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*Release of thymol from poly(lactic acid)-based antimicrobial films containing kenaf fibres as natural filler*

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## 24 **1 Introduction**

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

37  
38 Poly(lactic acid) (PLA) is a polyester synthesized from the renewable, bio-derived, monomer  
39 lactic acid and can be used as AM films and/or membranes for a different range of applications  
40 ([Auras, Harte, & Selke, 2004](#); [Karami, Rezaeian, Zahedi, & Abdollahi, 2013](#)). This GRAS  
41 (Generally Recognized As Safe) grade polymer can be used in contact with food and fabricated  
42 via conventional processing procedures ([Jamshidian, Tehrany, Imran, Jacquot, & Desobry, 2010](#)).  
43 The use of fillers in combination with PLA has been widely studied with aims to improve  
44 physicomaterial properties, reduce production costs and enhance biodegradability. Naturally  
45 derived additives or fillers such as starch and cellulose can be combined with PLA and other  
46 active agents such as AM and AOX compounds. For example, [Hwang et al. \(2013\)](#) studied the

47 migration of  $\alpha$ -tocopherol and resveratrol from poly(L-lactic acid) (PLLA)/starch blend films into  
48 ethanol and found that the neat PLLA containing  $\alpha$ -tocopherol had a lower release rate than the  
49 PLLA/starch blend films at 43°C in 100% ethanol simulant with diffusivity coefficients of  $89 \times$   
50  $10^{-11}$  and  $282 \times 10^{-11} \text{ cm}^2 \text{ s}^{-1}$  respectively. A similar trend was observed for resveratrol, a non-  
51 volatile AOX compound with diffusion rate of  $25 \times 10^{-11} \text{ cm}^2 \text{ s}^{-1}$  in neat PLLA and  $40 \times 10^{-11}$   
52  $\text{cm}^2 \text{ s}^{-1}$  in the PLA/starch films. [Fortunati et al. \(2012\)](#) prepared PLA AM films with 50 g/kg  
53 microcrystalline cellulose (MCC) and 10 g/kg silver nanoparticles by extrusion and injection  
54 molding techniques. The PLA films with silver nanoparticles and MCC had greater AM activity  
55 against *Escherichia coli* due to the presence of MCC; however, it had less AM activity than PLA  
56 films with silver nanoparticles. Although there are examples of the controlled release of AM and  
57 AOX substances from PLA materials ([Busolo & Lagaron, 2013](#); [Fernandez, Soriano, Hernandez-](#)  
58 [Munoz, & Gavara, 2010](#); [Hwang et al., 2013](#); [Iniguez-Franco et al., 2012](#); [Llana-Ruiz-Cabello et](#)  
59 [al., 2015](#)), few reports have combined PLA with natural fibres in order to control the release of  
60 active substances from composite films.

61  
62 Antimicrobial packaging systems can be categorised as either migratory or non-migratory  
63 systems. In the former, AM substances migrate from the packaging material into the headspace  
64 of the package and onto the food surface, whereas in the latter, AM substances are immobilised  
65 onto the packaging material which is placed in direct contact with the foodstuff to facilitate its  
66 activity ([Han, 2003](#)). The integration of PLA with AM substances has been investigated by a  
67 number of researchers ([Del Nobile et al., 2009](#); [Jin, 2010](#); [Qin et al., 2015](#); [Rhim, Hong, & Ha,](#)  
68 [2009](#)). Of the reported studies, many have investigated the inhibition of targeted microorganisms  
69 with little attention **having been** devoted to studying the release rate from the active systems.

70 This might be due to the preponderance of non-volatile and/or immobilised AM substances (e.g.  
71 nisin, chitosan, lysozyme and peptide) incorporated into PLA films rather than volatile AM  
72 substances ([Green, Fulghum, & Nordhaus, 2011](#); [Rhim, 2013](#); [Tawakkal, Cran, Miltz, & Bigger,](#)  
73 [2014](#)). Several AM substances have been incorporated directly into polymers including a range  
74 of volatile plant extracts such as basil, thymol, linalool, methyl cinnamate and cavacrol ([Cran,](#)  
75 [Rupika, Sonneveld, Miltz, & Bigger, 2010](#); [Del Nobile, Conte, Incoronato, & Panza, 2008](#);  
76 [Fernández-Pan, Maté, Gardrat, & Coma, 2015](#); [Rubilar et al., 2013](#); [Suppakul, 2004](#); [Suppakul,](#)  
77 [Sonneveld, Bigger, & Miltz, 2011](#); [Tawakkal, Cran, & Bigger, 2015](#)).

