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Antibacterial polymer composites based on low-density polyethylene and essential oils immobilized on various solid carriers

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ABSTRACT: In this study, we investigated the antibacterial modification of polymers with biologically active substances in essential oils [EOs; linalool, 4-allylanisole (ALY), and *trans*-anethole]. These compounds were thermoplastically incorporated into a low-density polyethylene matrix via solid inert carriers [wood flour (WF) and talc and molecular sieves] with physically immobilized EOs. The concentrations of the antibacterial modifiers on the carriers and in the resulting composites were determined with three chromatographic techniques (gas chromatography with mass spectrometry, pyrolysis and gas chromatography with mass spectrometry, and high-performance liquid chromatography). The effects of such modifications to the mechanical properties of the prepared composites were studied by stress–strain analysis. Interactions on the polymer matrix carriers were observed by scanning electron microscopy. The prepared composites were also tested for antibacterial activity against both Gram-negative and Gram-positive bacterial strains. The highest efficiency of isothermal immobilization was found for systems consisting of ALY and WF. This finding was in accordance with microbiological studies. The phase of immobilizing the EOs did not influence the mechanical properties of the studied composites. © 2015 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2015**, *132*, 42816.

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INTRODUCTION

Polymers are common in everyday life because of their superior physicochemical properties.[1] Widely used forms include polyolefins (polyethylene, polypropylene, and their copolymers) because of their low cost, great flexibility, good impact strength, oil and chemical resistance, high transparency, ease of manufacture, ability to be heat-sealed, and ease on which they can be printed.[2,3] Increasing demands on polymer materials have led to further development. Recently, the most emphasis has been put on the development of polymer composites that exhibit a required active function.[4] A polymer with resistance to microbial colonization is one such example of this active material function. The microbial contamination of food, cosmetic products, or medical devices is of great importance both economically and environmentally. An antibacterial polymer is a system consisting of a polymer matrix and an antibacterial agent that inhibits the growth of targeted microorganisms.[2] The antibacterial properties of the composite can be achieved by the direct incorporation of the antibacterial agent in the polymer matrix or through the immobilization of the antimicrobial agents on an inert carrier and their subsequent assimilation into the polymer. The resulting composites are now used in food packaging, medical devices, cosmetics, textile fibers, construction materials, and so on.

Antibacterial agents that are applicable as modifiers of polymer matrices include natural or synthetic-based substances. The past few decades have witnessed the development of various synthetic antibacterial agents for the antimicrobial modification of polymer materials.[5] Synthetic antibacterial agents used to modify low-density polyethylene (LDPE) and provide antibacterial activity against a wide range of microorganisms include organic acids (benzoic acid, sorbic acid, and propionic acid), acid anhydrides (benzoic anhydride and propionic anhydride), chelating agents (ethylenediaminetetraacetic acid), sorbates and

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

propionates, sanitizers (triclosan), parabens (ethyl paraben and propyl paraben), fungicides (benomyl and imazalil), and various metals, for example, silver (silver zeolite, silver nitrate).[5–7]

However, in recent years, because of great consumer awareness and concern regarding synthetic antibacterial agents, composites with natural compounds have gained potential.[8,9] The primary natural compounds used to modify LDPE include essential oils (EOs) derived from plants (e.g., basil, thyme, oregano, cinnamon, clove), enzymes obtained from animal sources (lysozyme), and bacteriocines from microbial sources (nisin, lacticin).[10,11] The active components of EO [e.g., linalool (LIN), methyl chavicol, thymol, and carvacrol] display a wide spectrum of antimicrobial activities exceeding many microorganisms; examples of the former include Gram-negative and Gram-positive bacteria,[12–15] forms of yeast,[11,16] and mold.[17,18]

It has been already reported that the natural antibacterial components of basil [basil EOs primarily contain LIN and 4-allylanisole (ALY) as the active volatile components responsible for their antibacterial activity] can be incorporated into LDPE-based polymers and retain their inhibitory effect against microbial growth (*Staphylococcus aureus, Listeria innocua, Escherichia coli,* and *Saccharomyces cerevisiae*) on culture media and on the surface of cheddar cheese. A preblended master batch of an ethylene vinyl acetate powder containing LIN or ALY has successfully been mixed with virgin LDPE pellets and manufactured into films with the same extruder.[11] It has also been demonstrated that LIN coated onto LDPE and nylon films exhibited inhibitory activity against the growth of *E. coli* in a liquid culture and on cheddar cheese.[9]

