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LDPE/Clay/Carvacrol Nanocomposites with Prolonged Antimicrobial Activity

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ABSTRACT: Over the past decade there is an immense effort to develop antimicrobial packaging systems, which incorporates natural biopreservatives, such as essential oils (EOs). The highly volatile nature of EOs, which is advantageous for their efficient diffusion and mode of action, presents a major obstacle for their incorporation with polyolefins via conventional high-temperature melt compounding and processing. This study presents a new approach to use organo-modified montmorillonite (MMT) clays, as active carriers for carvacrol (used as a model EO), aiming to minimize its loss throughout the polymer compounding. Different MMT clays are pretreated with carvacrol, resulting in the oil molecules intercalation in between the clay galleries and enhanced carvacrol thermal stability. These hybrids are incorporated within low-density polyethylene (LDPE) and the resulting films are characterized in terms of their nanostructure, thermal properties, and antimicrobial activity. The LDPE/(clay/carvacrol) nanocomposites exhibit excellent and prolonged antimicrobial activity against *E. coli* bacteria, while LDPE/carvacrol films loss their antimicrobial functions within several days. The superior antimicrobial behavior is ascribed to the significantly higher carvacrol content and its enhanced thermal stability within the films. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2015**, *132*, 41261.

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INTRODUCTION

Food-borne bacterial outbreaks remain a major cause for disease and mortality throughout the world.[1] Recent outbreaks over the past several years in Europe and the United States have demonstrated the devastating epidemiological and economical outcomes of these events. Thus, over the past decade there is an immense effort to develop antimicrobial packaging systems that can potentially improve food safety throughout the supply chain.[2] The rationale for incorporating antimicrobial activity into the packaging is to prevent microbial growth on food surfaces, where a large portion of spoilage and contamination occurs, improving food safety and quality as well as extending the food shelf life. This approach can reduce the addition of large quantities of food preservatives and antimicrobials that are usually incorporated into the bulk of the food.[3–6]

Different antimicrobial agents, including metal ions, organic acids and their salts, plant extracts, and essential oils, have been incorporated into different polymers.[7–11] Depending on both the nature of the antimicrobial agent and the incorporation method used, antimicrobial packaging can act by (i) direct contact leading to a sufficient active agent diffusion and, thus, concentration on the surface of the foodstuff, or (ii) by indirect contact in the case of volatile active agents.[12] There are several approaches for the introduction of antimicrobial activity into polymeric materials. These include incorporating antimicrobial agents directly into polymers, coating antimicrobials onto polymer surfaces, immobilizing antimicrobials by chemical grafting, or using polymers that possess intrinsic antimicrobial activity.[4,6,13] The resulting antimicrobial polymers have additional potential application in several other areas, particularly in medical devices, healthcare and hygiene, and water purification systems.

The use of antimicrobial agents such as essential oils is consistent with consumer demand for safe and natural products.[14–19] Therefore, this research aims to combine carvacrol, as a model essential oil, in a low-density polyethylene (LDPE) polymeric matrix. Carvacrol is a phenolic compound, which is the major component in the essential oils of both oregano and thyme,

Additional Supporting Information may be found in the online version of this article.

Table I. Characteristics of Commercially Available MMT Clays

Supplier	Name	Particle size (μm)	Modifier conc. (wt %)	XRD d-spacing (001) (A)	Organic modifier
Southern Clay	Cloisite Na+	2-13	7	11.7	none
	Cloisite 15A	2-13	43	31.5	dimethyl, dihydrogenated tallow, quaternary ammonium
	Cloisite 20A	2-13	38	24.2	
	Cloisite 10A	2-13	39	19.2	Dimethyl, benzyl, hydrogenated tallow, quaternary ammonium
Nanocor	Nanomer I30P	15-20	28-32	20	Octadecyl tallow, quaternary ammonium
	Nanomer I44PA	14-18	34-36	25	Dimethyl dihydrogenated tallow, quaternary ammonium
Laviosa	Dellite 72T	7-9	37-41	26.5	Dimethyl, dihydrogenated tallow, quaternary ammonium

with an antimicrobial activity against a variety of microorganisms, including bacteria, yeast, and mold.[20–30] Several studies over the past several years have demonstrated the incorporation of essential oils into different polymers and showed promising antimicrobial activity of these films.[3,17,29,31–44] However, this activity was studied for fresh samples and the challenges associated with the controlled and prolonged activity of these films were not studied.

