market value of the fruit. Nonsystemic synthetic fungicide "prochloraz" is used as a postharvest control measure for anthracnose in South Africa, Australia, and New Zealand.[4–6] Furthermore, copper oxychloride is also sprayed at the preharvest stage to control anthracnose.[7] However, these approaches have several disadvantages, such as time-consuming processes, waste disposal of the fungicide, and development of fungicide-resistant strains of microbes.[5,8,9] Above all, the rising consumer demand for chemical free food and the strict regulations enforced by the importing countries regarding the minimum residue levels present in the edible portion of the fruit also limit these strategies. The lack of effective postharvest treatments to control decay thus highlights the need for developing new effective control methods.[10,11]

Natural pesticides based on plant essential oils (EOs) are gaining popularity due to their antimicrobial, allelopathic, antioxidant, and biodegradable properties.[12,13] The antimicrobial activity of thyme oil (*Thymus vulgaris*) is well documented and is proven to inhibit the fungal and bacterial growth.[14–16] Moreover, thyme oil is a volatile essential oil which has GRAS status (generally regarded as safe) with an acceptable daily intake of 4.7 g/kg body weight according to Food and Drug Administration (FDA) United States.[17] However, the geographical areas where the plants grow can affect the major oil constituents (thymol and carvacrol) and hence its antimicrobial properties.[18] Sellamuthu *et al.*[19] reported that locally sourced thyme oil is highly effective against *C. gloeosporioides in vitro* or *in vivo* in South African avocado cultivars Hass and Fuerte. Fumigation of fruits with thyme oil is preferred than spraying or dipping since the vapor phase requires low concentration and EO volatiles do

not interfere with the fruit aroma.[20] They also showed that the thyme oil application in vapor phase in combination with modified atmosphere packaging enhanced activities of defense enzymes (PAL, chitinase, and peroxidase), antioxidant enzymes (catalase and superoxide dismutase) as well as high total phenols.[19] This study indicated that the effectiveness of EOs in controlling postharvest decay can be improved further by combining other postharvest treatments under controlled conditions. A number of recent studies have focused on extending the functional properties of polymeric films by adding different EOs to yield active packaging materials with antimicrobial activity.[21–23] The major benefit of incorporating EOs in the polymeric matrices is that they help to slow down the diffusion rate of antimicrobial agents, allowing a higher concentration of active compounds to interact with the fruit surface, where contamination has occurred or is likely to occur, for a prolonged period.[24] These packaging materials can be developed in the form of coatings, films, trays, releasing pads, or labels. Edible coatings and films of polysaccharides (cellulose derivatives, starch, chitin, and gums), proteins, or waxes containing EOs that can extend the shelf life of fresh produce are gaining a lot of interest from consumers currently.[25] Pires *et al.*[26] showed that the films with hake proteins and thyme oil have improved antioxidant properties at a loading of 0.25 oil/g of protein. While spray coating of fruits can be labor intensive, the commercial use of edible films is limited because these materials have poor mechanical and barrier properties as compared to synthetic polymers.[27]

Novel antimicrobial packaging materials based on synthetic polymers impregnated with EOs has been a topic of great research interest over the last few years. Synthetic polymers [e.g., polyethylene (PE), polypropylene (PP), polyamide (PA), poly(ethylene terephthalate) (PET), polystyrene (PS)] are effective carriers for active substances and these packaging products are of high commercial interest because of the advantages such as its functionality, low cost, and ease of processing. This also allows the combined application of natural antimicrobial agents and modified atmospheric packaging technologies to preserve quality and shelf life of fresh produce. Dias *et al.*[28] developed low-density PE (LDPE) based flavoring films with essential oils and lemon aroma for cereal products packaging. Torres and coworkers[29] recently produced linear LDPE (LLDPE) films containing thymol by supercritical impregnation and found that the thymol solubility in the polymer increased with applied pressure. Perez *et al.*[30] developed LDPE nanocomposite with organically modified montmorillonite and carvacrol which showed significant inhibition of bacterial (*Escherichia coli* and *Staphylococcus aureus*) growth. In another study, Ramos and coworkers[31] showed that the release of thymol and carvacrol from PP depended on the food stimulant and also the concentration of the active substances. According to their observations, PP films at 80 g of thymol/carvacrol were readily released into different food stimulants and the additives remained in the polymer even after 15 days. Guarda *et al.*[32] demonstrated slow releasing antimicrobial properties of biaxially oriented PP films with microencapsulated thymol and carvacrol. Most of the studies focus on the use of purified components of the EOs (e.g., thymol and carvacrol), which are expensive or fumigation of fresh produce with EOs combined with MAP while very few reports are on direct incorporation of locally available EOs as such into the polymer films.

This study focuses on the optimization of antifungal packaging film properties based on LDPE and thyme oil for avocados. Trilayer films were prepared by blown film extrusion with thyme oil blended with polymer in the middle layer. The mechanical, thermal, morphological, and barrier properties of the films were investigated thoroughly at varying concentrations of thyme oil. The antifungal activity of the prepared films was tested against *C. gloeosporioides* which is the most common anthracnose-causing fungi in avocado fruit.

