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Release of Thymol from Poly(lactic acid)-Based Antimicrobial Films Containing Kenaf Fibres as Natural Filler

Intan S. M. A. Tawakkal[1], Marlene J. Cran[2*] and Stephen W. Bigger[2]

1. College of Engineering and Science, Victoria University, PO Box 14428, Melbourne, 8001, Australia.

2. Institute for Sustainability and Innovation, Victoria University, PO Box 14428, Melbourne, 8001, Australia

Corresponding author: marlene.cran@vu.edu.au, ph. +61 3 9919 7642, fax. +61 3 9919 7696

Abstract

The migration of thymol, a natural antimicrobial (AM) substance, from poly(lactic acid) (PLA) films containing 300 g/kg kenaf fibres into food simulants is reported. Neat PLA and PLA/kenaf films containing 100 g/kg thymol were prepared *via* melt blending and heat pressing and were placed in contact with 150 mL/L and 950 mL/L ethanol/water mixtures at different temperatures. First-order kinetics, diffusion modelling and Fick's law modelling were used to describe the release. The release rate of thymol into 950 mL/L ethanol/water at different temperatures displays Fickian behavior with diffusion coefficient values between 1 and 100 × 10^{-11} m^2 s^{-1} with close to 100% of thymol being released. The release rate of thymol is temperature dependent and is affected by the percentage of ethanol in the simulant. In the case of neat PLA and PLA/kenaf films, a faster release occurred in 950 mL/L ethanol/water than in 150 mL/L ethanol/water with the composite film exhibiting a higher diffusion coefficient in each case.

Keywords: active packaging; antimicrobial; thymol; migration; poly(lactic acid).

1 Introduction

Controlled release systems in food packaging have experienced considerable growth recently due to developments in active packaging concepts such as the integration of antioxidant (AOX) and/or antimicrobial (AM) substances into packages in order to improve the quality and safety of food products. In these systems, low molecular mass compounds and/or substances are released from the package in a slow and controlled manner to maintain an adequate concentration of the substance in the packed food for a certain period of time. The release of substances that involve migration is the result of diffusion, dissolution and equilibrium processes (Crank, 1979). There are various factors that influence the migration of a substance from the packaging material including the film fabrication method, the volatility and polarity of the substance, the chemical interaction between the substance and polymer chains, hydrophobicity and hydrophilicity of the polymer as well as food properties and composition (Suppakul, Miltz, Sonneveld, & Bigger, 2003).

Poly(lactic acid) (PLA) is a polyester synthesized from the renewable, bio-derived, monomer lactic acid and can be used as AM films and/or membranes for a different range of applications (Auras, Harte, & Selke, 2004; Karami, Rezaeian, Zahedi, & Abdollahi, 2013). This GRAS (Generally Recognized As Safe) grade polymer can be used in contact with food and fabricated *via* conventional processing procedures (Jamshidian, Tehrany, Imran, Jacquot, & Desobry, 2010). The use of fillers in combination with PLA has been widely studied with aims to improve physicomechanical properties, reduce production costs and enhance biodegradability. Naturally derived additives or fillers such as starch and cellulose can be combined with PLA and other active agents such as AM and AOX compounds. For example, Hwang et al. (2013) studied the migration of α-tocopherol and resveratrol from poly(L-lactic acid) (PLLA)/starch blend films into ethanol and found that the neat PLLA containing α-tocopherol had a lower release rate than the PLLA/starch blend films at 43°C in 100% ethanol simulant with diffusivity coefficients of 89×10^{-11} and 282×10^{-11} cm^2 s^{-1} respectively. A similar trend was observed for resveratrol, a non-volatile AOX compound with diffusion rate of 25×10^{-11} cm^2 s^{-1} in neat PLLA and 40×10^{-11} cm^2 s^{-1} in the PLA/starch films. Fortunati et al. (2012) prepared PLA AM films with 50 g/kg microcrystalline cellulose (MCC) and 10 g/kg silver nanoparticles by extrusion and injection molding techniques. The PLA films with silver nanoparticles and MCC had greater AM activity against *Escherichia coli* due to the presence of MCC; however, it had less AM activity than PLA films with silver nanoparticles. Although there are examples of the controlled release of AM and AOX substances from PLA materials (Busolo & Lagaron, 2013; Fernandez, Soriano, Hernandez-Munoz, & Gavara, 2010; Hwang et al., 2013; Iniguez-Franco et al., 2012; Llana-Ruiz-Cabello et al., 2015), few reports have combined PLA with natural fibres in order to control the release of active substances from composite films.

Antimicrobial packaging systems can be categorised as either migratory or non-migratory systems. In the former, AM substances migrate from the packaging material into the headspace of the package and onto the food surface, whereas in the latter, AM substances are immobilised onto the packaging material which is placed in direct contact with the foodstuff to facilitate its activity (Han, 2003). The integration of PLA with AM substances has been investigated by a number of researchers (Del Nobile et al., 2009; Jin, 2010; Qin et al., 2015; Rhim, Hong, & Ha, 2009). Of the reported studies, many have investigated the inhibition of targeted microorganisms with little attention having been devoted to studying the release rate from the active systems.