However, many antibacterial agents are not easily incorporated into polymer matrices because of their volatility. For example, the effectiveness of the antibacterial modification of common polymer matrices with biologically active substances (BASs) of EO is low; hence, this modification requires special and expensive technologies. This drawback can be solved by the immobilization of antibacterial agents on inert carriers commonly used as fillers of plastics.[19–21] For instance, wood plastic composites are becoming important materials in industry, as they are a sustainable resource and are low in cost and recyclable. Numerous studies have been carried out on the influences of forms of wood flour (WF) incorporated in plastic matrices.[20,22] Talc (TC) has been used for many years as an appealing filler in a wide range of industries and in products such as pharmaceuticals, polymers, paint, lubricants, ceramics, and cosmetics.[23,24]

In this study, we focused on preparing and characterizing LDPE-based antibacterial composites with BAS [LIN, ALY and *trans*-anethole (ANE)] immobilized on three different inert carriers [molecular sieves (MSs), TC, and WF]. To the best of the authors' knowledge, research on the technology required for the immobilization of BAS on these inert carriers has never previously been published.

The main objective of this study was to find an interrelationship between the type of filler, BAS, and the resulting characteristics of the composite properties, primarily antibacterial ones. Additionally, the morphological characteristics and mechanical properties, such as the tensile strength, strain at break, and Young's modulus, were observed via scanning electron microscopy (SEM). Furthermore, emphasis was put on the quantitative analysis of BAS. This was measured with three techniques: pyrolysis and gas chromatography with mass spectrometry (Py/GC/MS), gas chromatography with mass spectrometry (GC/MS), and high-performance liquid chromatography (HPLC).

EXPERIMENTAL

Materials

LDPE (BRALEN RB 03-23) was produced by SLOVNAFT, a. s. (Bratislava, Slovak Republic). LIN (97%, CAS: 78-70-6), ALY

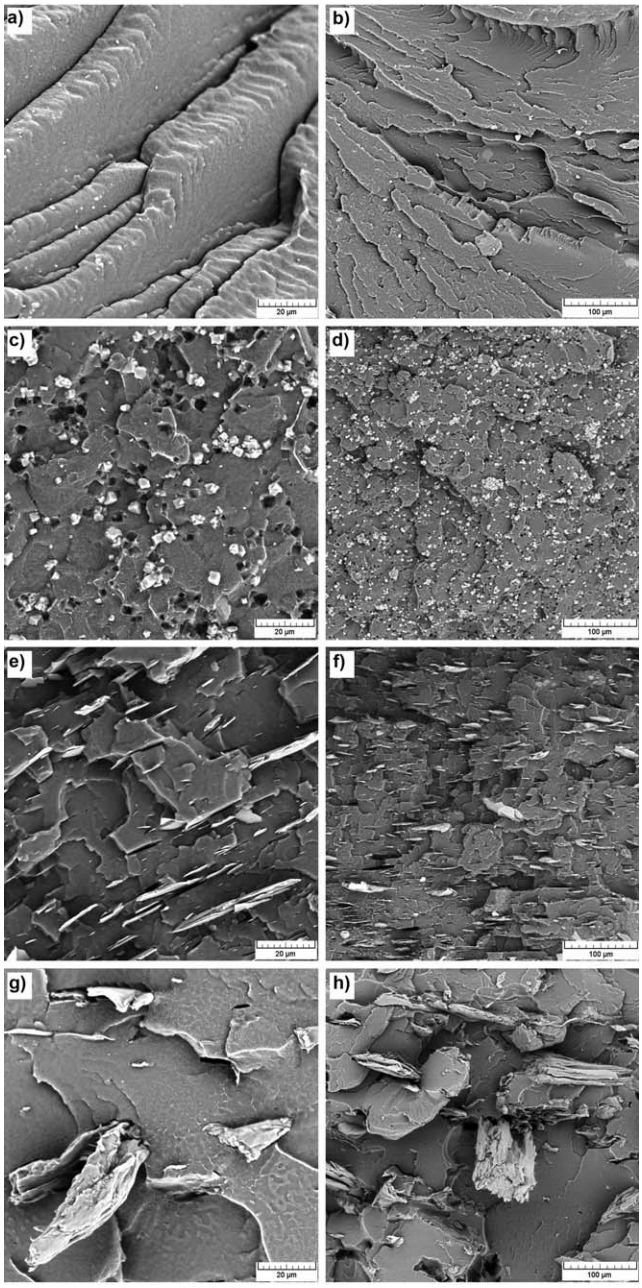

Figure 1. SEM images of the LDPE and LDPE composite samples at various resolutions: (a,b) pure LDPE, (c,d) LDPE/MS/20, (e,f) LDPE/TC/20, and (g,h) LDPE/WF/20.

