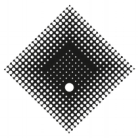
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Migration of Antimicrobial Agents from Starch-Based Films into a Food Simulant

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Abstract

The migration of antimicrobial (AM) agents carvacrol, thymol and linalool from heat pressed and coated starch-based packaging films into isooctane, a recommended fatty food simulant, was investigated. The AM agents were effectively released into isooctane and their overall release consistently obeyed first-order kinetics. When the test temperature was increased from 15 to 35°C, the diffusion coefficients increased from 6.3×10^{-13} to 12.9×10^{-13} m^{-2} s^{-1} for carvacrol, from 12.0×10^{-13} to 29.7×10^{-13} m^{-2} s^{-1} for thymol and from 9.5×10^{-13} to 19.0×10^{-13} m^{-2} s^{-1} for linalool from the heat pressed starch-based films. The diffusion coefficients of carvacrol, thymol and linalool from starch-based films coated with a methylcellulose/hydroxypropyl methylcellulose matrix containing the AM agent increased from 2.2×10^{-13} to 8.7×10^{-13} m^{-2} s^{-1}, from 2.7×10^{-13} to 6.1×10^{-13} m^{-2} s^{-1} and from 5.1×10^{-13} to 9.4×10^{-13} m^{-2} s^{-1} respectively between 15 and 35°C. The activation energy E_a, for the migration of carvacrol, thymol and linalool from the heat pressed films was found to be, 26.2, 33.6 and 25.5 kJ mol^{-1} respectively whereas the corresponding E_a values for the migration from the coated systems were 31.3, 3.0 and 22.5 kJ mol^{-1} respectively. The results suggest that the AM agents were effectively released into isooctane and that these systems show a potential for use as AM packaging materials.

Keywords: antimicrobial packaging; antimicrobial agent; biopolymer; carvacrol; linalool; thymol; food simulants; diffusion; migration.

1 Introduction

Consumer preference for preservative-free and high-quality food products that are packaged in materials that create a lower environmental impact has inspired research into the application of biopolymeric materials in antimicrobial (AM) packaging systems (López, Sánchez, Batlle & Nerín, 2007). When a volatile AM agent is incorporated into a package, it is released mainly by permeation and diffusion onto food surfaces to control pathogenic or spoilage microorganisms during the shelf life period (Suppakul, Sonneveld, Bigger & Miltz, 2011b). Antimicrobial packaging is among the more promising forms of active packaging (AP) systems aimed at protecting food products from microbial contamination. The latter are systems in which the product, the package and the environment interact to extend shelf life or improve microbial safety or sensory properties whilst simultaneously maintaining the quality of food products (Miltz, Passy & Mannheim, 1995). According to Rooney (1995), the additional preservation roles, rendered by AP systems to the packaged food product, differentiates them from traditional packaging systems that offer only protective functions against external influences. Numerous studies (Appendini & Hotchkiss, 2002; Han, 2005; López, Sánchez, Batlle & Nerín, 2007; Tovar, Salafranca, Sanchez & Nerin, 2005) have identified migratory and non-migratory as the two main categories of AM packaging systems. In migrating AM packaging systems, AM agents incorporated into the packaging material are released onto food surfaces and/or into the headspace of the packages to suppress microbial growth (Appendini & Hotchkiss, 2002; Han, 2003). The release rate of AM agents from the packaging material has a significant effect on the AM activity and potential applications of AM films in food packaging (LaCoste, Schaich, Zumbrunnen & Yam, 2005; Rardniyom, 2008). An AM agent incorporated into a packaging material is released onto food surfaces mainly by permeation and diffusion to control pathogenic and/or spoilage microorganisms

during the storage period (Buonocore, Del Nobile, Panizza, Corbo & Nicolais, 2003; Limm & Holifield, 1995).