78  
79 Thymol, an essential oil extract that has GRAS status, can be used in contact with food products  
80 and like other volatile AM substances, the migration of this substance into real food products is  
81 complex. In some studies, active AM films containing thymol were evaluated *in vitro* and *in vivo*  
82 against a wide spectrum of microorganisms such as bacteria, mould and yeast ([Kuorwel, Cran,](#)  
83 [Sonneveld, Miltz, & Bigger, 2011](#); [Wu et al., 2014](#)). In a recent study by [Petchwattana and](#)  
84 [Naknaen \(2015\)](#), extruded films of poly(butylene succinate) (PBS) containing thymol  
85 demonstrated AM activity against *Escherichia coli* and *Staphylococcus*. Moreover, the release  
86 rate of thymol from the PBS films into 950 mL/L ethanol/water was found to be  $5.9 \times 10^{-14} \text{ m}^2 \text{ s}^{-1}$ .  
87 <sup>1</sup>. [Ramos, Beltrán, Peltzer, Valente, and Garrigós \(2014\)](#) reported that the migration of thymol  
88 from polypropylene (PP) films into 950 mL/L ethanol at 40°C conformed to Fick's law with a  
89 diffusion coefficient of  $1.0 \times 10^{-14} \text{ m}^2 \text{ s}^{-1}$ . In general, an AM substance can be released into a  
90 food simulant by swelling-controlled release with three main steps involved: (i) the absorption of  
91 fluid (penetrant) from the food simulant which leads to the swelling effect of the polymer, (ii) the

92 active substance molecule being dissolved or dispersed in the polymeric matrix and (iii) the  
93 active substance migrating to the food simulant ([Del Nobile & Conte, 2013](#)).

94

95 The quantification of volatile AM substances in active films is an important consideration given  
96 that high pressure, shear forces and temperatures are required for film processing. According to  
97 [Raouche, Mauricio-Iglesias, Peyron, Guillard, and Gontard \(2011\)](#), a higher temperature of *ca.*  
98 160 to 190°C was needed to process PLA using extrusion which may easily degrade and  
99 evaporate the volatile AM substance during thermal processing. The retention of volatile  
100 additives such as thymol in PLA film was found to be approximately 70 to 80% after thermal  
101 fabrication ([Tawakkal, Cran, & Bigger, 2015](#)). Such retention percentages were significantly  
102 higher than those found in polyolefin film systems containing similar volatile additives. In  
103 comparison, [Ramos, Jiménez, Peltzer, and Garrigós \(2012\)](#) reported much lower retention of  
104 thymol and carvacrol (*ca.* 25 to 45% respectively) in PP formulations. The marked reduction in  
105 the retention of these additives in polyolefin films upon thermal processing may result from the  
106 reduced compatibility between the polymer matrix and the natural additives as well as the  
107 processing parameters such as temperature, time and screw rotation ([Del Nobile et al., 2009](#)).

108

109 There are clearly many examples of the release of a wide range of AM substances from  
110 homopolymers such as PLA. However, little attention has been devoted to evaluating the release  
111 of AM agents from ternary composite systems, particularly taking into account the possible  
112 swelling of polymeric materials immersed in food simulants as well as the effects of natural  
113 fillers on the release rate. Moreover, the release of volatile AM substances from polymeric  
114 materials also has been restricted to mainly hydrophobic and moderate hydrophilic polymeric

115 matrices ([Buonocore, Del Nobile, Panizza, Corbo, & Nicolais, 2003](#); [Herath, 2009](#)). The aim of  
116 the current work was therefore to investigate the release of a naturally derived AM agent from a  
117 novel ternary system comprised of PLA, kenaf fibers and thymol. In particular, the release of  
118 thymol from PLA and PLA/kenaf composite and its diffusion kinetics were studied.

119

## 120 **2 Experimental Section**

### 121 **2.1 Materials**

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

129

### 130 **2.2 Production of PLA/Kenaf/Thymol Films**

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

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

152

### 153 **2.3 Quantification of Thymol in PLA and PLA/Kenaf Films**

154 One gram of film sample was cut into pieces ( $0.25 \text{ cm}^2$ ) and immersed in a round bottom flask  
155 containing 150 mL of isooctane for solid-liquid extraction. Isooctane was used as it promotes the  
156 swelling of the polymer and a typical reflux extraction was performed at 100°C for 4-5 h to  
157 extract thymol from the films. The quantification was achieved with a gas chromatography (GC)  
158 instrument (Varian 8200C<sub>x</sub>) equipped with a fused silica capillary column (DB5; 30 m × 0.25  
159 mm i.d.; thickness 0.25 μm; J & W Scientific, USA). The conditions applied in the GC  
160 instrument were as follows: injected volume: 1.0 μL, initial column temperature: 80°C; heating



161 rate: 5°C min<sup>-1</sup> up to 120°C, held at this temperature for an additional 5 min; injector  
162 temperature: 250°C; FID detector temperature: 300°C; flow rate: 2 mL min<sup>-1</sup>; splitting; carrier  
163 gas: nitrogen. Standard solutions of thymol in isooctane at concentrations from 0.05 to 1.0 mg  
164 mL<sup>-1</sup> were prepared and used to produce a calibration curve. The experiments were performed in  
165 triplicate. The retention of thymol in the neat PLA and PLA/kenaf films following thermal  
166 processing was 73% and 62% respectively as reported by [Tawakkal, Cran, and Bigger \(2015\)](#).  
167 The loss of thymol observed in the present study for composite films seems to be at an  
168 acceptable level compared to petroleum-based (polyolefin) films containing volatile additives.