Table I. Mechanical Properties of the Composites

Sample	Additive concentration (%)	Young's modulus (MPa)	Tensile strength (MPa)	Strain at break (%)
LDPE	0	217/16	16.7/1.3	238/23
LDPE/MS	5	232/27	14.1/1.4	237/15
	10	251/22	13.4/1.2	227/22
	15	263/23	12.6/0.7	197/19
	20	324/24	10.6/0.8	192/12
LDPE/TC	5	243/26	13.8/1.0	218/20
	10	272/27	8.8/0.7	187/21
	15	318/23	8.4/0.6	168/11
	20	428/33	8.4/0.5	145/27
LDPE/WF	5	305/27	9.5/0.5	132/15
	10	352/24	8.2/0.5	39/4
	15	365/46	8.2/0.7	23/2
	20	410/23	8.7/0.9	12/1

[a]The values are shown as averages/standard deviations.

(98%, CAS: 140-67-0), ANE (99%, CAS: 4180-23-8), and MSs (4Å (CAS: 70955-01-0) were obtained from Fluka and Sigma Aldrich. TC-Ph Eur quality (CAS: 14807-96-6) was supplied by IPL Lukes (Uhersky Brod, Czech Republic). WF (spruce) was sourced independently. A fraction, with particle sizes ranging from 75 to 126 μm, was used in this study. The bacterial species, *S. aureus* (4516) and *E. coli* (4517) were obtained from the Czech Collection of Microorganisms, Masaryk University (Brno, Czech Republic). The media required for the microbiological studies (1% w/w peptone nutrient broth, plate count agar, soybean casein digest broth with lecithin and Tween 80) were purchased from HiMedia Laboratories Pvt., Ltd. (India).

Sample Preparation

First, the carriers were activated under isothermal conditions (MSs at 300°C for 16 h, TC at 220°C for 16 h, and WF at 40°C for 4 h). Afterward, BAS was immobilized onto the carriers by an isothermal process (25°C) in a closed glass flask containing the carriers and BAS in a ratio of mix equaling 1 : 1 w/v. Any unabsorbed BAS was evaporated for 24 h at various temperatures (LIN at 50°C, ALY at 50°C, and ANE at 75°C).

Samples of LDPE with various concentrations of additives (BAS immobilized on carriers) were prepared by a thermoplastic process in the following way: the virgin LDPE pellets were compounded with additives at concentrations of 0, 5, 10, 15, and 20 wt % in two-roll mills (Labtech, Ltd., Thailand) for 8 min. The temperatures of the rolls were set to 155 and 135°C, respectively. Then, the obtained products were compression-molded at 140°C for 5 min in a manual press into a film (up to 1 mm thick) and subsequently cooled under the pressure of 10 MPa for 5 min. A two-roll mill compounding technique was selected to prevent high shear stress and the subsequent dissipation of mechanical energy that could influence the immobilized highly volatile BAS. The prepared samples were homogeneous in all cases.

Samples were designated as LDPE/carrier/BAS, where the carrier is the type of inert carrier (MS, TC, or WF) and BAS is LIN, ALY, or ANE.

Characterization

SEM. SEM was carried out on the VEGAII LMU (TESCAN, Czech Republic) operating in a high-vacuum/secondary electron imaging mode at an accelerating voltage of 10 kV. Samples for performing We prepared the SEM analysis by breaking the test specimen in liquid nitrogen and then scanning the fractured surfaces.

Stress–Strain Analysis. The mechanical properties (Young's modulus, tensile strength, and strain at break) were tested with the aid of a tensile testing machine (M350-5CT, Testometric Co., Ltd., United Kingdom) according to the ČSN EN ISO 527-1-3:1997 standard at 23°C. The speed of the moving clamp was 100 mm/min. The specimens were conditioned at 50% relative humidity at 23°C for 88 h to reach equilibrium before further investigation. The values were calculated as averages determined over 10 specimens for each plastic film.

Additive Quantification. The amounts of LIN, ALY, or ANE in both carriers and LDPE composites were arrived at via three techniques: (1) GC/MS, (2) Py/GC/MS, and (3) HPLC. Initially, 5 g of sample was extracted by a Soxhlet extractor in 250 mL of methanol for 18 h. This extract was used for GC/MS and HPLC analysis.

GC/MS was performed on a Shimadzu GCMS-QP2010 Ultra device equipped with a fused silica capillary column (SLB-5 MS, 30 m $\times$ 0.25 mm, film thickness = 0.25 μm, Supelco). Helium was used as the carrier gas at a flow rate of 1.12 mL/min. The injector temperature was maintained at 200°C; the volume of the injected sample was 1 μL. Split injection was conducted at a split ratio of 1 : 100. The column temperature was initially held at 70°C for 1 min and then increased from 70 to 180°C at a rate of 10°C/min and held at 180°C for 7 min. The ion source was set at