The aim of the present research is to design LDPE/clay nanocomposites containing carvacrol with controlled and tunable antimicrobial activity. These polymeric systems can be potentially used as antimicrobial packaging materials. Polymer nanocomposites containing exfoliated organoclay platelets have been extensively studied for their enhanced mechanical, thermal, and physicochemical properties (at a low level of filler concentration) in comparison to neat polymers and conventional polymer composites.[2,45–52] In particular, these nanocomposites exhibit excellent barrier properties due to the presence of clay layers, which delay the diffusing molecule pathway, owing to tortuosity effects[2,9,53,54]. Recent studies reported antimicrobial activity of nanocomposites based on commercially available organoclays.[5,55–58] In some studies, direct melt compounding of the essential oil, e.g., carvacrol and thymol, with the polymer and the organoclays was used to produce antimicrobial films.[5,36] In the present study, we use nano-scale inorganic materials, e.g., organo-modified montmorillonite (MMT) clays, as functional carriers for carvacrol. Different MMT clays are pretreated with carvacrol to allow intercalation of the oil molecules in between the clay galleries. We postulate that as a result, the volatile carvacrol molecules will exhibit higher thermal stability and its migration from the films will be efficiently delayed. In order to increase the degree of clay exfoliation and dispersion level within the polymer matrix, addition of a compatibilizer, PE-grafted maleic anhydride (MA), is investigated as well. This study focuses on studying the effect of different MMT clays on the nanostructure and thermal properties of clay/carvacrol hybrids and their incorporation with LDPE by melt-compounding processes. The antimicrobial activity of the resulting LDPE/(clay/carvacrol) films is investigated against Gram-negative (*E. coli*) bacteria as function of storage time and temperature.

EXPERIMENTAL

Materials

Materials used in this study are a commercially available grade of LDPE, Ipethene 320 from Carmel Olefins Ltd. (Haifa, Israel) and PE-grafted maleic anhydride Fusabond E226 compatibilizer from DuPont (Geneva, Switzerland). Different MMT clays are obtained from Southern Clay (Gonzales, Texas, USA), Nanocor (Hoffman Estates, Illinois, USA), and Laviosa (Livorno, Italy) as detailed in Table I. Carvacrol (98 %) is purchased from Sigma Aldrich Chemicals, Israel.

Preparation of Clay/Carvacrol Hybrids

Carvacrol is incorporated into different commercially available MMT clays (see Table I) by shear mixing followed by ultrasonication (Vibra cell VCX 750 - Sonics & Materials Inc., USA) for 10 min at room temperature, at a mass ratio of 2 : 1, respectively. Following clay modification, the degree of clay intercalation is evaluated by small-angled X-ray diffraction (SAXS) and thermogravimetric analysis (TGA).

Small Angle X-ray Scattering (SAXS)

SAXS was performed using a small-angle diffractometer (Molecular Metrology SAXS instrument) with Cu Kα radiation from a sealed microfocus tube (MicroMax-002+S), two Göbel mirrors, and three-pinhole slits. Generator is powered at 45 kV and 0.9 mA. The scattered intensity I(h) is recorded in the interval $0.07 < h < 2.7\ nm^{-1}$, where h is the scattering vector defined as $h = (4\pi/\lambda)\ \sin(\theta)$, where 2θ is the scattering angle, and λ is the radiation wavelength (0.1542 nm).

Thermogravimetric Analysis (TGA)

Thermal measurements are performed using TGA Q5000 setup (TA instruments, USA) at a heating rate of 20°C/min under nitrogen atmosphere.

Preparation of LDPE/Clay–Carvacrol Films

LDPE with or without the addition of 5 wt % compatibilizer is melt compounded at 150°C with the carvacrol-modified clays at

Table II. Momentum Transfer (h) and d-Spacing Values of Clay/Carvacrol Hybrids and Corresponding Unmodified Clays

Clay type	h (peak) (1/A)	d (001) of clay/ carvacrol hybrids (A)	d (001) of unmodified clay[a] (A)	Δd (A)
Cloisite Na+	none	-	11.7	-
Cloisite 15A	0.16812	37.4	31.5	5.9
Cloisite 20A	0.17113	36.7	24.2	12.5
Cloisite 10A	0.16912	37.2	19.2	18
Nanomer I30P	0.17314	36.3	22	14.3
Nanomer I44PA	0.17013	36.9	26.9	10
Dellite 72T	0.17013	36.9	25.6	11.3

[a] d-spacing values for the unmodified clays are taken from the technical data sheets of the suppliers.

a mass ratio of 85/(5/10) using a 16 mm twin-screw extruder (Prism, England) L/D ratio of 25 : 1 with a screw speed of 150 rpm and feeding rate of 2 kg/hr. The resulting pellets are hot pressed at 130°C, under 150 bar to obtain thin films, approximately 120 μm thick.