169

#### 170 **2.4 Migration of Thymol into Food Simulants**

171 The release of thymol from neat PLA and PLA/kenaf films into aqueous food simulants was  
172 studied at different temperatures. The release was examined *via* a total immersion migration test  
173 ([EC, 1997](#)) using 950 and 150 mL/L ethanol/water. Ethanol is commonly used as food simulant  
174 to investigate the migration of AM substances from the PLA matrix. The 950 and 150 mL/L  
175 ethanol/water simulants are fatty and aqueous food simulants respectively. The sorption of  
176 ethanol by the PLA matrix may lead to the creation of voids and/or swelling of the matrix where  
177 it can penetrate the PLA chains and promote the migration of the active substance ([Mascheroni,  
178 Guillard, Nalin, Mora, & Piergiovanni, 2010](#)). In the case of the 950 mL/L ethanol/water  
179 simulant the experiments were performed at 30, 40, 50 and 60°C and for the 150 mL/L  
180 ethanol/water simulant experiments were performed at 60, 65, 75 and 83°C. In the latter  
181 experiments, the test temperatures were higher than recommended in the standard methods ([EC,  
182 1997](#)) in order to accelerate the migration of thymol. Studying the release rates of thymol from  
183 the neat PLA and PLA/kenaf films by using fatty and aqueous food simulants is important in

184 order to assess the *in vitro* and *in vivo* AM activity of these systems against targeted  
185 microorganisms.

186  
187 Samples of film weighing *ca.*0.5-0.6 g were immersed in 100 mL of simulant in a three-neck  
188 round bottom flask with the ratio of simulant volume per area film being *ca.* 2.7-4.7 mL cm<sup>-2</sup>.  
189 The flask was immersed in an oil bath that was placed on a magnetic stirrer heating plate and the  
190 simulant was gently agitated using magnetic stirring at 60 rpm. The flask was connected to a  
191 condenser and the stirring speed and temperature were fixed and monitored throughout the  
192 experiment. The amount of AM agent released from the films was monitored until equilibrium  
193 was attained. A 0.2 mL sample of the simulant solution was collected periodically during the  
194 experiment and 1  $\mu$ L aliquots were injected into the GC. The quantification of thymol in all  
195 simulants was performed using the same method and calibration curve described in the previous  
196 section. The thymol quantification was performed in triplicate.

197  
198 **2.5 Data Analysis**  
199 The migration of thymol from the PLA and PLA/kenaf composite films was analysed using three  
200 data analysis treatments: (i) overall kinetics, (ii) diffusion models in accordance with [Cran,](#)  
201 [Rupika, Sonneveld, Miltz, and Bigger \(2010\)](#) and [Kuorwel, Cran, Sonneveld, Miltz, and Bigger](#)  
202 [\(2013\)](#) and (iii) Fick's diffusion law model.

203  
204 **2.5.1 Overall Kinetics Analysis**  
205 By considering the overall diffusion process to be a single process that obeys first-order kinetics,  
206 equations describing the migration of an additive from a polymeric film into simulant with time

207 have been described by [Miltz \(1987\)](#) and [Crank \(1979\)](#). The release of the AM agent into the  
208 simulant was initially analysed for its fit to a first order kinetics model. In the case of a first-  
209 order system, equation (1) applies:

$$210 \quad \ln\left(1 - \frac{m_t}{m_\infty}\right) = -k_1 t \quad (1)$$

211 where  $m_t$  is the mass of additive released from the film at time  $t$ ,  $m_\infty$  is the amount of additive  
212 released from the film at equilibrium ( $t = \infty$ ) and  $k_1$  is the first-order rate constant. From equation  
213 (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  
214 first-order rate constants were calculated using equation (1) and the initial release rates,  $v_0$ , of the  
215 AM agent were calculated using equation (2) ([Kuorwel, Cran, Sonneveld, Miltz, & Bigger,](#)  
216 [2013](#)):

$$217 \quad v_0 = m_\infty k_1 \quad (2)$$

### 218 **2.5.2 Diffusion Model**

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

$$225 \quad \frac{m_t}{m_\infty} = 4 \left(\frac{Dt}{\pi l^2}\right)^{\frac{1}{2}} \quad (3)$$

226 where  $D$  is the diffusion coefficient and  $l$  is the thickness of the film. A plot of  $m_t/m_\infty$  versus  $t^{1/2}$   
227 should yield a straight line from which the diffusion coefficient can be obtained.