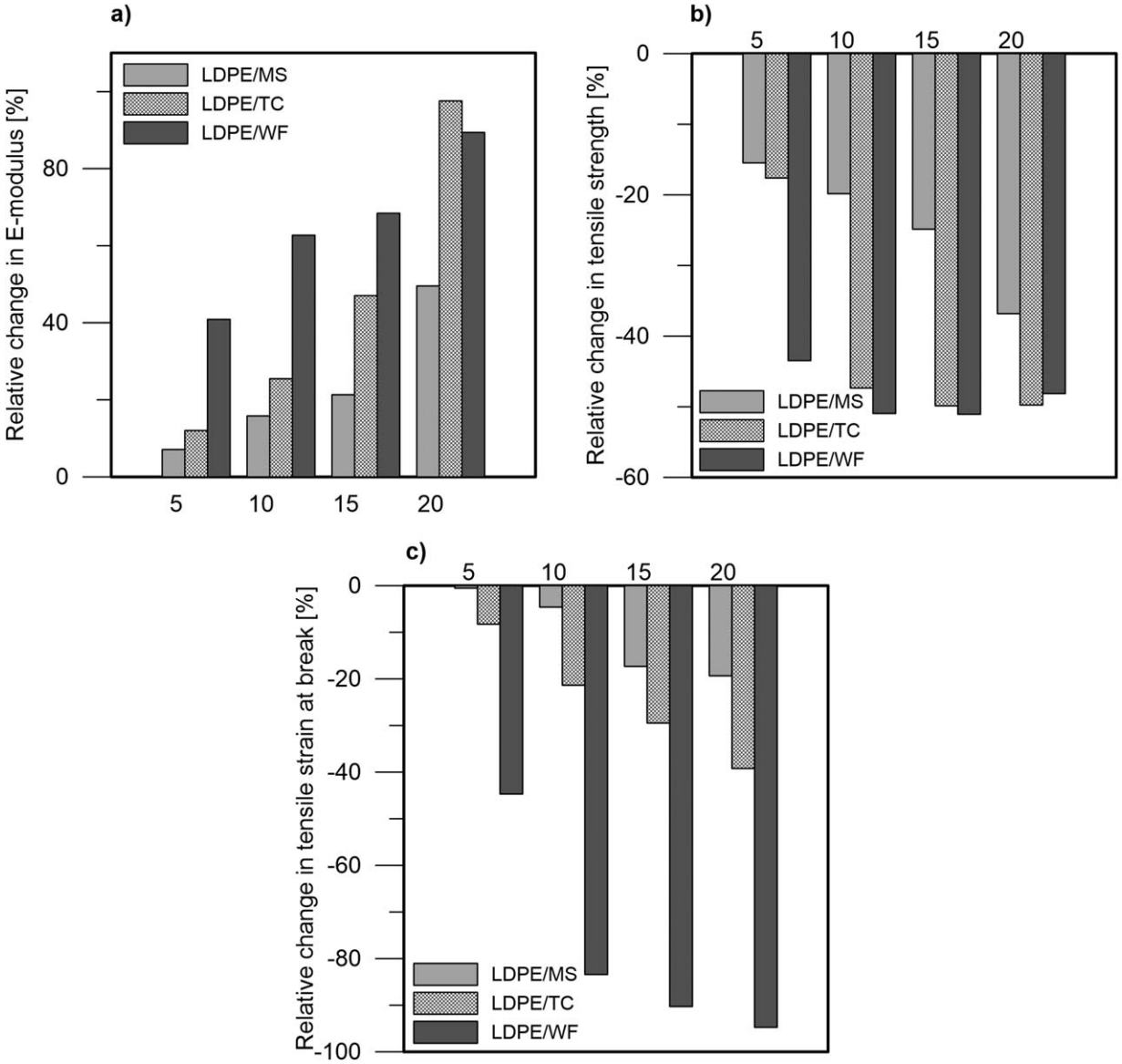

Figure 2. Relative changes in the mechanical properties of the prepared composites in comparison with the unmodified LDPE: (a) Youngs modulus, (b) tensile strength, and (c) strain at break.

200°C. The scan range was 35–350 *m*/*z* (mass/charge). The GC/MS interface temperature was set at 300°C.

HPLC analysis was carried out as a complementary technique for LIN, ALY, and ANE determination in the extracts of the LDPE composites. HPLC analysis was performed on a Thermo Fischer Scientific Accela HPLC modular system (Waltham, MA) equipped with an Accela 600 quarter pump and an Accela photodiode array detector. Chromatographic separation was carried out on a Thermo Scientific Accucore PFP analytical column (2.1 × 150 mm^2, 2.6 μm). The mobile phase was acetonitrile–water (55 : 45 v/v) with a flow rate of 400 μL/min, and the wavelength of detection was set at 210 nm. The column temperature was maintained at 22°C, and the injection volume for each sample and standard solution was 3 μL.