Differential Scanning Calorimetry (DSC)

The thermal properties of the resulting films are studied by DSC (DSC 1, Mettler-Toledo, Switzerland). Films are heated from room temperature to 250°C at a rate of 10°C/min under nitrogen atmosphere.

The crystallinity (w_c) of the polymer is calculated according to equation (1), where ΔH_m (J/g) is the latent heat of fusion of the sample, f_p is the LDPE weight fraction in the sample, and ΔH°_m. is the theoretical latent heat of fusion for 100% crystalline LDPE, 293 J/g.

$$w_c = \frac{\Delta H_m}{f_p \Delta H^\circ_m}. \quad (1)$$

Transmission Electron Microscopy (TEM)

The nanostructure of the films is studied using a Philips CM120 transmission electron microscope (TEM) operated at 120 kV accelerating voltage, using 400 mesh carbon-covered Cu grid Pk/100 (SPI Supplies West Chester, USA). Images are recorded digitally by a Gatan MultiScan 791 CCD camera using the Digital Micrograph 3.1 software. Ultra-thin sections of approximately 100 nm thick are prepared at −100°C with a Reichert E Ultracut microtome, using a glass knife.

Antimicrobial Activity

The antimicrobial activity of the films against *Escherichia coli* (ATCC 8739) bacteria is evaluated by two methods. The first method is based on measuring the bacterial inhibition zone around the polymeric films. This method gives a qualitative assessment for the antimicrobial activity of the film.[11,59] Discs (of 12 mm in diameter) are cut from the films and are located onto the surface of a Luria broth (LB) agar plate seeded with 10^8 colony forming units (CFU)/mL of bacteria culture. The plates are incubated at 37°C for 12 hr and antimicrobial activity is evaluated by observing a clear inhibition zone around the studied sample. Unmodified films are also assayed as a negative control.

The second method is quantitative and is based on the Japanese standard (JIS Z 2801 : 2000 "Antimicrobial products—test for antimicrobial activity and efficacy"). *E. coli* bacteria are grown overnight in Nutrient Broth media (NB, Sigma Aldrich, Israel) under shaking (250 rpm) at 37°C. In the following day, the overnight culture is diluted in a fresh NB medium to an optical density (OD) value of 0.1, which approximately corresponds to 10^8 CFU/mL, and grown for 1.5 hr to allow the cells to enter a logarithmic state until OD = 0.6 is reached. Then, the bacteria are diluted into NB 1% (1 : 100) to obtain a stock solution with a working concentration of 10^5 CFU/mL. Each film is placed in a well in a 6 well plate and incubated with 3 mL of the stock solution. The plates are incubated at 37°C (under agitation at 100 rpm) for 24 hr. After 24 hr samples are serially diluted in NB 1 : 100 (performed in 96 well plates). 20 mL drops are incorporated into NB bacto-agar (Becton Dickinson) plates to determine cell numbers. Plates are incubated at 37°C for 18–24 hr; CFU are counted and log reduction is calculated in comparison to control growth of *E. coli* in the NB 1 : 100 medium (10^8 CFU/mL). All determinations, including the growth controls, are performed in triplicates.

RESULTS AND DISCUSSION

Characterization of Clay/Carvacrol Hybrids

Small Angle X-ray Scattering (SAXS) studies are carried out in order to monitor the structural changes of the different MMT clays after their modification with carvacrol. The obtained momentum transfer h (1/A) and d-spacing values are summarized in Table II and compared to the corresponding d-spacing values of the unmodified clays.