This might be due to the preponderance of non-volatile and/or immobilised AM substances (e.g. nisin, chitosan, lysozyme and peptide) incorporated into PLA films rather than volatile AM substances (Green, Fulghum, & Nordhaus, 2011; Rhim, 2013; Tawakkal, Cran, Miltz, & Bigger, 2014). Several AM substances have been incorporated directly into polymers including a range of volatile plant extracts such as basil, thymol, linalool, methyl cinnamate and cavacrol (Cran, Rupika, Sonneveld, Miltz, & Bigger, 2010; Del Nobile, Conte, Incoronato, & Panza, 2008; Fernández-Pan, Maté, Gardrat, & Coma, 2015; Rubilar et al., 2013; Suppakul, 2004; Suppakul, Sonneveld, Bigger, & Miltz, 2011; Tawakkal, Cran, & Bigger, 2015).

Thymol, an essential oil extract that has GRAS status, can be used in contact with food products and like other volatile AM substances, the migration of this substance into real food products is complex. In some studies, active AM films containing thymol were evaluated *in vitro* and *in vivo* against a wide spectrum of microorganisms such as bacteria, mould and yeast (Kuorwel, Cran, Sonneveld, Miltz, & Bigger, 2011; Wu et al., 2014). In a recent study by Petchwattana and Naknaen (2015), extruded films of poly(butylene succinate) (PBS) containing thymol demonstrated AM activity against *Escherichia coli* and *Staphylococcus*. Moreover, the release rate of thymol from the PBS films into 950 mL/L ethanol/water was found to be 5.9×10^{-14} m^2 s^{-1}. Ramos, Beltrán, Peltzer, Valente, and Garrigós (2014) reported that the migration of thymol from polypropylene (PP) films into 950 mL/L ethanol at 40°C conformed to Fick's law with a diffusion coefficient of 1.0×10^{-14} m^2 s^{-1}. In general, an AM substance can be released into a food simulant by swelling-controlled release with three main steps involved: (i) the absorption of fluid (penetrant) from the food simulant which leads to the swelling effect of the polymer, (ii) the active substance molecule being dissolved or dispersed in the polymeric matrix and (iii) the active substance migrating to the food simulant (Del Nobile & Conte, 2013).

The quantification of volatile AM substances in active films is an important consideration given that high pressure, shear forces and temperatures are required for film processing. According to Raouche, Mauricio-Iglesias, Peyron, Guillard, and Gontard (2011), a higher temperature of *ca.* 160 to 190°C was needed to process PLA using extrusion which may easily degrade and evaporate the volatile AM substance during thermal processing. The retention of volatile additives such as thymol in PLA film was found to be approximately 70 to 80% after thermal fabrication (Tawakkal, Cran, & Bigger, 2015). Such retention percentages were significantly higher than those found in polyolefin film systems containing similar volatile additives. In comparison, Ramos, Jiménez, Peltzer, and Garrigós (2012) reported much lower retention of thymol and carvacrol (*ca.* 25 to 45% respectively) in PP formulations. The marked reduction in the retention of these additives in polyolefin films upon thermal processing may result from the reduced compatibility between the polymer matrix and the natural additives as well as the processing parameters such as temperature, time and screw rotation (Del Nobile et al., 2009).

There are clearly many examples of the release of a wide range of AM substances from homopolymers such as PLA. However, little attention has been devoted to evaluating the release of AM agents from ternary composite systems, particularly taking into account the possible swelling of polymeric materials immersed in food simulants as well as the effects of natural fillers on the release rate. Moreover, the release of volatile AM substances from polymeric materials also has been restricted to mainly hydrophobic and moderate hydrophilic polymeric matrices (Buonocore, Del Nobile, Panizza, Corbo, & Nicolais, 2003; Herath, 2009). The aim of the current work was therefore to investigate the release of a naturally derived AM agent from a novel ternary system comprised of PLA, kenaf fibers and thymol. In particular, the release of thymol from PLA and PLA/kenaf composite and its diffusion kinetics were studied.

2 Experimental Section

2.1 Materials

Poly(lactic acid) (7001D Ingeo^{TM}; specific gravity 1.24; melting temperature 152.5°C (Tawakkal, Cran, & Bigger, 2014)) was obtained from NatureWorks LLC, USA. Mechanically separated kenaf fibre (bast) was purchased from Ecofibre Industries, Australia. Thymol (T0501, purity of 99.5%) was purchased from Sigma Aldrich Pty. Ltd., Australia. Sodium hydroxide and acetic acid were purchased from Merck Chemicals, Australia. Un-denatured ethanol was purchased from Chem-Supply Pty Ltd., Australia. Isooctane (2,2,4-trimethylpentane, 36006) was purchased from Sigma Aldrich, Australia.

2.2 Production of PLA/Kenaf/Thymol Films

Kenaf fibres were soaked in 0.05 g/mL sodium hydroxide for 2 h at room temperature. The fibers were then filtered and washed with distilled water prior to acid treatment to affect neutralization by adding a few drops of acetic acid. The fibres were then filtered, washed and rinsed with distilled water to remove the acetic acid, the latter being confirmed using a pH meter (inoLab$^{®}$ pH7110, WTW GmbH, Germany). Finally, the fibres were dried overnight in an oven at 105°C. Prior to mixing, PLA resin and kenaf fibres were further dried in an oven at 60°C overnight before mixing with thymol at 100 g/kg concentration.