MATERIALS AND METHODS

Materials

Film-grade LDPE pellets (LT 033, MFI: 0.33 g/10 min, 921 kg m^{-3} density) were obtained from Sasol Polymers, South Africa. The pellets were pulverized before preparing films for easy processing. The preservative free EO additive, thyme oil (*Thymus vulgaris* L.), was obtained from Burgess and Finch (Vital Health Foods SA Distributor, South Africa and Dis-Chem (Pty) Ltd., South Africa).

Preparation of LDPE/Thyme Oil Films

Triple layer LDPE films of approximately 70–90 μm thickness were produced by using a coextrusion film blowing process [Scientific Laboratory Extruder LE25-30/CV]. The inner layer consisted of a blend of thyme oil and LDPE (at different thyme oil concentrations of 1, 2.5, and 5 wt %), whereas the outer layer was made of pure LDPE. The processing conditions were the following: screw speed = 60 rpm, temperature = 160°C, roller speed 3.6 m/min. To ensure a relatively high loading of oil, a specific amount of the thyme oil was mixed with pulverized LDPE and kept for approximately 24 h to ensure complete absorption of the oil. Film without thyme oil was used as control and was prepared under similar conditions as the films containing the active agents.

Film Characterization

The following techniques were used to characterize LDPE films with and without thyme oil. The surface morphology of cryo-fractured samples was studied using scanning electron microscopy (SEM; AURIGA, Carl Zeiss) at an accelerating voltage of 5 kV. FT-IR spectrometer equipped with a universal attenuated total reflection (ATR) accessory in the range of 4000–500 cm^{-1} at a resolution of 4 cm^{-1} was used to find out any interaction between thyme oil and LDPE matrix.

Thermogravimetric analyses (TGA) were conducted on a TG-Q500 analyzer (TA Instruments). Samples weighing approximately 10 mg were heated from room temperature to 900°C at a heating rate of 10°C/min under an air atmosphere. To obtain characteristic thermal stability indicators, such as the onset degradation temperature, two independent tests were carried out per sample, and the average value was reported. Differential scanning calorimetry (DSC) measurements were carried out on a DSC (TA Q2000 Instrument) in the temperature range of

−10 to +190°C under a nitrogen atmosphere (25 mL/min), using samples of approximately 7 mg. The samples were tested at the same heating and cooling rates of 10°C/min in three scans: heating, cooling, and heating. While the first heating scan erased the previous thermal history of the samples, the cooling curve was used to measure crystallization peak temperature T_c, the second heating scan was used for the determination of melting peak temperature T_m. Degree of crystallization (χ_c) was calculated according to the relation $\chi_c = [\Delta H_m/\omega\Delta H_f] \times 100$, where ω is the weight fraction of LDPE component, ΔH_m is the enthalpy of melting and ΔH_f is the heat of fusion of 100% crystalline LDPE (taken as 293 J/g).[33]

The effect of incorporation of thyme oil on the melt crystallization behavior of LDPE matrix was studied using polarized optical microscopy (POM). The thin sample was prepared by pressing the film between two cover glasses. The samples were then melted at 200°C on a Linkam hot stage and cooled to 120°C at 10°C/min. They were then held isothermally at 123°C and crystallized for 15 min during which images were taken by a Carl Zeiss POM.

Tensile tests to determine the modulus, yield strength, and elongation-at-break were carried out using an Instron 5966 tester (Instron Engineering Corp., USA) with a load cell of 10 kN and according to ASTM D882-02 standard. Initial grip separation was set at 100 mm and cross-head speed at 50 mm/min. The results presented are the average of at least six independent tests.

Water vapor and oxygen transmission rates (WVTR and OTR) of the films were determined by a MOCON PERMATRAN-WVR Model 3/33 and OXTRAN 2/21 MH instruments (MOCON, USA). The test area of the films was 50 cm^2 and thickness was measured as an average of five using digital Vernier calipers. Permeability cells were flushed using nitrogen and specimens were conditioned for 24 h prior testing. For WVTR measurements, temperature was fixed at 37.8°C and relative humidity of 100%, whereas OTR was tested at 23°C and relative humidity of 50%. The specimens were run until a steady-state was reached.

Head space volatiles from the film containing 5 wt % thyme oil was analyzed by Agilent 7890A gas chromatograph (GC) equipped with split/split-less inlet in combination with an Agilent 5973 N MSD after solid-phase micro extraction (SPME). A film disc (32 mm dia) was placed in a 50 mL beaker, sealed with an aluminum foil, and left for 24 h at 25°C. The analytes from the film was captured on the stationary phase by an SPME fiber assembly for 1 h *ca* 25°C and then desorbed into the GC injector for 25 min and analyzed using HP-5MS column (30 m × 0.25 mmi.d. × 0.25 μm, P = 65 kPa, He carrier gas). The volatile compounds were separated using a column with a split ratio of 25:1 and injector temperature of 250°C. The temperature program was 60–240°C at 3°C/min with total run time of 60 min for the separation of components. The mass spectra were taken at 70 eV, under positive electron impact ionization, with a mass range from 50 to 550 amu, solvent delay of 2 min, and transfer line of 300°C. Compound identification was confirmed by comparison of the mass spectra with NIST08 (National Institute of Standards and Technology 08). The analysis was done in triplicate and the average values are reported.