As can be inferred from Table II, the d-spacing values for of all clay/carvacrol hybrids (except for the Cloisite Na^+ clay) are higher in comparison to the corresponding neat clays, indicating intercalation of the carvacrol in between the MMT layers. The organic modification, i.e., quaternary ammonium salts, of the clays plays a vital role in the intercalation of the carvacrol molecules within the layered silicates and the resulting expansion of the clay galleries. Indeed, in the natural unmodified clay, Cloisite Na^+, no expansion in the d-spacing value is observed, demonstrating that the carvacrol cannot penetrate in between the clay galleries. Thus, SAXS results suggest effective intercalation of carvacrol within the organoclays, where the clay serves as a potential carrier for carvacrol molecules (EO model), and not as "passive" fillers as in previous studies.[5,31–33,36]

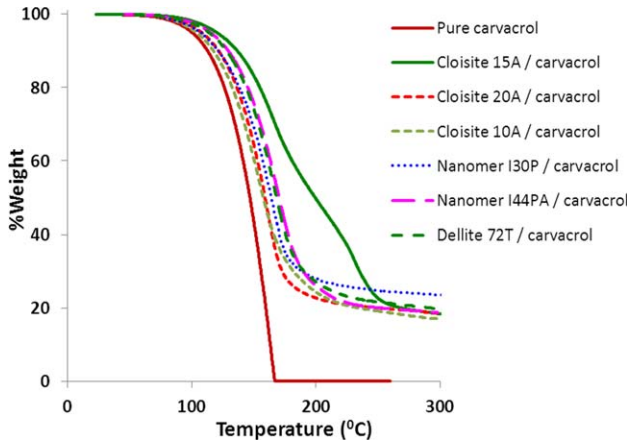

Figure 1. TGA curves of pure carvacrol and different clay/carvacrol hybrids. The composition of all hybrids is 1 : 2 mass ratio of clay and carvacrol, respectively. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

To further support the suggested concept of clays serving as functional carvacrol carriers, the thermal stability of the different clay/carvacrol hybrids is characterized by TGA. Figure 1 displays thermograms, i.e., weight loss versus temperature, of the different hybrids in comparison to neat carvacrol. The volatile carvacrol is observed to completely evaporate by ~170°C. The thermograms of the clay/carvacrol hybrids depict a distinct weight loss process, occurring in the range of 100–200°C (this specific weight loss process is not observed in the neat organoclays, see Figure S1, Supporting Information, which exhibit degradation of their organic moieties at higher temperatures). Thus, this weight loss is assigned to the evaporation of carvacrol molecules from the modified clays. It should be noted that evaporation of carvacrol from the hybrids occurs at significantly higher temperatures (>20°C) in comparison to the neat carvacrol. Specifically, the Cloisite 15A/carvacrol hybrids exhibit the highest thermal stability with delayed carvacrol loss of approximately 50°C and a two-stage weight loss process, which is not observed for the other hybrids. Thus, these results further indicate that clay carriers enhance the thermal stability of the carvacrol.

Characterization of LDPE/(Clay/Carvacrol) Films

Following melt compounding of LDPE with the different clay/carvacrol hybrids and compression molding, the resulting films are characterized in terms of their thermal properties, nanostructure, and antimicrobial activity.

Thermal Properties

Table III summarizes the differential scanning calorimetry (DSC) results for the different LDPE/(clay/carvacrol) systems, with and without the addition of the compatibilizer. The melting temperature and crystallinity values of the different films are presented as well as the results for neat LDPE, LDPE/clay, and LDPE/carvacrol films. Addition of neat clays or clay/carvacrol hybrids results in negligible effect on the melting temperature of the LDPE matrix. These results are consistent with previous studies.[5,60] Nevertheless, the crystallinity of the polymer is significantly higher in the LDPE/(clay/carvacrol) systems and this effect is further intensified upon the addition of the compatibilizer. The highest degree of crystallinity values, 53 % and 57 %, are obtained for films containing the compatibilizer and the Cloisite 15A/carvacrol and Cloisite 10A/carvacrol hybrids, respectively. It is well established that in semicrystalline polymers, such as PE, the two main parameters affecting crystallinity are the presence of heterogeneous nucleation sites and the changes in chain mobility.[5,45] Our results suggest that the clay platelets act as a nucleator for the initial heterogeneous nucleation process (these results are also observed for the corresponding reference LDPE/clay) and the subsequent growth of crystallites.[5,45] Furthermore, we hypothesize that the diffusional chain mobility is promoted by the carvacrol presence, which may enhance the ability of the LDPE chains to fold and crystallize. Addition of the compatibilizer to the compounding process, enhance the dispersion of the clay-carvacrol hybrids within the polymer matrix, which results in a greater exfoliation of the clay platelets and higher crystallinity.