The film samples were prepared firstly by melt-blending the components in an internal mixer (Haake PolyLab OS, Germany) at 155°C for 8 min and 50 rpm followed by heat pressing, according to a method previously reported by Tawakkal, Cran, and Bigger (2014). The PLA was added to the mixer first and the kenaf and thymol were introduced once the polymer was molten in order to avoid unnecessary loss of thymol. In the current study, a 300 g/kg loading of kenaf fibres was used to produce the PLA/kenaf composite that has moderate flexibility as well as high strength and stiffness compared with unfilled PLA which is more suitable for the production of rigid packaging applications (Tawakkal, Cran, & Bigger, 2014). In a second step, a laboratory press (L0003, IDM Instrument Pty. Ltd., Australia) was used to prepare films. The samples were preheated at 150˚C for 3 min without applying pressure until the material melted, and then pressed at the same temperature for 2 min under a force of 20 kN before quench cooling to 30˚C under pressure. The average thicknesses of the pressed neat PLA and PLA/kenaf films incorporated with thymol were 0.19 ± 0.03 and 0.25 ± 0.05 mm respectively. The film thickness was measured using a hand-held micrometer (Hahn & Kolb, Stuttgart, Germany).

2.3 Quantification of Thymol in PLA and PLA/Kenaf Films

One gram of film sample was cut into pieces (0.25 cm^2) and immersed in a round bottom flask containing 150 mL of isooctane for solid-liquid extraction. Isooctane was used as it promotes the swelling of the polymer and a typical reflux extraction was performed at 100°C for 4-5 h to extract thymol from the films. The quantification was achieved with a gas chromatography (GC) instrument (Varian 8200C_x) equipped with a fused silica capillary column (DB5; 30 m × 0.25 mm i.d.; thickness 0.25 µm; J & W Scientific, USA). The conditions applied in the GC instrument were as follows: injected volume: 1.0 µL, initial column temperature: 80°C; heating rate: 5°C min^{-1} up to 120°C, held at this temperature for an additional 5 min; injector temperature: 250°C; FID detector temperature: 300°C; flow rate: 2 mL min^{-1}; splitting; carrier gas: nitrogen. Standard solutions of thymol in isooctane at concentrations from 0.05 to 1.0 mg mL^{-1} were prepared and used to produce a calibration curve. The experiments were performed in triplicate. The retention of thymol in the neat PLA and PLA/kenaf films following thermal processing was 73% and 62% respectively as reported by Tawakkal, Cran, and Bigger (2015). The loss of thymol observed in the present study for composite films seems to be at an acceptable level compared to petroleum-based (polyolefin) films containing volatile additives.

2.4 Migration of Thymol into Food Simulants

The release of thymol from neat PLA and PLA/kenaf films into aqueous food simulants was studied at different temperatures. The release was examined *via* a total immersion migration test (EC, 1997) using 950 and 150 mL/L ethanol/water. Ethanol is commonly used as food simulant to investigate the migration of AM substances from the PLA matrix. The 950 and 150 mL/L ethanol/water simulants are fatty and aqueous food simulants respectively. The sorption of ethanol by the PLA matrix may lead to the creation of voids and/or swelling of the matrix where it can penetrate the PLA chains and promote the migration of the active substance (Mascheroni, Guillard, Nalin, Mora, & Piergiovanni, 2010). In the case of the 950 mL/L ethanol/water simulant the experiments were performed at 30, 40, 50 and 60°C and for the 150 mL/L ethanol/water simulant experiments were performed at 60, 65, 75 and 83°C. In the latter experiments, the test temperatures were higher than recommended in the standard methods (EC, 1997) in order to accelerate the migration of thymol. Studying the release rates of thymol from the neat PLA and PLA/kenaf films by using fatty and aqueous food simulants is important in order to assess the *in vitro* and *in vivo* AM activity of these systems against targeted microorganisms.

Samples of film weighing *ca.*0.5-0.6 g were immersed in 100 mL of simulant in a three-neck round bottom flask with the ratio of simulant volume per area film being *ca*. 2.7-4.7 mL cm^{-2}. The flask was immersed in an oil bath that was placed on a magnetic stirrer heating plate and the simulant was gently agitated using magnetic stirring at 60 rpm. The flask was connected to a condenser and the stirring speed and temperature were fixed and monitored throughout the experiment. The amount of AM agent released from the films was monitored until equilibrium was attained. A 0.2 mL sample of the simulant solution was collected periodically during the experiment and 1 µL aliquots were injected into the GC. The quantification of thymol in all simulants was performed using the same method and calibration curve described in the previous section. The thymol quantification was performed in triplicate.

2.5 Data Analysis

The migration of thymol from the PLA and PLA/kenaf composite films was analysed using three data analysis treatments: (i) overall kinetics, (ii) diffusion models in accordance with Cran, Rupika, Sonneveld, Miltz, and Bigger (2010) and Kuorwel, Cran, Sonneveld, Miltz, and Bigger (2013) and (iii) Fick's diffusion law model.