228

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

$$230 \quad \frac{m_t}{m_\infty} = 1 - \left(\frac{8}{\pi^2}\right) \exp\left(-\frac{\pi^2 Dt}{l^2}\right) \quad (4)$$

231

### 232 **2.5.3 Fick's Law Model**

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

$$237 \quad \frac{m_t}{m_\infty} = 1 - \frac{8}{\pi^2} \sum_{m=0}^{\infty} \frac{1}{(2m+1)^2} \exp\left[\frac{-D(2m+1)^2\pi^2 t}{l^2}\right] \quad (5)$$

238

### 239 **2.5.4 Diffusion Activation Energy**

240 The effect of temperature on the release rate of the AM agent was modelled in accordance with  
241 the Arrhenius equation ([Suppakul \(2004\)](#)):

$$242 \quad D = D_0 \exp\left(\frac{E_a}{RT}\right) \quad (6)$$

243 where  $D$  is the diffusion coefficient,  $D_0$  is the pre-exponential factor,  $E_a$  is the activation energy  
244 for the diffusion process,  $R$  is the ideal gas constant, and  $T$  is the absolute temperature.

245

## 246 3 Results and Discussion

### 247 3.1 Release of Thymol into Simulants

248 Figures 1(a) and 1(b) show plots of the mass fraction  $m_t/m_\infty$  versus time,  $t$  and the overall kinetic  
249 analysis for the release of thymol from neat PLA and PLA/kenaf films into 950 mL/L  
250 ethanol/water at the four temperatures studied. The thymol release reached equilibrium within  
251 *ca.* 9 h at the lowest temperature of 30°C (Figure 1(a)). A similar observation was found for PLA  
252 containing resveratrol where the system achieved equilibrium after *ca.* 14 h using the same food  
253 simulant at 33°C (Soto-Valdez, Auras, & Peralta, 2011). In comparison, the times required to  
254 achieve the equilibrium concentration of thymol in different polymeric systems with the same  
255 food simulant at 40°C were found to be 50 h for PBS/thymol films and 150 h for PP/thymol  
256 films (Ploypetchara, Suppakul, Atong, & Pechyen, 2014; Ramos, Beltrán, Peltzer, Valente, &  
257 Garrigós, 2014) and 2 h for starch/thymol films in isooctane at 35 °C (Kuorwel, Cran,  
258 Sonneveld, Miltz, & Bigger, 2013). In the current work, it was found that increasing the  
259 temperature to say 60°C, increases the release rate of thymol and equilibrium was attained within  
260 1.4 h. At the end of these experiments, *ca.* 87 to 100% and 82 to 95% of the thymol was released  
261 from the neat PLA and PLA/kenaf films respectively into 950 mL/L ethanol/water over the  
262 temperature range of 30 to 60°C.

263

264 >>>Insert Figure 1

265

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

269 950 mL/L ethanol/water consistently increased with an increase in temperature from 30 to 60°C.  
270 This is consistent with the findings of (Kuorwel, Cran, Sonneveld, Miltz, & Bigger, 2013) where  
271 an increase in temperature was found to have a significant effect on the migration of the additive  
272 from the film. From the results in Table 1 a similar observation can also be made in the case of  
273 the 150 mL/L ethanol/water simulant over the range of the four temperatures studied. However,  
274 in the latter case the rates are significantly lower compared with the 950 mL/L ethanol/water  
275 case. The experimental results were further analyzed by using the diffusion model in which the  
276 short-term and the long-term migration periods were considered.

277

278 >>> Insert Table 1

279

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

290

291 >>> Insert Figure 2

292 The results in [Table 1](#) confirm that the diffusion coefficients of thymol from the neat PLA and  
293 PLA/kenaf films into the [950 mL/L](#) ethanol/water increase with increasing temperature. As  
294 expected, the diffusion rates of the composite films are higher than those obtained for the neat  
295 PLA films. This may be attributed in part to the presence of the kenaf fibre filler in the polymer  
296 matrix that creates voids thereby enabling the release of thymol from the film. These findings are  
297 consistent with the result obtained for the retention of thymol [in a previous study](#) whereby  
298 [following processing](#) the composite films were found to retain less thymol than neat PLA film  
299 [\(Tawakkal, Cran, & Bigger, 2015\)](#). It is important to note that the values of the  $D$  parameter  
300 found for the neat PLA and PLA/kenaf films containing thymol are one order of magnitude  
301 higher than those observed for active low-density polyethylene (LDPE), PBS and PP containing  
302 a similar AM agent and using [950 mL/L](#) ethanol/water simulant [\(Cran, Rupika, Sonneveld,](#)  
303 [Miltz, & Bigger, 2010; Ployetchara, Suppakul, Atong, & Pechyen, 2014; Ramos, Beltrán,](#)  
304 [Peltzer, Valente, & Garrigós, 2014\)](#). Moreover, and as expected, active PLA films containing a  
305 non-volatile and thermally stable agent (i.e. resveratrol) prepared by [Soto-Valdez, Auras, and](#)  
306 [Peralta \(2011\)](#) exhibit diffusion coefficients that are one order of magnitude lower than those of  
307 PLA films containing a volatile AM agent such as thymol. Similar trends in the diffusion  
308 coefficients of thymol were observed for the neat PLA and PLA/kenaf films when immersed in  
309 the [150 mL/L](#) ethanol/water simulant. However, the diffusion coefficients for the films  
310 immersed in the [150 mL/L](#) ethanol/water simulant were consistently lower than those pertaining  
311 to the [950 mL/L](#) ethanol/water simulant. The observed decrease in the diffusivity may be  
312 explained by the lack of affinity between thymol and water that leads to the limited solubility of  
313 thymol in water. Moreover, PLA is a hydrophobic polymer but ethanol is sufficiently non-polar  
314 to facilitate the swelling of the PLA matrix [\(Sato, Gondo, Wada, Kanehashi, & Nagai, 2013\)](#),

315 dissolve the thymol and release it into the bulk of the simulant. According to [Manzanarez-López,](#)  
316 [Soto-Valdez, Auras, and Peralta \(2011\)](#), ethanol is also an aggressive solvent for PLA which can  
317 penetrate into PLA chains and release the active substance.