Thermogravimetric analysis (TGA) is employed to study the thermal stability of carvacrol and the effect of the different clays on carvacrol loss. Figure 2 presents the TGA curves for the different LDPE/(clay/carvacrol) systems. Addition of clay/carvacrol hybrids rather than neat carvacrol to LDPE has a profound effect on carvacrol loss and its thermal stability.

In LDPE/carvacrol films, carvacrol is completely lost at ~160°C, while in the LDPE/(clay/carvacrol) systems, carvacrol loss occurs at higher temperatures ~170–190°C. Moreover, the thermograms can be used for determining carvacrol content in the film following melt compounding and compression molding. All LDPE/(clay/carvacrol) films exhibit higher carvacrol content (5–6 wt %) in comparison to the reference LDPE/carvacrol films, which contained only ~3 wt %. Thus, these results confirm that the clay platelets function as active carriers of the volatile carvacrol and their role in enhancing its thermal stability during high-temperature processes. It should be emphasized that the different organoclays, studied in this work, exhibit similar effects on the thermal stability as well as on carvacrol loss.

Figure 3 presents thermograms of LDPE/(Cloisite 15A/carvacrol) systems with and without compatibilizer addition. The PE-grafted-MA compatibilizer has a pronounced effect on the thermal stability of the system. Both, the loss of carvacrol and LDPE degradation, are observed to occur at higher temperatures in comparison to LDPE/(Cloisite 15A/carvacrol) system with no compatibilizer. It should be noted that the carvacrol content (following melt processing) is lower when the compatibilizer is added. This behavior may be ascribed to the enhanced dispersion of the clay/carvacrol hybrids within the LDPE matrix. Thus, the improved interaction between the LDPE and hybrids may result is a higher extent of exfoliation in these nanocomposites, which in turn reduce the carvacrol confinement within the clay galleries. In order to assert this hypothesis, the LDPE/(Cloisite 15A/carvacrol) films are further studied by transmission electron microscopy (TEM). Figure 4 displays TEM images of cryogenic crosssectioned LDPE/(Cloisite 15A/carvacrol) films with and without compatibilizer addition. The images clearly demonstrate different intercalation and exfoliation levels of the modified clays in the LDPE matrix. The compatibilizer addition

Table III. Melting Temperature and Degree of Crystallinity of Different LDPE/(Clay/Carvacrol) Systems, as Well as the Results for Neat LDPE, LDPE/Clay and LDPE/Carvacrol Films, Used as for Reference

Sample name	Tm (°C)	Crystallinity (%)	Tm (°C)	Crystallinity (%)
w/o compatibilizer	Without compatibilizer		With compatibilizer	
LDPE neat	110	35	109.5	43
LDPE/Cloisite 15A	112	49		
LDPE/carvacrol	111	48		
LDPE/(Cloisite 15A/carvacrol)	110	41	109	53
LDPE/(Cloisite 20A/carvacrol)	109	43	111	47
LDPE/(Cloisite 10A/carvacrol)	110	48	109	57
LDPE/(Nanomer I30P/carvacrol)	110	46	111	46
LDPE/(Nanomer I44PA/carvacrol)	111	47	109	50
LDPE/(Dellite 72T/carvacrol)	110	51	110	50

is observed to enhance the intercalation and exfoliation of modified clays (see Figure 4a), while in the film without compatibilizer, more closely packed organo-silicate stacks can be seen (Figure 4b). Accordingly, compatibilizer addition increases the dispersion and exfoliation of the clay/carvacrol hybrids, while decreasing the postprocessing embedded carvacrol content within the films.

It should be noted that films with other organoclays, studied in this work, exhibit a similar morphology to that observed for the LDPE/(Cloisite 15A/carvacrol) films.

Antimicrobial Activity of the Films

Antimicrobial studies are carried using the inhibition zone method[20,21] against *E. coli* (ATCC 8739) bacteria. Typical results of these tests for LDPE/(clay/carvacrol) films are depicted in Figure 5. A clear zone of bacteria growth inhibition is observed around the LDPE/(clay/carvacrol) film (Figure 5b), while for the reference neat LDPE films no zone of inhibition could be detected in the agar test (Figure 5a).