2.5.1 Overall Kinetics Analysis

By considering the overall diffusion process to be a single process that obeys first-order kinetics, equations describing the migration of an additive from a polymeric film into simulant with time have been described by Miltz (1987) and Crank (1979). The release of the AM agent into the simulant was initially analysed for its fit to a first order kinetics model. In the case of a first-order system, equation (1) applies:

$$\ln\left(1-\frac{m_t}{m_\infty}\right)=-k_1 t \qquad (1)$$

where m_t is the mass of additive released from the film at time t, m_∞ is the amount of additive released from the film at equilibrium ($t = \infty$) and k_1 is the first-order rate constant. From equation (1), a plot of $\ln(1 - m_t/m_\infty)$ *versus* time should be a straight line with a slope of $-k_1$. The apparent first-order rate constants were calculated using equation (1) and the initial release rates, v_0, of the AM agent were calculated using equation (2) (Kuorwel, Cran, Sonneveld, Miltz, & Bigger, 2013):

$$v_0 = m_\infty k_1 \qquad (2)$$

2.5.2 Diffusion Model

In the diffusion model, the release of the AM agent from the film into the simulant is considered in two stages, namely the short-term and the long-term (Crank, 1979; Miltz, 1987). This diffusion model is based on a geometry whereby the release is considered to occur from both sides on the film. The diffusion data were analysed using equation (3) for short-term migration and the corresponding rate constants were calculated using equation (4) for long-term migration. Short-term migration is defined as the time for which $m_t/m_\infty < 0.6$:

$$\frac{m_t}{m_\infty}=4\left(\frac{Dt}{\pi l^2}\right)^{\frac{1}{2}} \qquad (3)$$

where *D* is the diffusion coefficient and *l* is the thickness of the film. A plot of m_t/m_∞ *versus* $t^{½}$ should yield a straight line from which the diffusion coefficient can be obtained.

For long-term migration $m_t/m_\infty > 0.6$ and equation (4) applies:

$$\frac{m_t}{m_\infty} = 1 - \left(\frac{8}{\pi^2}\right) exp\left(-\frac{\pi^2 Dt}{l^2}\right) \tag{4}$$

2.5.3 Fick’s Law Model

The diffusion coefficient of the AM agent can be determined from its release *versus* time data, by fitting these data to Fick’s second law. Equation (5) can be derived from Fick’s second law in the case of one dimensional diffusion from a limited volume of film that is in contact with an infinite volume of solution (Crank, 1979).

$$\frac{m_t}{m_\infty} = 1 - \frac{8}{\pi^2}\sum_{m=0}^{\infty}\frac{1}{(2m+1)^2} exp\left[\frac{-D(2m+1)^2\pi^2 t}{l^2}\right] \tag{5}$$

2.5.4 Diffusion Activation Energy

The effect of temperature on the release rate of the AM agent was modelled in accordance with the Arrhenius equation (Suppakul (2004)):

$$D = D_0 exp\left(\frac{E_a}{RT}\right) \tag{6}$$

where *D* is the diffusion coefficient, D_o is the pre-exponential factor, E_a is the activation energy for the diffusion process, *R* is the ideal gas constant, and *T* is the absolute temperature.

3 Results and Discussion

3.1 Release of Thymol into Simulants

Figures 1(a) and 1(b) show plots of the mass fraction m_t/m_∞ *versus* time, t and the overall kinetic analysis for the release of thymol from neat PLA and PLA/kenaf films into 950 mL/L ethanol/water at the four temperatures studied. The thymol release reached equilibrium within *ca*. 9 h at the lowest temperature of 30°C (Figure 1(a)). A similar observation was found for PLA containing resveratrol where the system achieved equilibrium after *ca*. 14 h using the same food simulant at 33°C (Soto-Valdez, Auras, & Peralta, 2011). In comparison, the times required to achieve the equilibrium concentration of thymol in different polymeric systems with the same food simulant at 40°C were found to be 50 h for PBS/thymol films and 150 h for PP/thymol films (Ploypetchara, Suppakul, Atong, & Pechyen, 2014; Ramos, Beltrán, Peltzer, Valente, & Garrigós, 2014) and 2 h for starch/thymol films in isooctane at 35 °C (Kuorwel, Cran, Sonneveld, Miltz, & Bigger, 2013). In the current work, it was found that increasing the temperature to say 60°C, increases the release rate of thymol and equilibrium was attained within 1.4 h. At the end of these experiments, *ca*. 87 to 100% and 82 to 95% of the thymol was released from the neat PLA and PLA/kenaf films respectively into 950 mL/L ethanol/water over the temperature range of 30 to 60°C.