318  
319 The lower concentration of ethanol in the 150 mL/L ethanol/water simulant may lead to a slight  
320 extent of swelling as well as hydrolysis of the PLA ([Manzanarez-López, Soto-Valdez, Auras, &](#)  
321 [Peralta, 2011](#)). Interestingly this slight swelling effect of PLA matrix appears to be more  
322 significant than the swelling effect of the kenaf fibres. The PLA/kenaf composites are semi-  
323 hydrophilic materials due to the presence of kenaf fibre that acts as a hydrophilic filler. One may  
324 therefore expect the rate of diffusion of thymol from these composite systems (PLA/kenaf films)  
325 to increase as the water content of the simulant increases. [Taib, Ramarad, Mohd Ishak, and Todo](#)  
326 [\(2009\)](#) prepared PLA/kenaf composites containing polyethylene glycol (PEG) and reported that  
327 when the composite was immersed into the water, the fibres absorb water leading to expansion of  
328 the fibres. Such an effect may create internal stress in the adjacent matrix and resulting in the  
329 formation of microcracks. However, the rate of diffusion of thymol from the PLA/kenaf films  
330 was in fact found to be lower in the 150 mL/L ethanol/water simulant than in the 950 mL/L  
331 ethanol/water simulant. It can therefore be suggested that a complex and strong interaction exists  
332 between the kenaf, PLA and thymol in the composite systems. The PLA matrix may act as a  
333 coating agent to the kenaf fibre filler, preventing the water from swelling the kenaf fibres and  
334 releasing thymol that is associated with the fibres. Such an interaction between the PLA, kenaf  
335 fibre and thymol was reported previously where these composite systems were studied using  
336 Fourier transform infrared (FTIR) spectroscopic and thermogravimetric (TG) analyses  
337 ([Tawakkal, Cran, & Bigger, 2015](#)). In that study, the activation energy for the thermal release of



338 thymol, using a 3D diffusion kinetic model, was found to be  $46 \text{ kJ mol}^{-1}$  in the case of neat PLA  
339 containing thymol and  $65 \text{ kJ mol}^{-1}$  for the PLA/kenaf composite system.

340

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

353

354 >>> Insert [Figure 3](#)

355

356 Using the diffusion coefficients obtained from [the](#) diffusion model, the effect of temperature on  
357 the diffusion coefficient for the release of thymol into [950](#) and [150 mL/L](#) ethanol/water was  
358 determined and Arrhenius plots of the data are shown in [Figure 4](#). The activation energy for the  
359 diffusion process,  $E_a$ , was calculated from the slope of [the](#)  $\ln(D)$  versus  $1/T$  plot in each case (see  
360 [Figure 4](#)) in accordance with the Arrhenius equation (see equation (6)). The activation energies

361 for the release of thymol from the neat PLA and PLA/kenaf films were found to be: 90.6 and  
362 76.2 kJ mol<sup>-1</sup> in 950 mL/L ethanol/water and 98.7 and 84.8 kJ mol<sup>-1</sup> in 150 mL/L ethanol/water  
363 respectively. Significant differences were therefore found to exist amongst the  $E_a$  values for  
364 these active neat PLA and PLA/kenaf films immersed in the 950 and 150 mL/L ethanol/water  
365 simulants.

366

367 >>> Insert [Figure 4](#)

368

369 The  $E_a$  values for the neat PLA films are significantly higher than those found for PLA/kenaf  
370 films and this is attributed mainly to the strong interaction between the PLA and thymol that  
371 presumably is not as strong in the presence of the kenaf filler. Furthermore, the  $E_a$  values  
372 increase with an increase in water content of the simulant and this is consistent with the  
373 observations made in relation to the diffusion coefficients discussed above (see [Table 1](#)). The  $E_a$   
374 values obtained in the present study are all considerably lower than those reported elsewhere for  
375 the diffusion of butylated hydroxytoluene (BHT) from PLA/BHT films into 950 mL/L  
376 ethanol/water which was 164.7 kJ mol<sup>-1</sup> ([Ortiz-Vazquez, Shin, Soto-Valdez, & Auras, 2011](#)).  
377 This significant difference may be explained by the difference in molecular interaction and  
378 hydrogen bonding that exists between the polymeric matrix and the AM additive in these  
379 systems ([Kuorwel, Cran, Sonneveld, Miltz, & Bigger, 2013](#)). As expected, the trend in  $E_a$  values  
380 for the various systems when calculated using the Fick's law model are consistent with those  
381 calculated by the diffusion model given that the latter model has been derived from the former  
382 model. The activation energies using the Fick's law model for the release of thymol from the  
383 neat PLA and PLA/kenaf films were found to be: 83.3 and 80.9 kJ mol<sup>-1</sup> in 950 mL/L

384 ethanol/water and 118.5 and 96.3 kJ mol<sup>-1</sup> in 150 mL/L ethanol/water respectively. These slight  
385 differences in the  $E_a$  values that were obtained using the different models might be due to the use  
386 of the diffusion coefficients derived from the short-term experimental data in the construction of  
387 the Arrhenius plot.