As carvacrol is a volatile compound, antimicrobial studies are performed over a period of 100 days following films preparation in order to assess their activity as a function of time. The samples are stored at 4°C and the inhibition zone studies are performed at regular intervals (weekly). Figure 6 summarizes the results of the LDPE/(clay/carvacrol) systems in comparison to LDPE/carvacrol films. All LDPE/(clay/carvacrol) exhibit a prolonged antimicrobial activity with insignificant changes in the inhibition zone diameter values. On the contrary, the LDPE/carvacrol films, in which the carvacrol is directly melt-compounded with LDPE, depict a sharp decrease in their activity and within 2 weeks no zone of inhibition is observed. These results demonstrate the vital role of the clay platelets in preserving the antimicrobial properties of the films and their function as active carriers of the volatile carvacrol molecules.

In addition to the inhibition zone tests, which provide qualitative assessment of the antimicrobial properties of the films; quantitative antimicrobial studies of different LDPE/(clay/carvacrol) films are executed by inoculating the films with *E. coli* bacteria suspensions (10^8 CFU/mL, for 18–24 hr, at 37°C), after which CFU are counted and log reduction is calculated in comparison to control growth of *E. coli*. Figure 7 summarizes the results of these experiments for fresh and 1 month old LDPE/

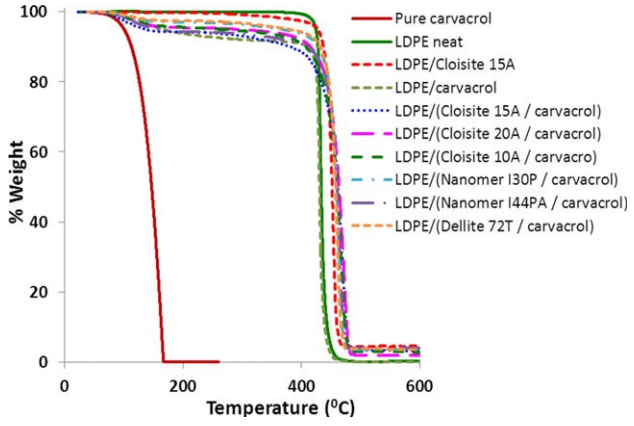

Figure 2. Thermograms of different LDPE/(clay/carvacrol) systems, as well as the results for neat carvacrol, neat LDPE, LDPE/clay, and LDPE/carvacrol films, used as for reference. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

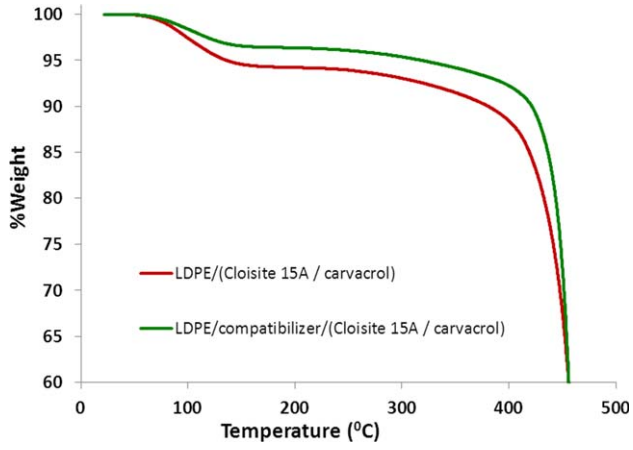

Figure 3. Thermograms of LDPE/(Cloisite 15A/carvacrol) systems with and without compatibilizer addition. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