>>>Insert Figure 1

The initial release rates and the overall rate constants for the release of thymol that were calculated from the data in Figure 1(b) are presented in Table 1. For both neat PLA and PLA/kenaf films, the initial release rates and the overall rate constants for thymol release into

950 mL/L ethanol/water consistently increased with an increase in temperature from 30 to 60°C. This is consistent with the findings of (Kuorwel, Cran, Sonneveld, Miltz, & Bigger, 2013) where an increase in temperature was found to have a significant effect on the migration of the additive from the film. From the results in Table 1 a similar observation can also be made in the case of the 150 mL/L ethanol/water simulant over the range of the four temperatures studied. However, in the latter case the rates are significantly lower compared with the 950 mL/L ethanol/water case. The experimental results were further analyzed by using the diffusion model in which the short-term and the long-term migration periods were considered.

>>> Insert Table 1

Figures 2(a) and 2(b) show the respective plots of m_t/m_∞ *versus* $t^{1/2}$ for the short-term release of thymol and $\ln(1 - m_t/m_\infty)$ *versus* t for the long-term release of thymol from the neat PLA and PLA/kenaf films into 950 mL/L ethanol/water at 30°C. A similar behavior was observed at each of the temperatures that were studied and the linearity of the plots suggests that the data conform well to the diffusion model given in equation (3) for short-term release. Values of the apparent diffusion coefficient at each of the various temperatures were determined from the gradients of these plots and are also presented in Table 1. For the long-term release, the linearity of the $\ln(1 - m_t/m_\infty)$ *versus* t plots also confirms the data reliably fit the model presented in equation (4). Indeed, all results exhibited good linear correlation with correlation coefficients (r^2 values) greater than 0.97.

>>> Insert Figure 2

The results in Table 1 confirm that the diffusion coefficients of thymol from the neat PLA and PLA/kenaf films into the 950 mL/L ethanol/water increase with increasing temperature. As expected, the diffusion rates of the composite films are higher than those obtained for the neat PLA films. This may be attributed in part to the presence of the kenaf fibre filler in the polymer matrix that creates voids thereby enabling the release of thymol from the film. These findings are consistent with the result obtained for the retention of thymol in a previous study whereby following processing the composite films were found to retain less thymol than neat PLA film (Tawakkal, Cran, & Bigger, 2015). It is important to note that the values of the *D* parameter found for the neat PLA and PLA/kenaf films containing thymol are one order of magnitude higher than those observed for active low-density polyethylene (LDPE), PBS and PP containing a similar AM agent and using 950 mL/L ethanol/water simulant (Cran, Rupika, Sonneveld, Miltz, & Bigger, 2010; Ploypetchara, Suppakul, Atong, & Pechyen, 2014; Ramos, Beltrán, Peltzer, Valente, & Garrigós, 2014). Moreover, and as expected, active PLA films containing a non-volatile and thermally stable agent (i.e. resveratrol) prepared by Soto-Valdez, Auras, and Peralta (2011) exhibit diffusion coefficients that are one order of magnitude lower than those of PLA films containing a volatile AM agent such as thymol. Similar trends in the diffusion coefficients of thymol were observed for the neat PLA and PLA/kenaf films when immersed in the 150 mL/L ethanol/water simulant. However, the diffusion coefficients for the films immersed in the 150 mL/L ethanol/water simulant were consistently lower than those pertaining to the 950 mL/L ethanol/water simulant. The observed decrease in the diffusivity may be explained by the lack of affinity between thymol and water that leads to the limited solubility of thymol in water. Moreover, PLA is a hydrophobic polymer but ethanol is sufficiently non-polar to facilitate the swelling of the PLA matrix (Sato, Gondo, Wada, Kanehashi, & Nagai, 2013), dissolve the thymol and release it into the bulk of the simulant. According to Manzanarez-López, Soto-Valdez, Auras, and Peralta (2011), ethanol is also an aggressive solvent for PLA which can penetrate into PLA chains and release the active substance.

The lower concentration of ethanol in the 150 mL/L ethanol/water simulant may lead to a slight extent of swelling as well as hydrolysis of the PLA (Manzanarez-López, Soto-Valdez, Auras, & Peralta, 2011). Interestingly this slight swelling effect of PLA matrix appears to be more significant than the swelling effect of the kenaf fibres. The PLA/kenaf composites are semi-hydrophilic materials due to the presence of kenaf fibre that acts as a hydrophilic filler. One may therefore expect the rate of diffusion of thymol from these composite systems (PLA/kenaf films) to increase as the water content of the simulant increases. Taib, Ramarad, Mohd Ishak, and Todo (2009) prepared PLA/kenaf composites containing polyethylene glycol (PEG) and reported that when the composite was immersed into the water, the fibres absorb water leading to expansion of the fibres. Such an effect may create internal stress in the adjacent matrix and resulting in the formation of microcracks. However, the rate of diffusion of thymol from the PLA/kenaf films was in fact found to be lower in the 150 mL/L ethanol/water simulant than in the 950 mL/L ethanol/water simulant. It can therefore be suggested that a complex and strong interaction exists between the kenaf, PLA and thymol in the composite systems. The PLA matrix may act as a coating agent to the kenaf fibre filler, preventing the water from swelling the kenaf fibres and releasing thymol that is associated with the fibres. Such an interaction between the PLA, kenaf fibre and thymol was reported previously where these composite systems were studied using Fourier transform infrared (FTIR) spectroscopic and thermogravimetric (TG) analyses (Tawakkal, Cran, & Bigger, 2015). In that study, the activation energy for the thermal release of thymol, using a 3D diffusion kinetic model, was found to be 46 kJ mol^{-1} in the case of neat PLA containing thymol and 65 kJ mol^{-1} for the PLA/kenaf composite system.