The antimicrobial effectiveness of the films was determined according to Kristo *et al.*[34] and Sánchez-González *et al.*[35] with slight modification. *C. gloeosporioides* was obtained from the Fruit and Vegetables Technology Laboratories, Tshwane University of Technology, South Africa. The *C. gloeosporioides* isolates were cultured and maintained on malt extract agar (MEA) (Merck, South Africa) and incubated at 25°C for 12–13 days. Spore suspension was prepared according to Bill *et al.*[36] and the mycelia fragments were removed from suspension by filtering through three layers of muslin cloth. Spore were counted using a hemocytometer and adjusted to 1 × 10^5 spore/mL. Aliquots of MEA (10 g) were poured into Petri dishes. After the culture medium solidified, diluted spore solution (50 µL/plate) was inoculated on the surface. The different test films (neat LDPE, LDPE/thyme oil 1 wt %, LDPE/thyme oil 2.5 wt %, and LDPE/thyme oil 5 wt %) of the same diameter as the Petri dishes (65 mm) were placed on the inoculated surface. Plates were then covered with parafilm to avoid dehydration and stored for 7 days at 25°C. To the end, the agar was removed aseptically from Petri dishes and placed in sterile plastic bag with 100 mL of sterile distilled water. The bag was homogenized for 2 min in a Stomacher blender. Serial dilutions were made and then poured onto MEA plates, incubated for 5 days at 25°C before colonies were counted. All tests were run in duplicates.

RESULTS AND DISCUSSION

Figure 1 shows the SEM images of trilayer films obtained for neat LDPE and LDPE with thyme oil additives. Homogeneous surface morphologies were observed for neat LDPE and the samples with 1 wt % of thyme oil. However, the surface characteristic of the films changed with the addition of higher amounts of thyme oil [parts (c) and (d) of Figure 1]. The film containing 2.5 and 5 wt % thyme oil had rougher surface than the films containing 1 wt % thyme oil. The surface of the films with higher concentrations of thyme oil showed crater-like dips on its surface [Figure 1(c,d)]. This could be due to the partial vaporization of thyme oil from the polymer matrix during extrusion process. This may also be due to the evaporation of thyme oil under high-energy electron beam during SEM analysis. Similar results were observed by Ramos *et al.*[37] for PP films incorporated with carvacrol and thymol additives.

The infrared spectra of neat film and films with different concentrations of thyme oil are shown in Figure 2. The characteristic peaks of LDPE were observed at 2958, 2849, 1472, and 720 cm^{-1} which respectively attributed to C—H asymmetric and symmetric stretching, C—H bending and rocking vibrations of the linear aliphatic polymer chain.[38] The FTIR spectra of other films were very similar to that of LDPE film since the thyme oil concentrations were comparatively low. This showed that thyme oil did not have any noticeable effect on the structure of LDPE. None of the spectra revealed the carbonyl vibration at 1740–1700cm^{-1} which could account for most of the oxidation products formed during the thermo-oxidative degradation of PE.[39]

The thermal stability of all the films was studied by TGA in air atmosphere. Figures 3(a,b) respectively shows the weight loss

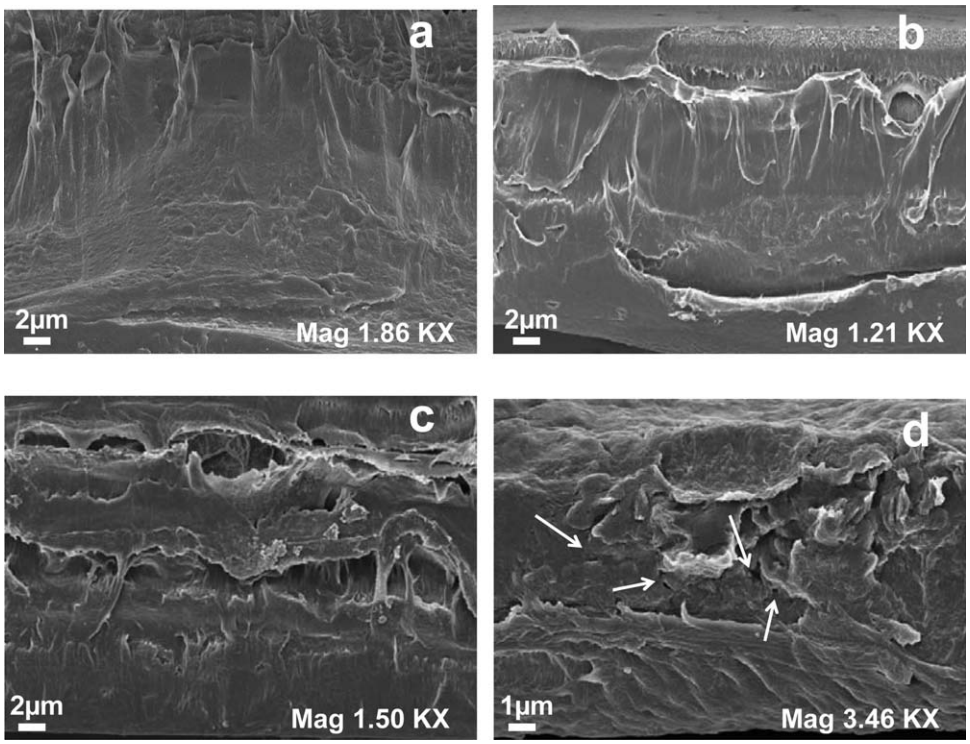

Figure 1. FTIR spectra of (a) LDPE, (b) LDPE/thyme oil (1 wt %), (c) LDPE/thyme oil (2.5 wt %), and (d) LDPE/thyme oil (5 wt %).