Unextracted samples were used for Py/GC/MS analysis. Py/GC/MS analysis was conducted via a Multi-Shot Pyrolyzer EGA/PY-3030D connected to the Shimadzu GCMS-QP2010 Ultra device. In this case, the sample was pyrolytically decomposed, and evolved gas analysis was carried out by the GC/MS technique. Samples of approximately 3 mg were weighed into a pyrolysis cup. The samples were pyrolyzed at 300°C; the pyrolysis time was fixed at 3 min. Separation was carried out on an Ultra Alloy-PY2 capillary column (30 m × 0.25 mm × 0.5 μm). The GC injector temperature was 300°C, and the interface between the pyroprobe and GC was maintained at 300°C. The mass spectrometry temperature was maintained at 250°C, and it scanned over a range of 33–450 *m*/*z*. The oven temperature of the GC was held at 60°C for 3 min; this was followed by continuous heating (6°C/min) to 200°C. Then, the final temperature was held for 10 min to ensure that no heavy molecules remained in the column. Helium was used as a carrier gas with a column flow of 1.01 mL/min, and the split injector ratio equaled 1 : 100.

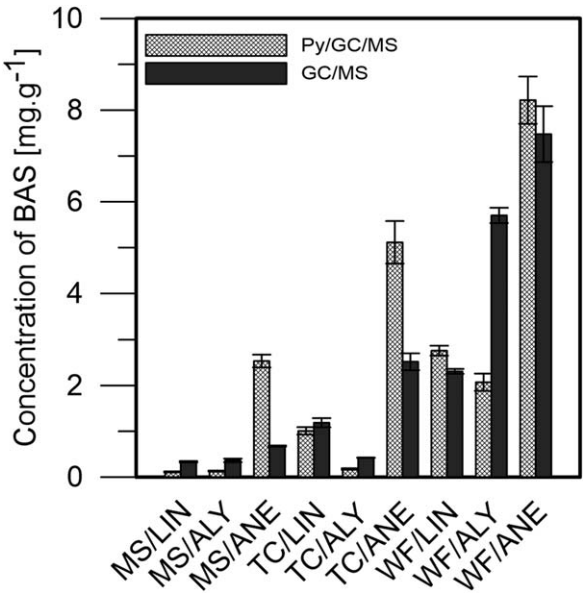

Figure 3. Concentration of BAS on inert carriers after the immobilization process.

The LIN, ALY, and ANE contents of the samples were calculated from standard calibration dependences.

All of the chromatography results represent averages from three independent analyses.

Study of the Antibacterial Properties. Testing was carried out with a procedure based on ISO 22196:2007. The bacteria used in this study were *S. aureus* and *E. coli*. For these measurements, only the LDPE/inert carrier/BAS 20 composites were used. Bacterial suspensions were prepared at concentrations of 5.6×10^6 and 2.8×10^7 cfu/mL for *E. coli* and *S. aureus*, respectively. The dimensions of each specimen were 25 x 25 mm^2, and the cover polyethylene film dimensions were 20×20 mm^2.

The effectiveness of the plastic films was evaluated according to the following equation:

$$R_a = (U_t - U_0) - (A_t - U_0) = U_t - A_t \quad (1)$$

where R_a is the antibacterial activity, U_0 is the average of the common logarithm for the viable bacteria in cells·(cm^{-2}) recovered from the untreated test specimens (pure LDPE) immediately after inoculation, U_t is the average of the common logarithm for the viable bacteria in cells·(cm^{-2}) recovered from untreated test specimens after 24 h, and A_t is the average of the common logarithm for the viable bacteria in cells·(cm^{-2}) recovered from treated test specimens after 24 h.

RESULTS AND DISCUSSION

SEM

Figure 1 presents SEM images of the pure LDPE sample and its prepared composites. The pure LDPE [Figure 1(a,b)] had a homogeneous morphology and fractured surface. Three different carriers/fillers of differing shapes and properties were used in this studies. We found that MS was cubic in shape [Figure 1(c,d)] and ranged in size from 5 to 10 μm. In the case of TC, plate-type particles [Figure 1(e,f)] were observed with lengths of approximately 1 μm to over 40 μm. Figure 1(c–f) indicates that TC and MS were uniformly dispersed in the LDPE matrix without obvious aggregates. Nevertheless, some voids were identified in all of the LDPE/TC or LDPE/MS composites. These voids could be induced by the debonding of the TC or MS particles from the LDPE matrix as a consequence of their poor adhesion. These findings were in agreement with the results of other authors.[25,26] We observed that the particles of WF appeared to be irregular in shape. Distinct gaps between the WF and LDPE matrix are clearly shown in Figure 1(g,h). This phenomenon indicates that there was poor adhesion between the two phases; this might have been due to the low dispersion of hydrophilic WF in the nonpolar LDPE. Similar results have been reported in many research papers.[22,27,28]

Stress–Strain Analysis

Table I summarizes values for the mechanical properties of the prepared composites. As shown, the standard deviation was below 10% in all cases.