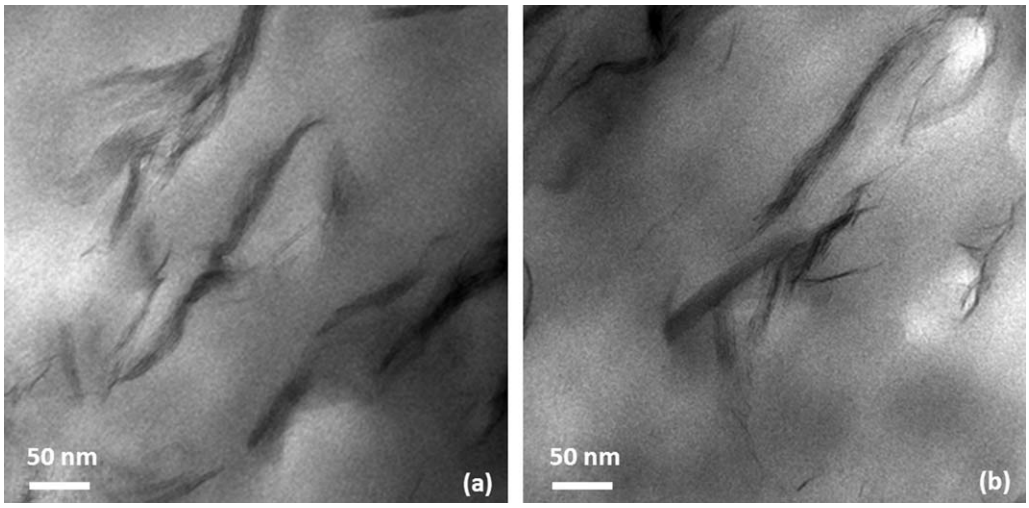

Figure 4. TEM images of LDPE/(Cloisite 15A/carvacrol) films (a) with and (b) without compatibilizer addition.

(clay/carvacrol) films (stored at room temperature). All freshly produced films have reduced *E. coli* cells to an undetectable level, demonstrating the effective bactericidal activity of carvacrol within the melt-compounded films. However, storage time has a profound effect on the antimicrobial potency of the films. LDPE/carvacrol films completely loss their antimicrobial activity within a month, in agreement with the inhibition zone studies (see Figure 6). The performance of the LDPE/(clay/carvacrol) films varies in *E. coli* reduction levels depending on organoclay type. Some of the films fully retain their antimicrobial potency (e.g., Cloisite 20A), while others exhibit 2–4 log reductions in viable bacteria population. Addition of the compatibilizer has an adverse effect on the prolonged antimicrobial properties of the films, possibly due to their lower carvacrol content (following melt processing), as discussed in previous sections. For example, for fresh LDPE/(Cloisite C15A/carvacrol) films, compatibilizer addition has no effect on the antimicrobial activity and full bactericidal activity against *E. coli* is attained. However, for 1 month old films, low and medium antimicrobial activity levels are observed with and without compatibilizer addition, respectively.

These results prove the clay role as active carriers for the highly volatile carvacrol. It should be emphasized that storing films at 4°C (relevant storage temperatures for dairy products and some fresh fruits and vegetables) preserve their full bactericidal activity against *E. coli* for several months (data not shown).

CONCLUSIONS

Nano-scale inorganic materials, i.e., organo-modified MMT clays, are designed as functional carriers for carvacrol. Different MMT clays are pretreated with carvacrol to allow intercalation of the oil molecules in between the clay galleries and enhanced carvacrol thermal stability, confirmed by XRD and TGA studies,