(TG) and the derivative (DTG) curves obtained for the samples. The DTG curves demonstrate the beginning, maximum, and final decomposition temperatures of the materials. The TGA weight loss curves [Figure 3(a)] showed that the polymeric structure started to decompose at approximately 330°C with a peak temperature around 400°C and ended around 475°C. The degradation pattern of LDPE changed with the additive indicating the availability of thyme oil in the film after processing. The onset degradation temperature of LDPE slightly increased with the addition of 1 and 2.5 wt % of thyme oil, whereas the film with 5 wt % thyme oil showed a decrease. The DTG curves given in Figure 3(b) demonstrated more clearly the difference in thermal stability of different films. The peak maximums of decomposition temperature observed were 404, 408, 411, and 397°C for neat and films with 1, 2.5, and 5 wt % of thyme oil. The results showed that the presence of thyme oil although increases the thermal stability of the film at lower concentrations, at higher concentrations unevenly distributed phase could reduce the morphological integrity, and hence the thermal stability. Suppakul *et al.*[40] also observed a lower decomposition temperature for LDPE/linalool film when compared to the additive free film.

Thermal properties of the films were studied by DSC analysis and the results are summarized in Table I. Figures 4(a,b) respectively represent the cooling and second heating thermograms obtained. As it can be seen, melting and crystallization temperatures did not show noticeable differences among different samples. However, ΔH_m of the film containing 5 wt % thyme oil was lower than other films. This indicated higher crystallinity for the neat film and films with thyme oil up to 2.5 wt % loading. The χ_c calculated were 32.8, 34.8, 38.2, and 33.0% revealing highest crystallinity for LDPE film with 2.5 wt % thyme oil. The films with 5 wt % of thyme oil showed crystallinity close to that of neat LDPE. These results contradict the decrease in crystallinity of PP containing carvacrol, thymol, and other antioxidants reported by Ramos *et al.*[31] and Alin *et al.*[41] In the case of film with high thyme oil content, the lower value of χ_c is due to the poor dispersion of thyme oil in LDPE matrix.

To get more insight into the crystallization behavior of the films and to confirm the DSC results, the spherulite evolution during the melt crystallization at 123°C for the neat LDPE and other films was monitored as a function of time and the observations

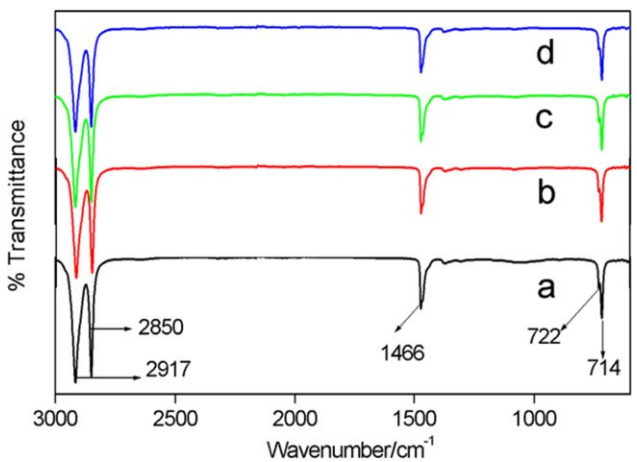

Figure 2. Scanning electron micrographs showing surface morphology of (a) LDPE, (b) LDPE/thyme oil (1 wt %), (c) LDPE/thyme oil (2.5 wt %), and (d) LDPE/thyme oil (5 wt %). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