The release rate of the AM agent from the packaging material is primarily influenced by factors that include the film fabrication method, the properties of the AM agent (such as volatility and polarity), the chemical interaction between the AM agent and polymer chains, changes in the packaging film that might be induced by the AM agent incorporated into the film, properties such as hydrophobicity and hydrophilicity of the polymer, food composition, properties such as water activity (a_w) and pH of the food, as well as environmental factors such as storage conditions, primarily temperature and relative humidity (Suppakul, Sonneveld, Miltz & Bigger, 2003; Weng & Hotchkiss, 1993). In most cases, it is time consuming and expensive to determine the migration of AM agent into the food because most foodstuffs are comprised of a complex mixture of substances such as water, carbohydrates, fats, lipids, proteins, vitamins, fibres and minerals (Cran, Rupika, Sonneveld, Miltz & Bigger, 2010). Thus, migration studies are usually performed using food simulants (Dopico, Lopez-Vilarino & Gonzalez-Rodriguesz, 2003). Different food simulants have been identified in the European food-packaging regulations (EC, 1997) for migration testing. The food simulants for various food products include: water (for water-based products); 3% (v/v) acetic acid in water (for acidic products); 50% (v/v) ethanol in water (for dairy products); olive oil; sunflower oil; synthetic fat simulant HB 307; 95% ethanol in water and isooctane for fatty products (EC, 1997; USFDA, 2007). Very recently, new simulants have been recommended by the European Communities (EC Regulation 10/2011) for different food products. This regulation is aimed to be implemented gradually and will become compulsory from January 1, 2016 (EC, 2011). Furthermore, the compatibility of an AM agent with different types of

84 foods or food simulants is an important factor that must be considered when designing AM
85 packaging systems (Rardniyom, Miltz, Bigger, Cran & Sonneveld, 2008).
86
87 Given the current interest in the use of both starch-based materials and natural AM agents in
88 packaging applications, the objective of this study was to investigate the migration of
89 carvacrol, thymol or linalool incorporated into or coated onto starch-based films. The fatty
90 food simulant isooctane recommended by the US Food and Drug Administration (USFDA,
91 2007) was used in the experimental work, as it is likely to mimic the packaging environment
92 of a fatty product like Cheddar cheese. The temperature dependency of the AM agents'
93 migration into isooctane was also investigated.
94
95 **2 Materials and Methods**
96 **2.1 Materials**
97 The materials used in the present study were a commercial, chemically modified
98 thermoplastic starch (TPS), a high-amylose corn starch and a commercial, starch-based film
99 comprising a thermoplastic starch blended with an aliphatic polyester (APTPS). The TPS
100 material has been specifically designed for the production of extruded or thermoformed
101 packaging products. The APTPS is a biodegradable material based on a blend of
102 thermoplastic starch, aliphatic polyesters and natural plasticisers. Methylcellulose (MC,
103 18,804-2); hydroxypropyl methylcellulose (HPMC, 42,321-1) and polyethylene glycol (PEG,
104 20,236-3) were purchased from Aldrich Chemical Company Inc., Milwaukee, WI, USA. The
105 AM agents were thymol (TO501), linalool (L2602) and carvacrol (W224502) with quoted
106 purities of 99.5%, 97% and 98% respectively. All of the AM agents were purchased from
107 Sigma-Aldrich Pty. Ltd., Sydney, Australia. Analytical reagent (AR) grade glycerol was
108 purchased from Merck, Australia.

2.2 Preparation of Starch-Based Film by Heat Pressing Under Compression

The preparation of the TPS starch-based films was achieved by heat pressing under compression in accordance with the method previously used by Mistry (2006). Master batches were prepared by gradually adding the starch-based material to a plasticiser made of a mixture of water and glycerol. The final composition of the formulation was 61% (w/w) starch-based material, 10% (w/w) water and 25% (w/w) glycerol. Each of the three natural AM agents, thymol, carvacrol and linalool were thoroughly blended with separate samples of the starch-based material at a formulation concentration of 4% (w/w). A sample weighing *ca.* 15 g of the resultant mixture was placed between two Mylar™ films positioned between two aluminium platens and then pressed in a laboratory press (IDM Instruments Pty. Ltd., Australia, model No. L0003). The temperature of the upper and lower platens of the press was maintained at 125°C for 5 min under a pressure of 30 kPa. The platens were then quench-cooled, removed from the press and the films were peeled away from the Mylar™ film. A starch-based material without any AM agent was similarly prepared and used as the control. The sample film thickness was measured immediately after it was peeled from the moulding film, using a hand-held micrometer with a precision of 0.001 mm (Mitutoyo, Japan). Films thickness was measured at five different positions and an average thickness was calculated from these readings. After measuring the thickness, the films were wrapped in an aluminium foil to prevent loss of the AM agent before being used.