Fick's second law model was also used to calculate the diffusion coefficient by minimizing the sum of the squared errors (SSE) of the measured and calculated value (see Table 1). To determine the fit of the experimental data, equation (5) was used and a non-linear regression function was applied to the data. Figure 3 shows plots of m_t/m_∞ *versus* t for the diffusion of thymol from PLA/kenaf film into 950 mL/L ethanol/water at 30°C where the data have been fitted using the Fick's second law model. The satisfactory fit of the experimental data suggests that the diffusion kinetics of thymol into 950 mL/L ethanol/water from the composite can also be adequately described by Fick's model. The SSE values for all the release models studied are also presented in Table 1. In general, the short-term diffusion model presents the best fit for the release kinetics with the lowest SSE values for all systems studied. This is followed by the Fickian diffusion model, with the first order model and long-term diffusion models showing some higher SSE values for some systems.

>>> Insert Figure 3

Using the diffusion coefficients obtained from the diffusion model, the effect of temperature on the diffusion coefficient for the release of thymol into 950 and 150 mL/L ethanol/water was determined and Arrhenius plots of the data are shown in Figure 4. The activation energy for the diffusion process, E_a, was calculated from the slope of the ln(D) *versus* 1/T plot in each case (see Figure 4) in accordance with the Arrhenius equation (see equation (6)). The activation energies for the release of thymol from the neat PLA and PLA/kenaf films were found to be: 90.6 and 76.2 kJ mol^{-1} in 950 mL/L ethanol/water and 98.7 and 84.8 kJ mol^{-1} in 150 mL/L ethanol/water respectively. Significant differences were therefore found to exist amongst the E_a values for these active neat PLA and PLA/kenaf films immersed in the 950 and 150 mL/L ethanol/water simulants.

>>> Insert Figure 4

The E_a values for the neat PLA films are significantly higher than those found for PLA/kenaf films and this is attributed mainly to the strong interaction between the PLA and thymol that presumably is not as strong in the presence of the kenaf filler. Furthermore, the E_a values increase with an increase in water content of the simulant and this is consistent with the observations made in relation to the diffusion coefficients discussed above (see Table 1). The E_a values obtained in the present study are all considerably lower than those reported elsewhere for the diffusion of butylated hydroxytoluene (BHT) from PLA/BHT films into 950 mL/L ethanol/water which was 164.7 kJ mol^{-1} (Ortiz-Vazquez, Shin, Soto-Valdez, & Auras, 2011). This significant difference may be explained by the difference in molecular interaction and hydrogen bonding that exists between the polymeric matrix and the AM additive in these systems (Kuorwel, Cran, Sonneveld, Miltz, & Bigger, 2013). As expected, the trend in E_a values for the various systems when calculated using the Fick's law model are consistent with those calculated by the diffusion model given that the latter model has been derived from the former model. The activation energies using the Fick's law model for the release of thymol from the neat PLA and PLA/kenaf films were found to be: 83.3 and 80.9 kJ mol^{-1} in 950 mL/L ethanol/water and 118.5 and 96.3 kJ mol^{-1} in 150 mL/L ethanol/water respectively. These slight differences in the E_a values that were obtained using the different models might be due to the use of the diffusion coefficients derived from the short-term experimental data in the construction of the Arrhenius plot.

3.2 Film Appearance

Figure 5 shows the images of neat PLA and PLA/kenaf films after the release of thymol into 150 and 950 mL/L ethanol/water at the different temperatures studied. A considerable change in color for the neat PLA and PLA/kenaf films was observed after the release experiments. These color changes are due to the effects of temperature as well as the presence of water in the simulants. The color is observed to change from clear to opaque for the neat PLA immersed in 150 mL/L ethanol/water with an increase in opacity with increasing temperature. A similar observation was made in the case of the 950 mL/L ethanol/water simulant. The opacity of materials may be attributed to a polymer hydrolytic degradation process which can be related to crystallization of the PLA matrix as well as moisture absorption. The water molecules diffuse through the films, promoting hydrolysis and leads to the formation of low molecular degradation by-products (Ramos et al., 2014). In addition, color changes from brown to light brown are also observed for the composites immersed in 150 and 950 mL/L ethanol/water. For the PLA/kenaf composites immersed in 150 mL/L ethanol/water, the PLA surrounding the fibres dissolved or delaminated from the film surface revealing the kenaf fibers and this effect was less apparent in the 950 mL/L ethanol/water simulant.

>>> Insert Figure 5

4 Conclusions

The results of this study suggest that thymol is readily released from neat PLA and PLA/kenaf films into 150 and 950 mL/L ethanol/water simulants and the process can be described by an overall first-order kinetics model that can be used to determine the initial release rate. The short- and long-term diffusion models also adequately describe the release of thymol from these systems and the results are consistent with those obtained using a Fick's law analysis approach. The diffusion coefficient data suggest that the addition of the kenaf filler to the PLA matrix facilitates the release of thymol from the matrix and that the diffusion follows an Arrhenius relationship with temperature. Furthermore, an increase in polarity of the simulant that results from an increase in its water content decreases the propensity of thymol to be released from the composite system. Nonetheless, it is apparent that active PLA/kenaf composites containing natural AM agents such as thymol are potential candidates to be explored further for use as active packaging systems. Such systems have the advantage of being derived from natural sources, contain a naturally-derived AM agent and, as such, are expected to be more susceptible to biodegradation than their synthetically-based counterparts.