A complex stress–strain analysis of the prepared composites (without and with immobilized BAS on fillers used) was done. The results reveal that there was no effect of the BAS (immobilized on the MS, TC, or WF) presence on the mechanical properties of the composites. Thus, only the composites with immobilized BAS will be discussed further. Moreover, the direct immobilization of highly volatile BAS into the LDPE matrix would be difficult and ineffective. The effect of various carriers is expressed as a relative change (in comparison with unmodified LDPE) in the mechanical properties in Figure 2. The unmodified LDPE was characterized by a lower Young's modulus value and higher tensile strength and strain at break values than the modified composites. All of the composites with various carriers showed enhanced Young's modulus values throughout the concentration range. However, we observed that increasing the concentration of fillers on the composites caused a reduction in the values for the tensile strength and strain at break. A similar result was observed herein when the effect of TC on the mechanical properties of high-density polyethylene composites was investigated. An increase in the Young's modulus was observed alongside a rise in the TC content. The tensile strength value decreased in conjunction with an increase in the filler content in the composite.[29]

The most significant increase in the Young's modulus was observed for the system LDPE with a content of 20% TC. Composites with MS and TC exhibited similar mechanical properties; this was caused by their physical and chemical similarity. The rise in Young's modulus in the composites containing WF corresponded with studies that have investigated the properties of composites made from linear low-density polyethylene (LLDPE) reinforced with WF.[30] The LDPE/WF composites displayed significant drops in the tensile strength and strain at break values; this was likely to have been caused by the high particle size and incompatibility of WF with the polymer matrix, as reported in many studies.[31] We affirmed that the resulting mechanical properties were set by the compatibility of the filler and the polymer matrix. MS and TC were more