2.3 Coating and Drying of Starch-Based Films

The coating solution was prepared from the MC and HPMC materials. Methylcellulose and HPMC were added slowly to absolute ethanol and heated, with stirring, on a magnetic hotplate. The heating was discontinued when the temperature reached 65°C. With continuous agitation, a mixture of PEG and distilled water, as a plasticiser, was added slowly to the MC-

134 HPMC dispersion whilst the dispersion cooled down. This resulted in the formation of a
135 uniformly clear coating solution or gel (Rardniyom, 2008). The AM agent was then added to
136 the coating solution to form the final coating material with the AM agent at a target level of
137 3% (w/w). The coating medium was applied to the starch-based material using a hand drawn
138 glass roller and the film was then dried under ambient conditions (temperature 21°C, RH
139 38%) for 24 h (Cooksey, 2005). To control the thickness of the coating, the starch-based
140 material was taped onto a 30 × 30 cm glass plate and the edges were framed using 3M™
141 masking tape. Each of the three solutions containing the natural AM agents: carvacrol,
142 linalool and thymol were coated separately onto the starch-based material. Similarly, a
143 coating solution without AM agent was also prepared and applied to the starch-based
144 substrates as the control. The film thickness was measured in accordance with the method
145 described earlier.
146
147 **2.4 Quantification of AM Agents in Starch-Based Films**
148 The heat pressed starch-based film samples of approximately 5 × 5 cm in dimension were
149 immersed in a sealed vessel of 100 mL isooctane, placed in an incubator shaker (Innova^{TM}
150 4230, New Brunswick Scientific, USA) and maintained at 37°C. The concentration of AM
151 agent that was extracted from the film into 100 mL of isooctane as a function of time was
152 analysed by gas chromatography. An auto-sampler (Varian 8200 C_x) attached to a Varian Star
153 3400-C_x GC system equipped with a fused silica capillary column (DB-5: 30 m × 0.25 mm
154 i.d., film thickness 0.25 μm, J & W Scientific, USA) was used. The conditions applied in the
155 GC were as follows: injected volume 1.0 μL; initial column temperature 80°C; heating rate
156 5°C min^{-1} up to 120°C, held at this temperature for an additional 10 min; injector temperature
157 250°C; FID detector temperature 300°C; flow rate of splitless nitrogen carrier gas 15 mL
158 min^{-1}. The actual concentration of the AM agents retained in the MC-HPMC coatings after

159 drying was determined on the basis of total dry weight of the film. The experiments were
160 performed in triplicate.
161
162 **2.5 Migration of Antimicrobial Agents into Food Simulant**
163 The study of the release of AM agents from heat pressed starch-based film samples into
164 isooctane as a fatty-food simulant was performed at three temperatures: 15, 25 and 35°C by
165 the total immersion migration method (EC, 1997; USFDA, 2007). Film samples weighing *ca.*
166 0.5 g were immersed in 100 mL of isooctane in a tightly sealed vessel that was gently
167 agitated (60 rpm) in an incubator shaker (InnovaTM 4230, New Brunswick Scientific, USA).
168 The release of AM agents from the MC-HPMC coated APTPS films was also investigated at
169 each of the three temperatures. In each case, immersion of the starch-based films or coated
170 films did not adversely affect the integrity of the materials. The amount of AM agent released
171 from the moulded starch-based films and/or MC-HPMC coatings were monitored until
172 equilibrium was attained. The amount of AM agent released from these systems at any time
173 was analysed by GC in accordance with the conditions described above. The release
174 experiments were performed in triplicate.
175
176 **2.6 Data Analysis**
177 The migration of AM agents from the starch-based film was analysed using two data analysis
178 treatments: the overall kinetics and the diffusion models, in accordance with Cran et al.
179 (2010). Equations describing the migration of AM agents from a polymeric film with time
180 have been derived and suggested by Miltz (1987) and Crank (1975). The release of AM
181 agent into the food simulant was initially analysed for the fit to first-order kinetics model.
182 For this model, equation (1) is used:
183

$$\ln(1 - m_t/m_\infty) = -k_1 t \quad (1)$$

where m_t is the amount of AM agent released from the film at any time t, m_∞ is the amount of AM agent released from the film at equilibrium and k_1 is the first-order rate constant. From equation (1), a plot of $\ln(1 - m_t/m_\infty)$ versus time over the entire time domain of the experiment should produce a straight line whose slope is equal to $-k_1$.

From equation (1) the initial rate of release of the AM agent, v_0, at time $t = 0$, can be derived and is given by:

$$v_0 = m_\infty k_2 \quad (2)$$

For the kinetic approach to data analysis, the rate constants were calculated using equation (1) and the initial release rates of AM agent were calculated using equation (2).