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Figure Captions

Figure 1. Plots of : (a) the mass fraction m_t/m_∞ *versus* t, and (b) $\ln(1 - m_t/m_\infty)$ *versus* t for the release of thymol from: (i) PLA and (ii) PLA/kenaf films into 950 mL/L ethanol/water at: ■ 30°C, □ 40°C,● 50°C and ○ 60°C where m_t is the mass of thymol released from the film at time t and m_∞ is the amount of thymol released from the film at equilibrium ($t = \infty$).

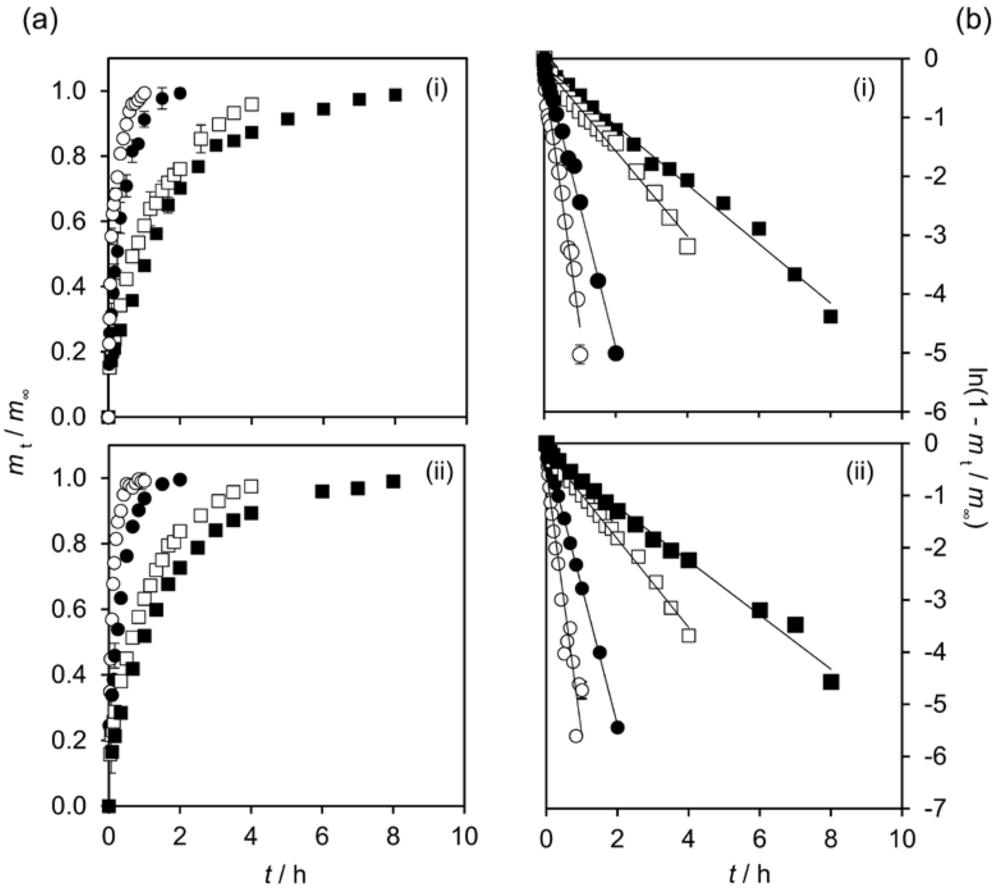

Figure 2. Plots of: (a) mass fraction m_t/m_∞ *versus* $t^{1/2}$ and (b) $\ln(1 - m_t/m_\infty)$ *versus* t for the release of thymol from: (i) PLA and (ii) PLA/kenaf films into 950 mL/L ethanol/water at 30°C where m_t is the mass of thymol released from the film at time t and m_∞ is the amount of thymol released from the film at equilibrium ($t = \infty$).