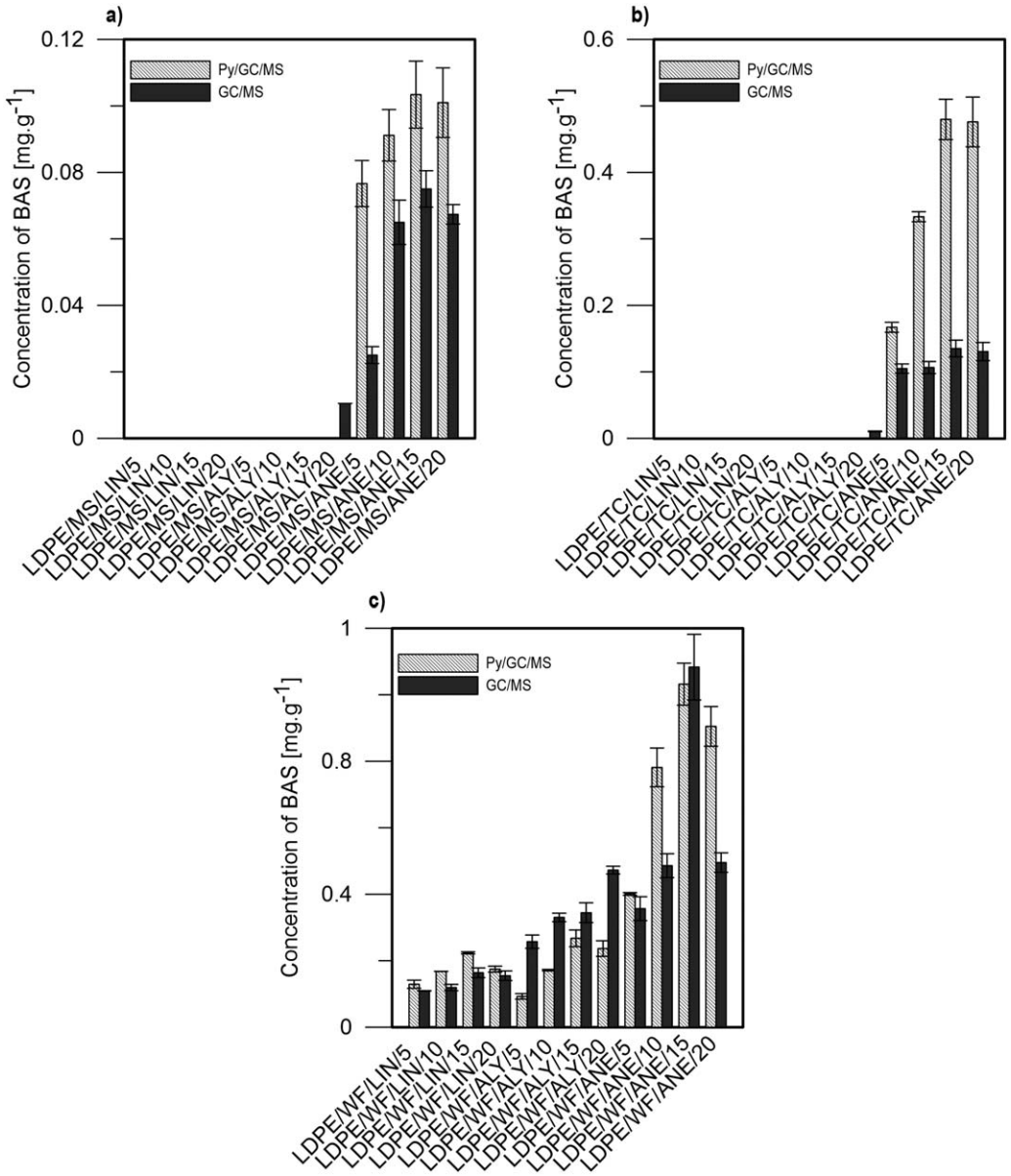

Figure 4. Concentration of BAS in the LDPE composites with a 20 wt % concentration of (a) MS, (b) TC, and (c) WF.

compatible with the polymer matrix than WF; this was also confirmed by SEM.

EO Quantification

Figure 3 presents the concentrations of BAS in various inert carriers. As shown in Figure 3, data were obtained by two methods: (1) the direct analysis of solid samples by Py/GC/MS and (2) GC/MS analysis of the extracted sample. The concentration of BAS was measured in the range from 0.1 mg/g to a maximum of 8.3 mg/g. The resulting concentrations of BAS measured by Py/GC/MS were comparable or higher than GC/MS, as these two methods had different yields. The highest differences in the concentrations of BAS measured by both methods were observed in samples of W/ALY, TC/ANE, and MS/ANE.

The highest concentration of all of the BAS was identified in the case of WF, whereas the lowest concentration of BAS was recognized in MS. According to this study, the solute polarity is a predominant controlling factor that influences sorption. The authors compared LIN and limonene; because of the higher polarity of LIN, it was less well adsorbed by nonpolar polymers such as polyethylene.[32] Other studies have also recognized that sorption by nonpolar polymers increases in conjunction with the hydrophobicity of the carrier.[33,34] Herein, confirmation was made that BAS absorbed more easily in inert carriers of similar polarity. The concentration of all of the BAS in hydrophilic WF was higher than in hydrophobic MS and TC.

Figure 4(a–c) shows the concentration of BAS in LDPE determined by two techniques (GC/MS and Py/GC/MS). The residual BAS concentration in LDPE registered from approximately 0 to a maximum

of 1.1 mg/g. Composites with WF [Figure 4(c)] were detected to contain the highest concentration of each selected BAS.

An interesting comparison of BAS determination with GC-based techniques (GC/MS and Py/GC/MS) with HPLC is presented in the Supporting Information (Figure S1).

The presence of LIN and ALY in all of the inert carriers and composites was generally low; this was probably caused by the higher volatility at low temperatures. The opposite effect was identified in the case of ANE, which exhibited the greatest ability to bind with all of the carriers. ANE was also the only BAS that was detected in composites containing MS and TC after incorporation into LDPE. The likely reason for this phenomenon was that ANE possessed a higher boiling point than LIN and ALY. This notion corresponded with research in which an observation was made that samples of BAS with higher boiling points were more capable of condensing and remaining within the polymer matrix.[35] In addition, this explanation for the highest concentration of ANE in inert carriers and composites was supported by the fact that ANE had a higher cohesive energy density (511 J/cm^3) than LIN (273 J/cm^3) and ALY (344 J/cm^3). These findings were in agreement with the study herein, where two similar terpenes were compared (carvone and limonene) with different polarities. It has been shown that the less polar limonene is not only absorbed at a faster rate but also diffuses more rapidly; this is probably due to its lesser cohesive forces.[36]

The cohesive energy density of BAS was calculated according to the following equation:[37]

$$e_{coh} = \left(H_{vap} - RT\right) V_m^{-1} \quad (2)$$

where e_{coh} is the cohesive energy density of BAS (J/cm^3), H_{vap} is the enthalpy of vaporization of BAS (J/mol), R is the ideal gas constant (J/K.mol), T is thermodynamic temperature (K), and V_m is the molar volume of BAS (cm^3/mol).