388

### 389 **3.2 Film Appearance**

390 Figure 5 shows the images of neat PLA and PLA/kenaf films after the release of thymol into 150  
391 and 950 mL/L ethanol/water at the different temperatures studied. A considerable change in  
392 color for the neat PLA and PLA/kenaf films was observed after the release experiments. These  
393 color changes are due to the effects of temperature as well as the presence of water in the  
394 simulants. The color is observed to change from clear to opaque for the neat PLA immersed in  
395 150 mL/L ethanol/water with an increase in opacity with increasing temperature. A similar  
396 observation was made in the case of the 950 mL/L ethanol/water simulant. The opacity of  
397 materials may be attributed to a polymer hydrolytic degradation process which can be related to  
398 crystallization of the PLA matrix as well as moisture absorption. The water molecules diffuse  
399 through the films, promoting hydrolysis and leads to the formation of low molecular degradation  
400 by-products (Ramos et al., 2014). In addition, color changes from brown to light brown are also  
401 observed for the composites immersed in 150 and 950 mL/L ethanol/water. For the PLA/kenaf  
402 composites immersed in 150 mL/L ethanol/water, the PLA surrounding the fibres dissolved or  
403 delaminated from the film surface revealing the kenaf fibers and this effect was less apparent in  
404 the 950 mL/L ethanol/water simulant.

405

406 >>> Insert Figure 5

#### 407 **4 Conclusions**

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

422

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428 samples.

429

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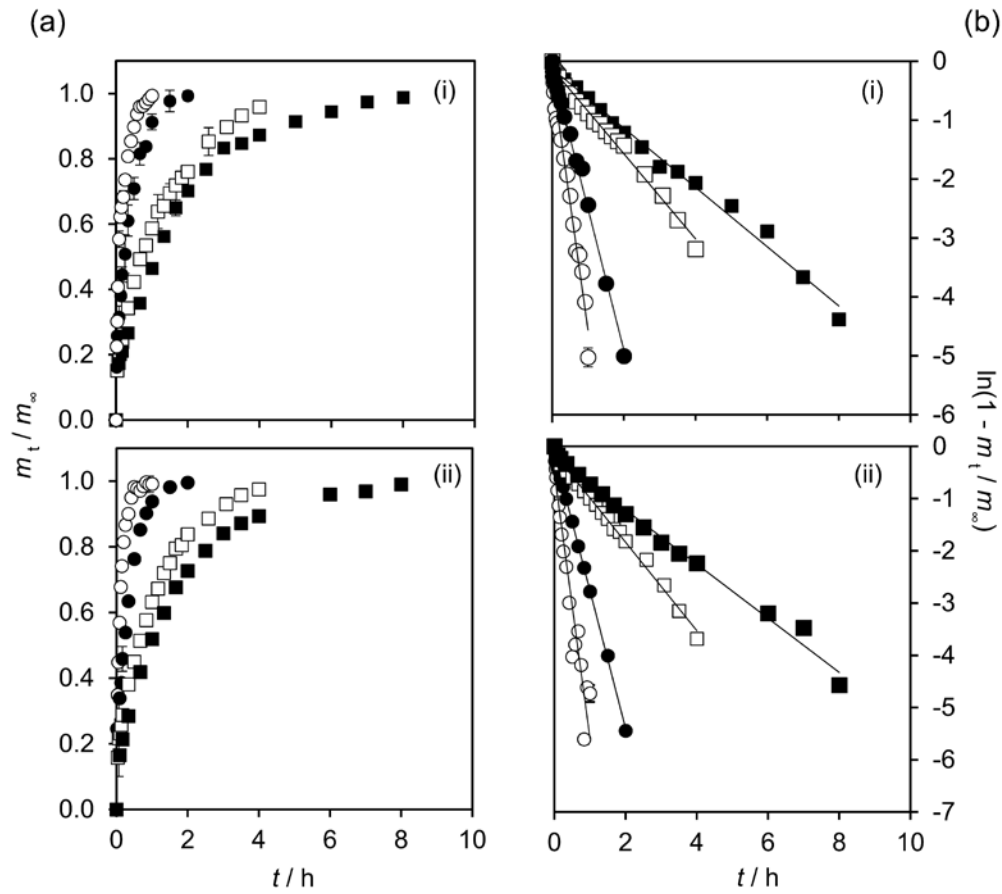
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560

561 **Figure Captions**

562

563 **Figure 1.** Plots of : (a) the mass fraction  $m_t/m_\infty$  versus  $t$ , and (b)  $\ln(1 - m_t/m_\infty)$  versus  $t$  for  
564 the release of thymol from: (i) PLA and (ii) PLA/kenaf films into 950 mL/L ethanol/water at: ■  
565 30°C, □ 40°C, ● 50°C and ○ 60°C where  $m_t$  is the mass of thymol released from the film at time  
566  $t$  and  $m_\infty$  is the amount of thymol released from the film at equilibrium ( $t = \infty$ ).