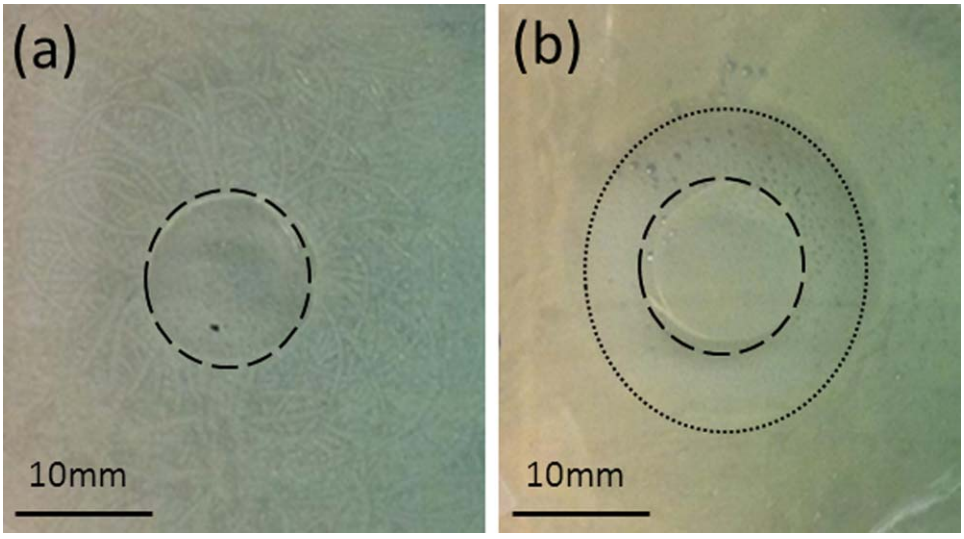

Figure 5. Optical image of the zone of inhibition for *E. coli* for: (a) Control neat LDPE film; (b) LDPE/(Cloisite C15A/carvacrol) film, depicting a clear inhibition zone around the polymer film. The film margins and inhibition zone are marked for clarity. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

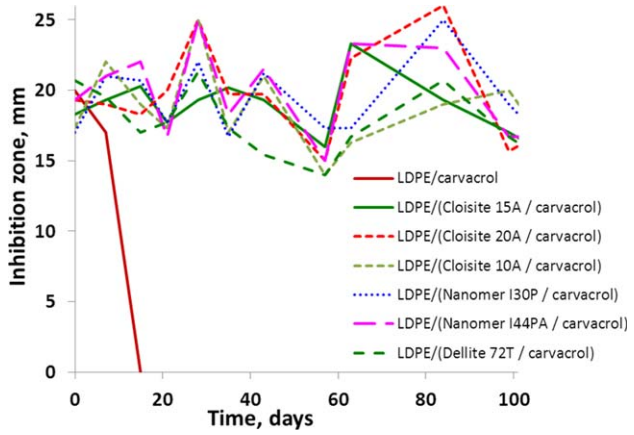

Figure 6. Antimicrobial activity of melt-compounded LDPE/carvacrol and LDPE/(clay/carvacrol) films, measured by the inhibition zone method as a function of film storage time. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

respectively. These clay/carvacrol hybrids are incorporated within LDPE by melt compounding processes and addition of a compatibilizer (PE-grafted MA) is investigated as well. The resulting films are characterized in terms of their nanostructure and thermal properties and compared to films prepared by direct addition of neat carvacrol to the LDPE matrix. All LDPE/(clay/carvacrol) films exhibit significantly higher carvacrol content and enhanced thermal stability in comparison to the reference LDPE/carvacrol films. Thus, these results confirm that the clay platelets function as active carriers of the volatile carvacrol and their role in preserving the oil molecules during high-temperature processes. The compatibilizer affects the dispersion level of clay/carvacrol hybrids within the LDPE; however, this in turn reduces the carvacrol confinement within the clay galleries. The LDPE/(clay/carvacrol) nanocomposites exhibit excellent and prolonged antimicrobial activity against *E. coli* bacteria, while LDPE/carvacrol films lost their antimicrobial functions within several days. To the best of our knowledge, this is the first study reporting prolonged antimicrobial potency of polyolefines/clay nanocomposites. This work demonstrates the vital role of the clay platelets in preserving the antimicrobial properties of the films and their function as active carriers of the volatile carvacrol molecules.

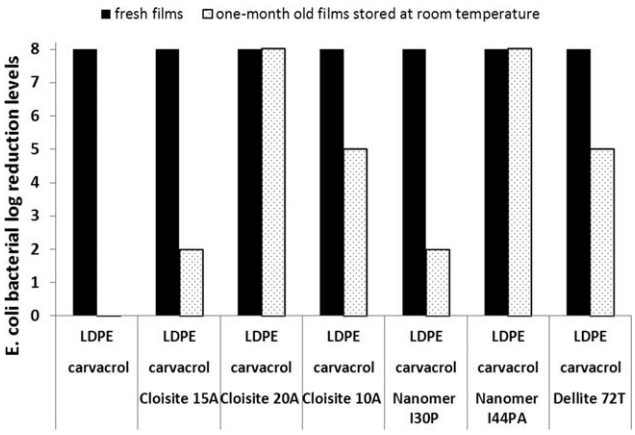

Figure 7. Antimicrobial activity of melt-compounded LDPE/carvacrol and LDPE/(clay/carvacrol) films, measured by inoculating the films with *E. coli* bacteria suspensions (10^8 CFU/mL, for 18–24 hr, at 37°C), after which CFU are counted and log reduction is calculated in comparison to control growth of *E. coli*. Results are presented for both fresh and a month old films (stored at room temperature).

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