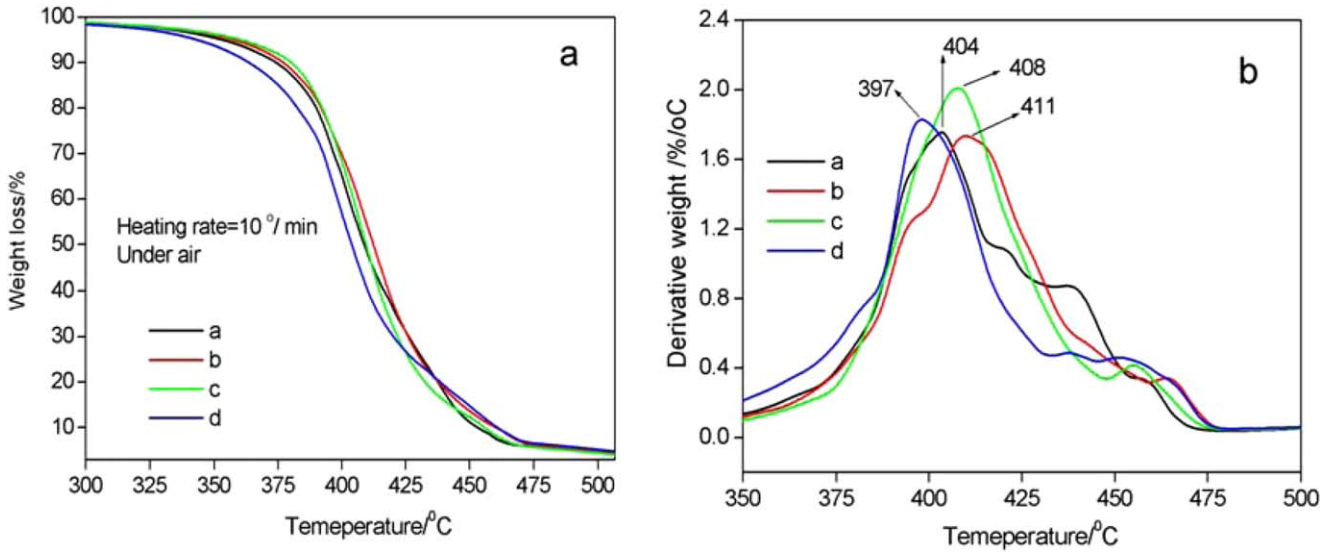

Figure 3. (a) TGA and (b) DTG curves of (a) LDPE, (b) LDPE/thyme oil (1 wt %), (c) LDPE/thyme oil (2.5 wt %), and (d) LDPE/thyme oil (5 wt %). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

are shown in Figure 5. From the POM images, it is clear that the nucleation was delayed in the film with 5 wt % of thyme oil in comparison to other films. On the other hand, faster nucleation with higher nuclei density was observed in the film with 2.5 wt % thyme oil. It supports the fact that the essential oil droplets dispersed in the LDPE matrix served as nucleating agents for LDPE crystallites. In higher concentrations, thyme oil droplet could aggregate which did not facilitate faster nucleation process. These observations are in agreement with the DSC results.

From the DSC and POM results, we speculate the existence of secondary crystallization process in the films with 2.5 wt % of thyme oil. The presence of well-dispersed oil droplets in the film may initiate heterogeneous nucleation during cooling which leads to the formation of subsidiary thinner crystal lamellae. The secondary crystallization is always associated with the fractionated low molar mass material and results in crystal imperfections. The fraction of imperfect crystals may not be high enough to reflect the effect of spherulite growth impingement on the crystallization temperature.[42,43] At higher concentrations of thyme oil, the higher fraction short branched chains could effectively hinder crystal growth, giving rise to more defects. As a result, the degree of crystallinity drops with increased thyme oil concentration. Further detailed kinetic studies are needed to confirm our suggestions.

Tensile tests were performed with all the materials studied to evaluate the influence on ductile properties. Results from elastic modulus (MPa), ultimate tensile stress (MPa), and elongation at break (%) are shown in Table II. The addition of thyme oil to LDPE matrix resulted in slight modifications of tensile properties. Decrease in elastic modulus (around 20%) was observed for the film containing 5 wt % thyme oil when compared to the neat LDPE film. This could be explained, again, by matrix inhomogeneity created by the presence of thyme oil in high concentration. All the films containing thyme oil showed a reduced elongation at break than the neat LDPE. This could be correlated to the dispersed oil phase and subsequent formation of imperfect crystal lamellae in LDPE matrix. Such secondary crystallization of amorphous region may produce opposing effects such as reduction in strength or elongation. This behavior has been reported for polypropylene- and polyethylene-based samples containing carvacrol active agent.[31,44]

The WVTR, OTR, and the permeation values normalized to thickness of the prepared films are listed in Tables III and IV. From the data which show very close values for the permeation rates, it is clear that the films with thyme oil did not show pronounced decrease in barrier properties in comparison to the neat film. It was interesting to note that the films with high concentration of thyme oil had barrier similar to other films.

There are three factors affecting the permeability of a material toward water vapor such as the degree of crystallinity, the polarity, and the structure of the polymer.[45]

Table I. Thermal Characteristics of Films Obtained from DSC Analysis

Sample	T_c (°C)	T_m (°C)	ΔH_m (J/g)	χ_c (%)
LDPE	111.5 ± 0.3	124.4 ± 0.5	96.4 ± 2.9	32.8 ± 1.9
LDPE/thyme oil (1 wt %)	111.8 ± 0.05	124.0 ± 0.05	102.3 ± 0.1	34.8 ± 0.5
LDPE/thyme oil (2.5 wt %)	111.6 ± 0.12	123.8 ± 0.05	111.9 ± 4.8	38.2 ± 1.5
LDPE/thyme oil (5 wt %)	111.4 ± 0.01	123.5 ± 0.04	96.8 ± 1.5	33.0 ± 0.5