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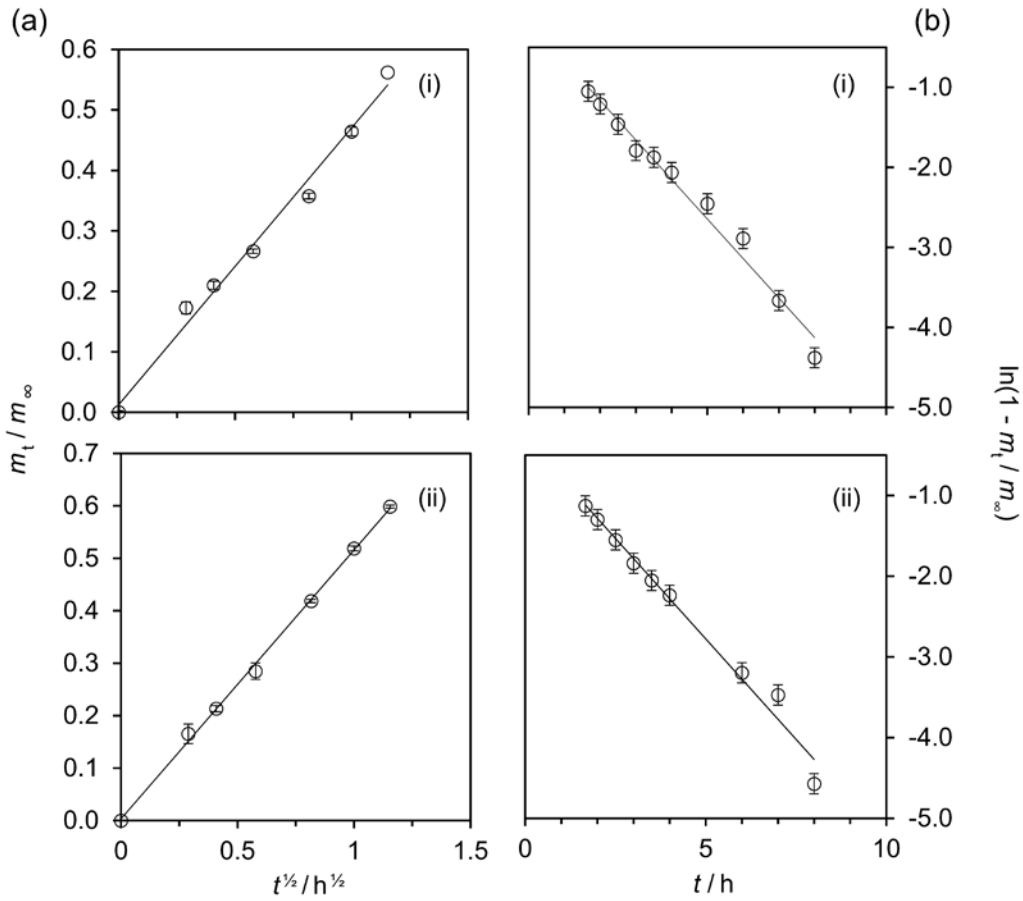
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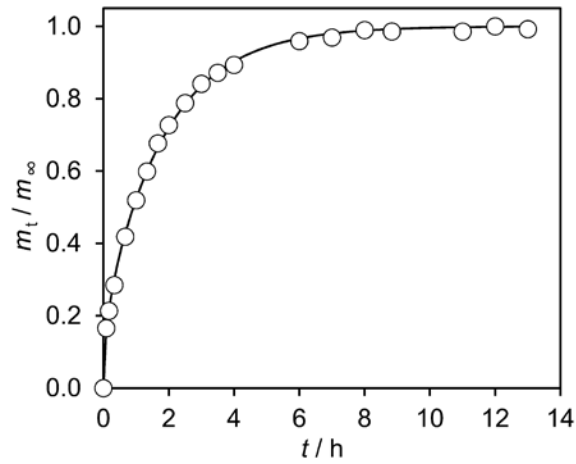


572 **Figure 2.** Plots of: (a) mass fraction  $m_t/m_\infty$  versus  $t^{1/2}$  and (b)  $\ln(1 - m_t/m_\infty)$  versus  $t$  for the  
 573 release of thymol from: (i) PLA and (ii) PLA/kenaf films into 950 mL/L ethanol/water at 30°C  
 574 where  $m_t$  is the mass of thymol released from the film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