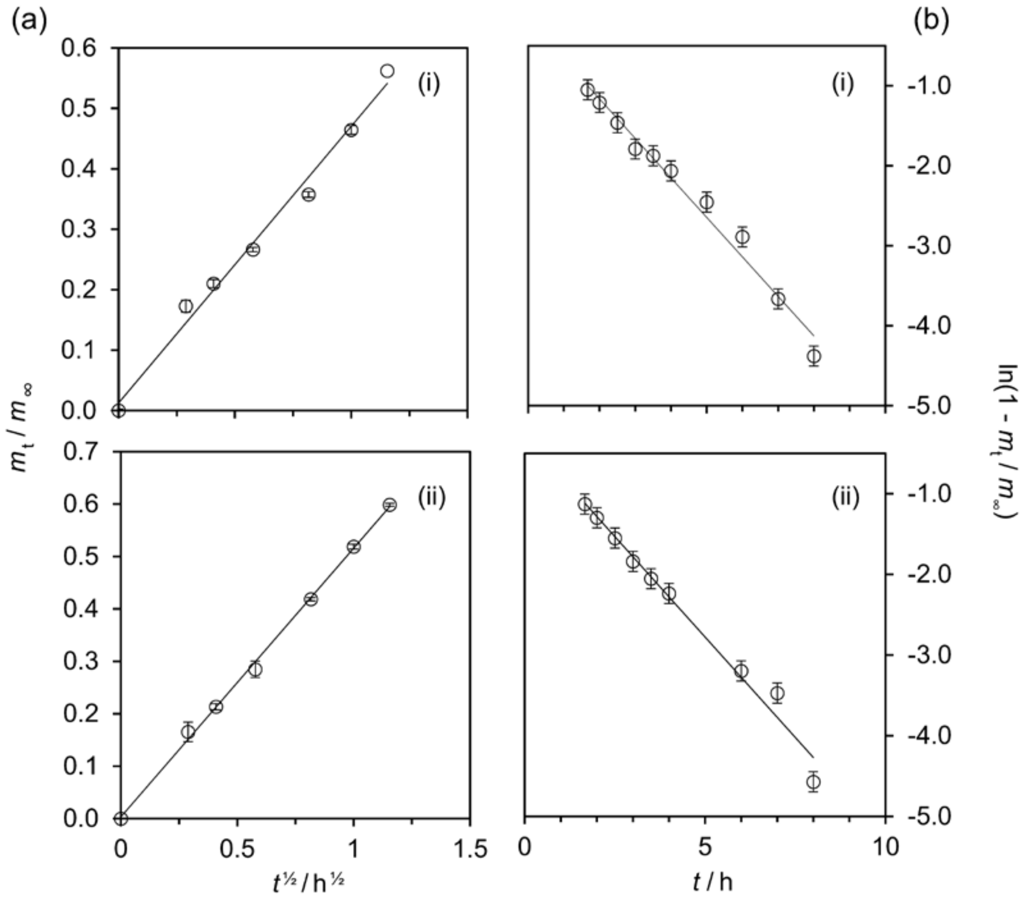

Figure 3. Plot of the mass fraction m_t/m_∞ *versus* t for the release of thymol from the PLA film into 950 mL/L ethanol/water at 30°C fitted using the Fick's law model where m_t is the mass of thymol released from the film at time t, and m_∞ is the amount of thymol released from the film at equilibrium ($t = \infty$).

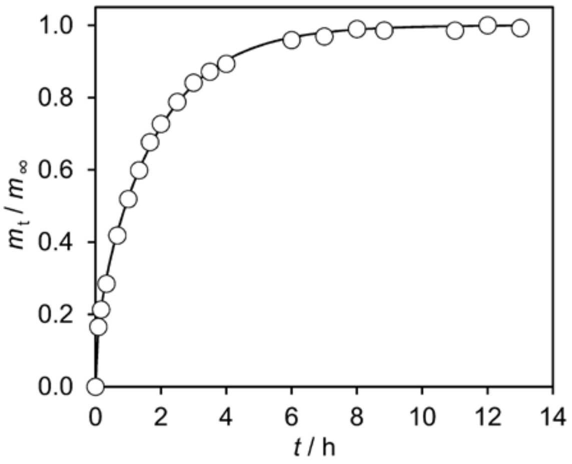

Figure 4. Arrhenius plots of ln(D) versus 1/T for the release of thymol into: (a) 950 mL/L ethanol/water and (b) 150 mL/L ethanol/water from: ● PLA film and ○ PLA/kenaf film where D is the diffusion coefficient and T is the absolute temperature.

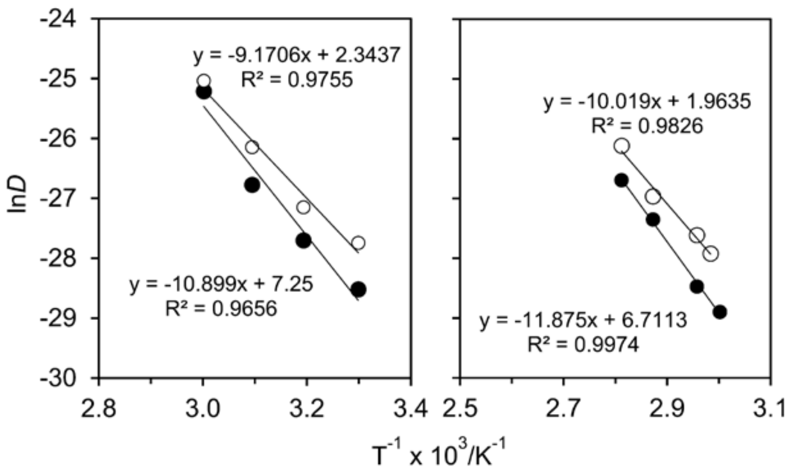

Figure 5. Images of PLA and PLA/kenaf films after the release of thymol into 150 and 950 mL/L ethanol/water stimulants at different temperatures.