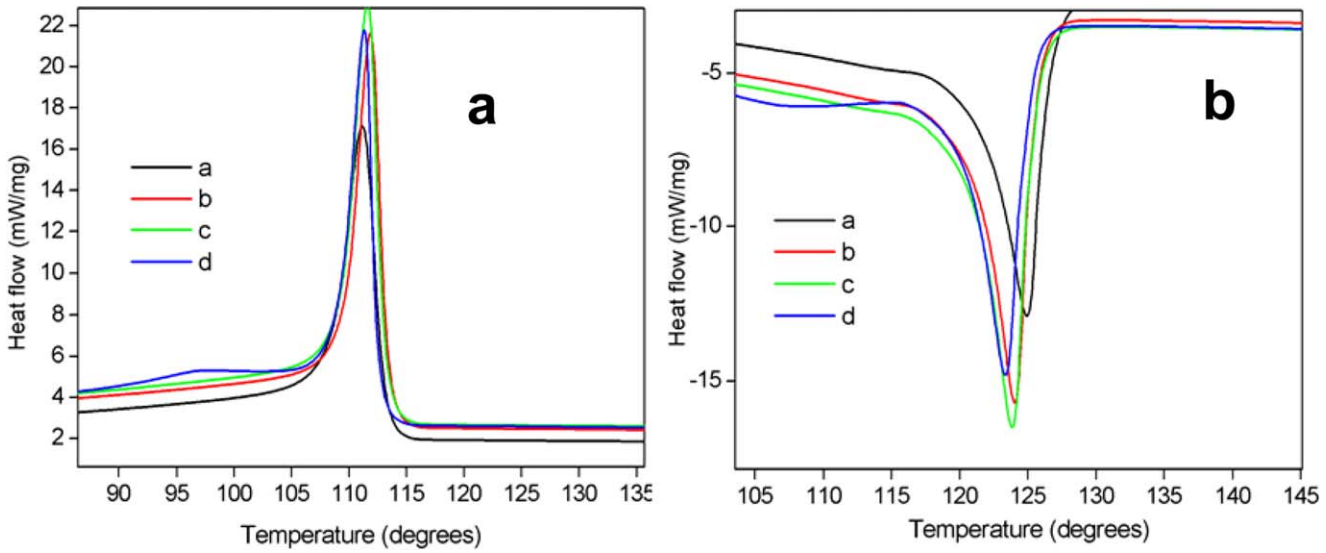

Figure 4. (a) DSC cooling and (b) second heating scans of (a) LDPE, (b) LDPE/thyme oil (1 wt %), (c) LDPE/thyme oil (2.5 wt %), and (d) LDPE/thyme oil (5 wt %). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

According to DSC results, LDPE with 5 wt % of thyme oil showed similar crystallinity as neat LDPE but lower than that of the films with 1 and 2.5 wt % of thyme oil. Moreover, the increased porosity of the polymer matrix at 5% thyme oil (from SEM results) could also be a detrimental factor to barrier property. However, since we could not see a significant difference in permeation rates of different films, we believe that the dispersed hydrophobic thyme oil droplets in a phase-separated structure filled the free volume within the polymer matrix thus compensating for the loss of crystalline regions. Hence the film could hinder the passage of water vapor and oxygen molecules as effectively as the other films with higher crystallinity. Pires *et al.*[26] and Tanaka *et al.*[45] have also demonstrated that the water vapor transfer process depends on the hydrophilic–hydrophobic ratio of

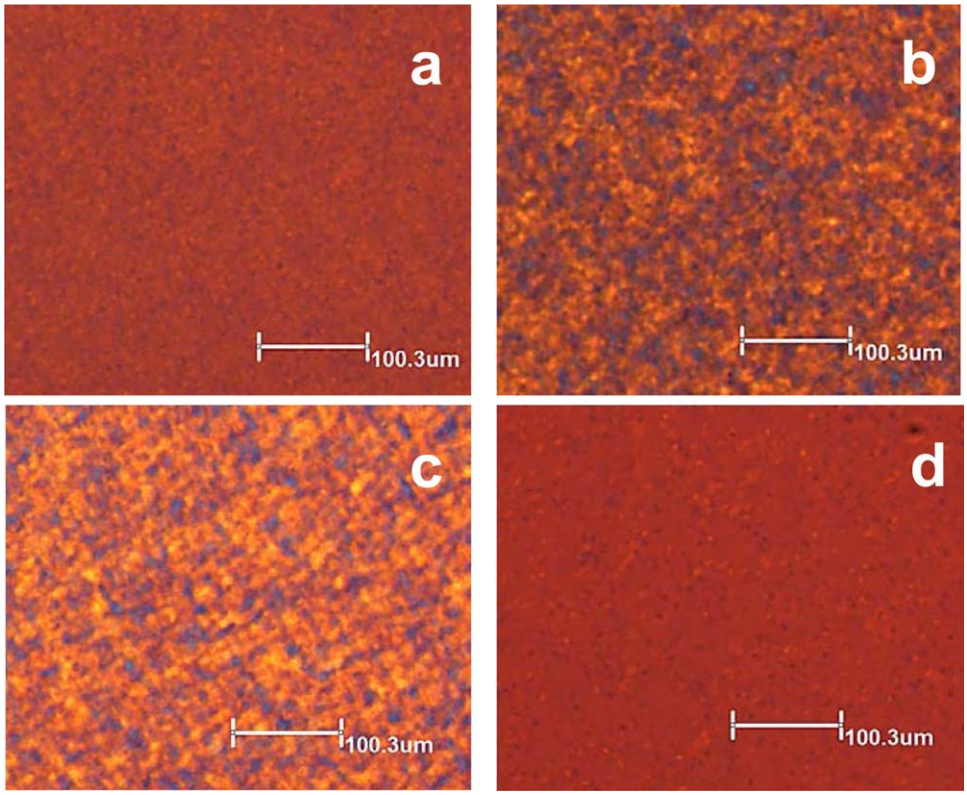

Figure 5. POM images of (a) LDPE, (b) LDPE/thyme oil (1 wt %), (c) LDPE/thyme oil (2.5 wt %), and (d) LDPE/thyme oil (5 wt %) during isothermal cooling at 123°C after 1.8 min. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table II. Tensile Properties of Neat LDPE Film and Other Films Containing Thyme Oil