In the diffusion model, the analysis of the AM agent release from the film into the food simulant is considered in two parts: the short-term and the long-term migration equations. For the short-term migration $m_t/m_\infty < 0.6$:

$$m_t/m_\infty = 4(Dt/\pi l^2)^{1/2} \quad (3)$$

where D is the diffusion coefficient and l is the film thickness. A plot of m_t/m_∞ versus $t^{1/2}$ should yield a straight line from which the diffusion coefficient can be obtained. For the long-term migration, $m_t/m_\infty > 0.6$, equation (4) is used:

$$m_t/m_\infty = 1 - (8/\pi^2)\, exp\, (-\pi^2 Dt/l^2) \quad (4)$$

Rearranging equation (4) yields:

$$\ln(1 - m_t/m_\infty) = \ln(8/\pi^2) - k_2 t \quad (5)$$

where k_2 is the rate constant. From equation (5), a plot of $\ln(1 - m_t/m_\infty)$ versus time should yield a straight line with slope of $-k_2$. In the case of the diffusion model, the diffusion coefficients were calculated using equation (3) for short-term migration and the rate constant was calculated using equation (5) for long-term migration.

It is important to note that equations (3) to (5) are based on a two-sided diffusion model whereby migration occurs from both sides of the film and this model was applied to both the heat pressed and the coated films. In the case of the coated films it is expected that the observed AM diffusion occurs primarily from the coating layer. Nonetheless, it is expected that AM diffusion will also occur within and originate from the APTPS (polyester/starch composite) layer, albeit that this diffusion will be impaired. Indeed, the solvent swelling of this material would be expected to facilitate the AM diffusion process. It is therefore expected that the AM diffusion will be asymmetric with respect to each side of the coated film system and that overall the diffusion model for this system will be complex; neither one sided nor two sided. In order to analyse the results and obtain comparative data the two sided

diffusion model was applied in all cases. Thus the diffusion data that are reported for the coated systems should be treated as apparent diffusion parameters that are suitable solely for the purpose of comparing the characteristics of the difference systems. The linearity of the data when fitted using the two-sided model suggests that the choice in model is adequate for this purpose.

The effect of temperature on the release of AM agents, was determined from the Arrhenius equation (Rardniyom, 2008; Suppakul, 2004). The activation energy of diffusion, E_a, was obtained from D values at different temperatures using equation (6):

$$D = D_0 \exp(-E_a/RT) \quad (6)$$

where D_0 is the pre-exponential factor, R is the ideal gas constant, and T is the absolute temperature.

3 Results and Discussions

3.1 Quantification of Antimicrobial Agents

The average thickness of the heat pressed starch-based films incorporated with thymol, carvacrol and/or linalool was found to be 164 μm, 131 and 185 μm respectively. A GC analysis of these indicated that the average concentration of carvacrol, thymol or linalool retained in the film after heat pressing was 1.12±0.05%, 1.18±0.03% and 1.04±0.06% (w/w) respectively. The significant loss of the AM agents observed in the present study can be attributed to their high volatility when subjected to the temperature of 125°C during the heat

254 pressing process. The high loss of these volatile additives is also consistent with the
255 observations made by Rupika et al. (2005) who reported a major loss of carvacrol (*ca*. 3.9%
256 (w/w) final concentration) and thymol (*ca*. 2.6% (w/w) final concentration) as a result of
257 thermal volatilisation during processing when a 5% (w/w) target concentration of AM agent
258 was used in the LDPE film formulations. Suppakul et al. (2011a) also reported a high loss of
259 linalool and methylchavicol upon thermal processing into LDPE film.
260
261 In the starch-based films coated with MC-HPMC, the residual carvacrol, thymol or linalool,
262 concentrations in the coatings of the dried films were close to the respective formulation
263 concentrations of 3% (w/w) with an average retention of *ca*. 95%. Therefore, the respective
264 average concentration of these AM agents, on the basis of the total weight of the dry film,
265 was 1.43±0.03% (w/w) for all agents. The high retention of AM agent in the coatings can be
266 attributed to the low temperature used during the coating process. The significant retention of
267 AM agents coated onto the starch-based films in the present study are consistent with the
268 results obtained by Rardniyom (2008) who reported considerable retention (96.2%) of
269 carvacrol in ethylacrylate-methylmethacrylate coatings.
270
271 **3.2 Release of AM Agents into Food Simulants**
272 The migration into isooctane (a fatty food simulant) of the AM agents from the starch-based
273 films prepared by heat pressing or from the MC-HMPC coatings was studied at three
274 different temperatures (15, 25 and 35°C). Figure 1(a) shows the plots of mass fraction (m_t/m_∞)
275 of carvacrol released from the heat pressed films into the simulant versus time at the three
276 temperatures. The migration of carvacrol from the MC-HPMC coated samples into isooctane
277 is shown in Figure 1(b). Similar behaviour was observed for thymol and/or linalool migration
278 into isooctane at these temperatures for the heat pressed and the MC-HPMC coated films (not