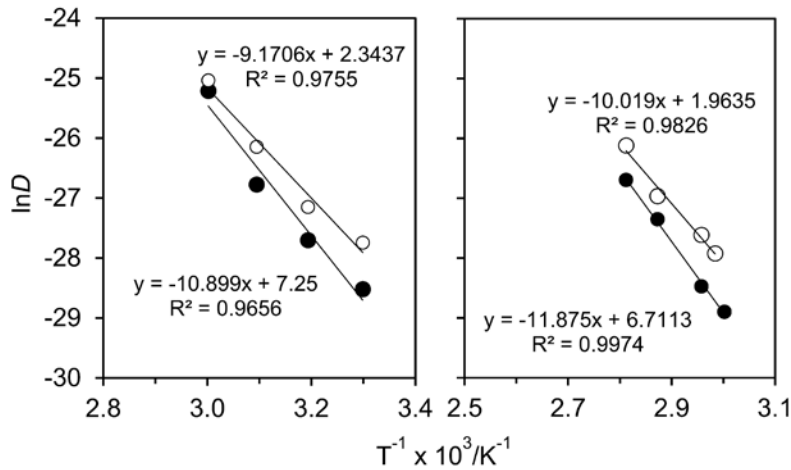
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583 **Figure 3.** Plot of the mass fraction  $m_t/m_\infty$  versus  $t$  for the release of thymol from the PLA  
 584 film into 950 mL/L ethanol/water at 30°C fitted using the Fick's law model where  $m_t$  is the mass  
 585 of thymol released from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



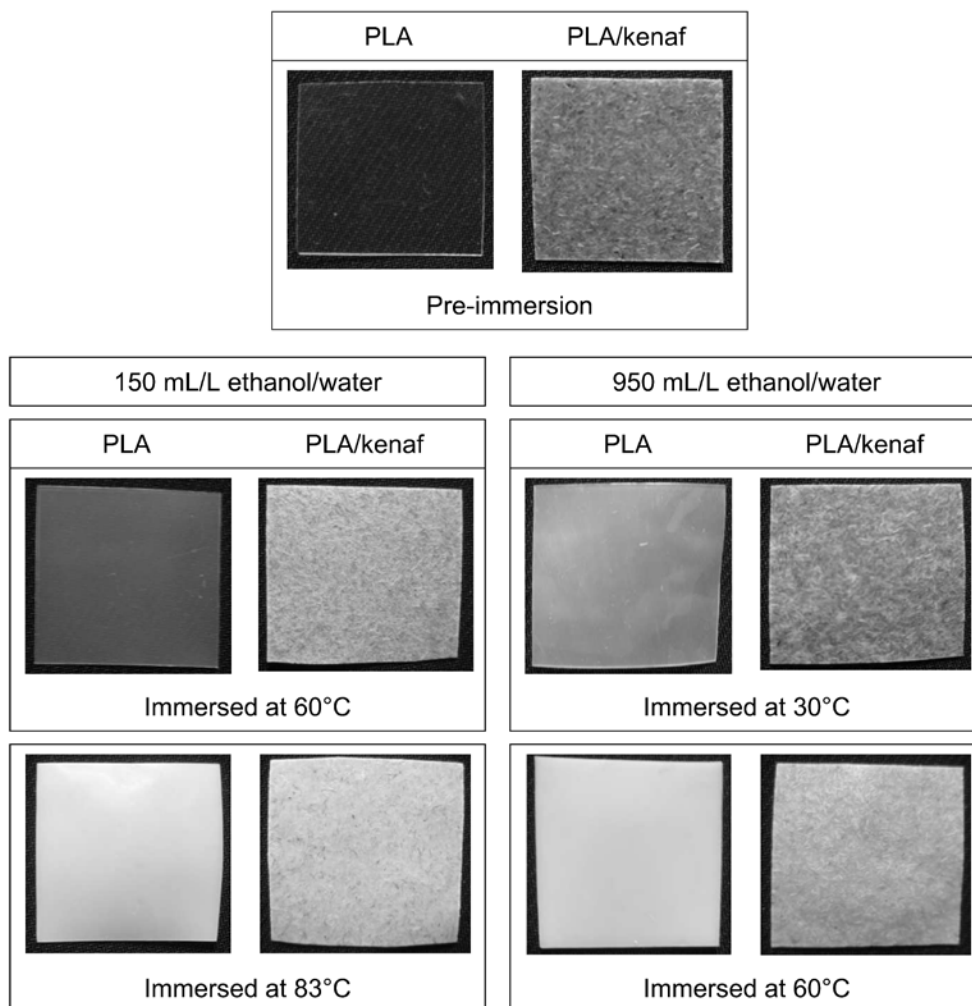
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**Figure 4.** Arrhenius plots of  $\ln(D)$  versus  $1/T$  for the release of thymol into: (a) 950 mL/L  
 ethanol/water and (b) 150 mL/L ethanol/water from: ● PLA film and ○ PLA/kenaf film where  $D$   
 is the diffusion coefficient and  $T$  is the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



593  
 594

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



597