Sample	Elastic modulus (MPa)	Ultimate tensile stress (MPa)	Elongation at break (%)
LDPE	273 ± 54	24.8 ± 4.1	601.6 ± 35
LDPE/thyme oil (1 wt %)	228 ± 9.8	15.0 ± 1.0	510.8 ± 18.3
LDPE/thyme oil (2.5 wt %)	256.7 ± 36.3	16.0 ± 0.15	528.1 ± 11.9
LDPE/thyme oil (5 wt %)	231.9 ± 29.8	15.1 ± 1.6	545 ± 19.6

Table III. WVPR Data for Neat LDPE and LDPE/Thyme Oil Films Measured at 37.8°C and 100% Relative Humidity

Sample	Thickness (mm)	WVTR g/[m^2 day]	Permeation g mm/[m^2 day]
LDPE	0.071 ± 0.001	6.4 ± 0.4	0.46 ± 0.03
LDPE/thyme oil (1 wt %)	0.072 ± 0.001	6.4 ± 0.3	0.46 ± 0.02
LDPE/thyme oil (2.5 wt %)	0.067 ± 0.007	6.8 ± 0.7	0.45 ± 0.01
LDPE/thyme oil (5 wt %)	0.086 ± 0.008	5.1 ± 0.5	0.44 ± 0.01

Table IV. OTR Data for Neat LDPE and LDPE/Thyme Oil Films Measured at 23°C and 50% Relative Humidity

Sample	Thickness (mm)	OTR (cc/[m^2 day])	Permeation (cc-mm/[m^2 day])
LDPE	0.068 ± 0.01	18.5 ± 0.5	1.2 ± 0.15
LDPE/thyme oil (1 wt %)	0.070 ± 0.002	16.2 ± 0.6	1.1 ± 0.02
LDPE/thyme oil (2.5 wt %)	0.073 ± 0.001	15.5 ± 0.9	1.1 ± 0.09
LDPE/thyme oil (5 wt %)	0.080 ± 0.002	16.0 ± 0.2	1.2 ± 0.01

the film constituents and hence the incorporation of hydrophobic oils in films decreases the WVTR and increases the barrier properties. According to Dias *et al.*,[28] the presence of oil phase could increases the tortuosity factor in polymer matrix.

Table V shows the levels of active volatile components released from film with 5 wt % of thyme oil, reached in the headspace after 24 h of storage at room temperature. According to the results given in peak area percentages, the major volatiles identified in the head space composition were thymol, caryoplyllen, and carvacrol; the antimicrobial properties of which are well-known and well-documented.[46,47] Among the volatiles, caryoplyllen was found in higher concentration indicating its higher molecular diffusion rate in the absence of any food stimulants. The addition of thyme oil to LDPE films is therefore expected to modify the packaging environment due to the release of the antimicrobial active agents and inhibit the microbial growth on packaged food stuff through active interaction.

Figure 6 shows the results of the antimicrobial tests performed against *C. gloeosporioides* on neat and films containing thyme oil additive. The effect of films on the survival of the microbe at a dilution of 10^{-3} is presented in Figure 7. From the results, it was evident that films containing 5 wt % of thyme oil was the most effective against the fungi showing higher degree of inhibition. The neat LDPE showed significantly lower effect on the inhibition of growth of *C. gloeosporioides*. The film with 1 wt % of EO found to be not active which might be due to the lower concentration of oil. Films with 5 wt % of thyme oil prevented the growth of the pathogen with efficiency up to 99%. These results demonstrated substantial antifungal activity of the LDPE films prepared by incorporating the thyme oil which could be attributed to the release of antimicrobial volatiles such as thymol, caryoplyllen, and carvacrol. Films with additives concentrations 5 wt % were found to be enough to achieve an adequate inhibition of the anthracnose-causing microorganism.

Different researchers have demonstrated the effectiveness of LDPE films containing essential oils, such as clove, basil, oregano, and cinnamon against different strains of bacteria.[20–22,30,48,49] The results from this study showed that the incorporation of thyme oil to LDPE matrix during blown film extrusion considerably

Table V. Head Space Analysis of Active Ingredient of Volatiles Released from the LDPE Film Containing 5 wt % of Thyme Oil

Active compounds from thyme oil	RT	A%
Thymol	17.7	0.69 ± 0.23
Caryoplyllen	20.0	1.11 ± 0.67
Carvacrol	19.5	0.30 ± 1.20

A% = percentage flame ionization detector (FID) peak area.