However, according to the results of antibacterial tests, it was not noticeable that ANE demonstrated the highest inhibitory activity against the selected bacteria, even though its concentration was higher than that of ALY. These conclusions corresponded with a study that examined the antibacterial activity of compounds of EO against standard tested strains and isolated strains of microorganisms expressed as minimum inhibitory concentrations. We found that ALY exhibited more antibacterial activity than ANE and LIN.[38]

Studies of the Antibacterial Properties

The antibacterial activity of the prepared samples was studied via a procedure based on ISO 22196:2007. The samples were tested against representatives of both Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacterial strains. The pure LDPE showed no antibacterial activity in both cases. The same results were observed for all of the BAS-free LDPE composites (LDPE/MS, LDPE/TC, and LDPE/WF). It is known that both MS and TC are biologically inert inactive materials.[39,40] On the other hand, WF is a suitable substrate for a microbial colonization under certain conditions, but it does not possess any antimicrobial activity.[41]

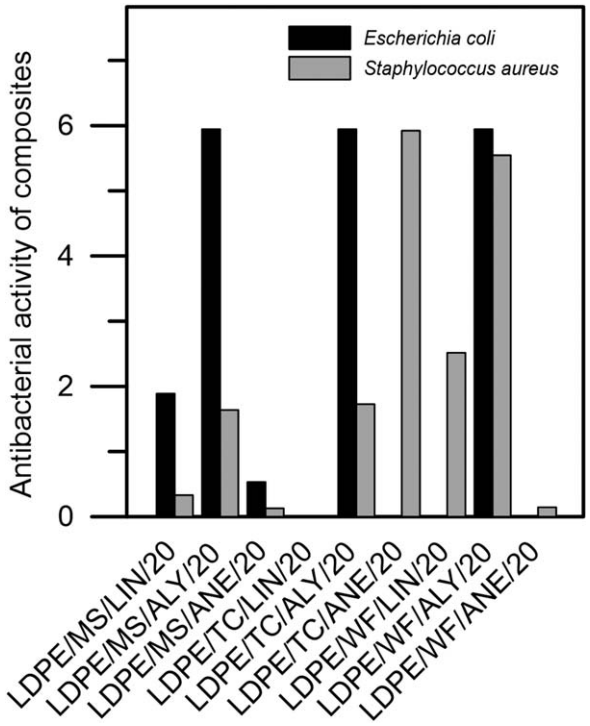

Figure 5. Antibacterial activity of the prepared composites against *S. aureus* and *E. coli*.

The results from the antimicrobial assessment are given in Figure 5.

We observed that the prepared composites were better at inhibiting the growth of the Gram-negative strain, with the exception of LDPE/WF/ANE, LDPE/WF/LIN, and LDPE/TC/ANE. Similar results were reported by Koga *et al.*,[42] who studied the bactericidal activities of EOs from basil against a range of bacteria. In contrast, Joshi[43] investigated the antibacterial activity of EOs from basil against various Gram-positive and Gram-negative bacteria. He reported that the Gram-negative bacteria were more resistant to such EOs than the Gram-positive ones. However, this particular study applied different representatives of Gram-positive and Gram-negative strains than in the research detailed herein and in the work by Koga *et al.*[42]

The LDPE/TC/ALY, LDPE/WF/ALY, and LDPE/MS/ALY composites proved the most effective against the growth of *E. coli*. It might have been that these composites exhibited antimicrobial activity even at concentrations below 20%. Nevertheless, the composites containing additives (TC/LIN,WF/LIN, TC/ANE, and WF/ANE) displayed no antibacterial activity against the growth of *E. coli*.

We observed that the LDPE/TC/ANE composite showed the greatest inhibitory effect against Gram-positive bacteria. In contrast, *S. aureus* was even not affected by LDPE/TC/LIN because of the low concentration caused by the ineffective immobilization of LIN, as shown in Figure 4.

Generally, R_a was observed at its height in composites containing ALY. These results were ascribed to its physical properties. Components with phenolic structures, including ALY, were

highly active against the test microorganisms, despite their relatively low solubility in water.[44] The antimicrobial action of phenolic compounds was related to the inactivation of cellular enzymes, which depended on the rate of penetration of the substance into the cell or was caused by changes in the membrane permeability. Increased membrane permeability is a major factor in the mechanism of antimicrobial action, where compounds may disrupt membranes and cause a loss of cellular integrity and eventual cell death.[45] In accordance with a study performed by Suppakul *et al.*,[11] the LDPE/ALY composites exhibited a greater efficiency of inhibition against *E. coli* than the composites with LIN.

CONCLUSIONS

Natural BASs (LIN, 4-allylanisol and *trans*-anethol) were thermoplastically incorporated into the matrix of LDPE. The crucial step of incorporating these substances was based on their immobilization on various carriers (WF, MSs, and TC). The antibacterial activity investigated, in accordance with ISO 22196, showed that the Gram-negative *E. coli* was more inhibited than the Gram-positive *S. aureus*. The greatest antimicrobial activity was exhibited by composites with ALY immobilized on WF, although promising results were observed even for the MSs and TC. The results from SEM and the mechanical properties of composites confirmed little compatibility existed between WF and the polymer matrix.

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