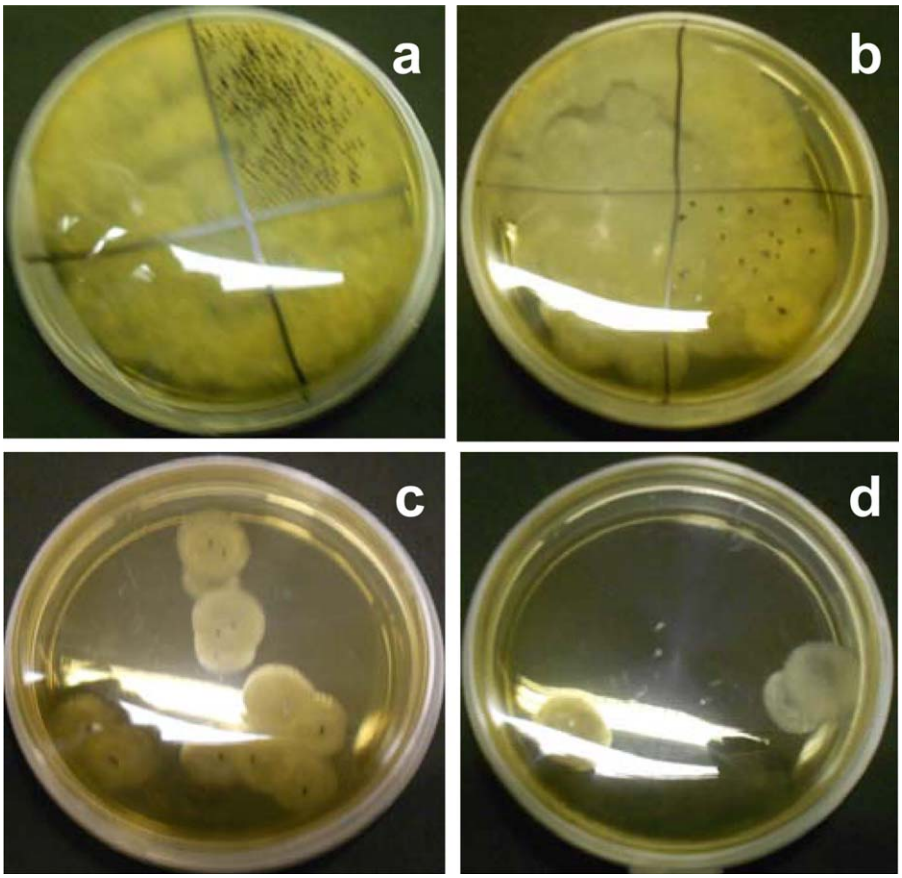

Figure 6. Antifungal activity of neat LDPE film and films containing thyme oil on *C. gloeosporioides* (a) LDPE, (b) LDPE/thyme oil (1 wt %), (c) LDPE/thyme oil (2.5 wt %), and (d) LDPE/thyme oil (5 wt %). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

reduced the microbial growth of *C. gloeosporioides* and hence it could be a potential packaging material for avocados for maintaining the quality and improving the shelf life.

CONCLUSIONS

Trilayer LDPE films containing different concentration of thyme oil were successfully prepared by blown film extrusion. From DSC results, film prepared with 2.5 wt % of thyme oil showed highest values for maximum decomposition temperature and degree of crystallinity which might be due to the uniform dispersion of thyme oil in polymer matrix. Faster nucleation with higher nuclei density was also observed in the film with 2.5 wt % thyme oil in POM images which indicated that the dispersed thyme oil droplets served as nucleating agents for LDPE crystallites. At higher concentrations above 2.5 wt %, thyme oil droplets have a tendency to aggregate which did not facilitate faster nucleation process. The addition of thyme decreased both elastic modulus and elongation at break in comparison to neat LDPE due to the matrix inhomogeneity created by the presence of thyme oil in high concentration. The WVTR of all the films were similar in spite of the decrease in crystallinity of the material at higher thyme oil concentration because of the compensating effect from water repellent thyme oil that filled the free volume of the polymer matrix. The films containing 5 wt % thyme oil showed substantial inhibition activity against *C. gloeosporioides* which a fungi that causes postharvest disease "anthracnose" in avocados and other fruits. This result indicated efficient thyme oil release properties of the film. The results of this study demonstrate the promising potential of LDPE films with incorporated thyme oil at concentrations of 5 wt % as active packaging materials for preserving foodstuffs that are susceptible to fungal infections.

Product development on a pilot scale, assessment of antimicrobial activity on fungi inoculated and naturally infected avocado fruits, and full life cycle assessment of the packaging material is currently underway in our laboratories.

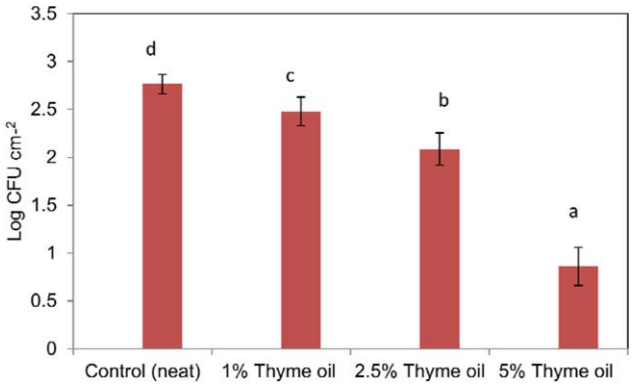

Figure 7. Effect of neat LDPE film and films containing thyme oil on the survival of *C. gloeosporioides*. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

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