279 shown). From Figure 1(a) and (b), it can be seen that carvacrol is readily released into
280 isooctane from both film forms.
281
282 It is evident from Figure 1(a) that the higher the temperature, the faster is the migration rate
283 of carvacrol, as could have been anticipated. At the lowest temperature of 15°C, the release of
284 carvacrol into isooctane reaches equilibrium within *ca.* 9000 s. For thymol and linalool at
285 this temperature equilibrium is achieved within *ca*. 7200 s (data not shown). Increasing the
286 temperature to 35°C increased the release rate of carvacrol and equilibrium was attained
287 within *ca.* 7200 s (see Figure 1(a)). In the heat pressed films containing thymol or linalool
288 similar migration profiles were demonstrated and the time to reach equilibrium at 35°C was
289 *ca*. 5400 s for both AM agents. The increased release rate of the AM agents from the starch-
290 based films at the higher temperatures is attributed to the enhanced mobility of the AM
291 molecules at the elevated temperatures (Zhu, Shentu, Liu & Weng, 2006). From Figure 1(b) it
292 can be seen that the release of carvacrol from the MC-HPMC coatings also increases with the
293 increase in temperature as again could have been anticipated. Similar trends were obtained
294 for the release of thymol or linalool into isooctane at 15, 25 and 35°C (data not shown).
295
296 The release data for the AM agents in the heat pressed and MC-HPMC coated films shown in
297 Figure 1 were further analysed in terms of an overall kinetic model and a diffusion model.
298 The overall kinetic analysis plots for the release of carvacrol from the heat pressed and the
299 MC-HPMC coated films at 15, 25 and 35°C, are shown in Figures 2(a) and 2(b) respectively.
300 In all cases the data fit an overall kinetic model with an expected increase in the release rate
301 with increasing temperature. Similar trends were observed for the migration of thymol and
302 linalool from their respective substrate films at these temperatures (data not shown). The
303 initial release rate, v_0 and the overall rate constant for release, k_1 that were obtained from the

304 analysis of the data by the kinetic model for the two kinds of films are presented in the Table
305 1.
306 The results shown in Table 1 along with the plots in Figure 2 demonstrate that an overall
307 first-order kinetics model adequately describes the release of the three AM agents into
308 isooctane from the starch-based systems. In the case of both kinds of film, the initial release
309 rate and the overall rate constant consistently increased with the increase in temperature from
310 15°C to 35°C. This observation is consistent with that of Han and Floros (1997) who have
311 stated that an increase in temperature has a significant effect on the migration of AM agents
312 from films.
313
314 The data from the kinetic model show an average increase in the initial release rate of about
315 310% and an average increase in the rate constant of about 200% for all samples over the
316 temperature range 15 to 35°C. These increases are somewhat lower than what could be
317 expected from the principal that the rate of a chemical or physical process doubles
318 approximately every 10°C rise in temperature. This deviation from the expected release
319 behaviour may be due to hydrogen bonding effects between the AM agents and the different
320 polymer matrices and/or due to tortuosity effects created within either of these matrices that
321 reduce the sensitivity to changes in temperature. As one would expect, the rate constant for
322 the migration of carvacrol, thymol and linalool from the MC-HPMC coatings are higher than
323 those obtained for the heat pressed samples. This observation may be attributed to the
324 differences in the concentration and different locations of AM agents in the two kinds of film
325 matrices. The experimental results were also analysed by the diffusion model of migration.
326 To apply this model, the migration of AM agents into the food simulant from the two kinds of
327 films was considered in two domains: the short-term and the long-term migration (Crank,
328 1975; Miltz, 1987).

329

330 Figures 3(a) and 3(b) show plots of m_t/m_∞ versus $t^{1/2}$ for the short-term release of carvacrol at
331 25°C from the heat pressed starch-based film and of $\ln(1 - m_t/m_\infty)$ versus t for the long-term
332 release respectively. Similar behaviour to that depicted in Figure 3 was also observed for the
333 release of carvacrol into isooctane at 15 and 35°C. Similar results to those shown in Figure 3
334 were found for the heat pressed starch-based films containing thymol or linalool. The
335 linearity of the plots at $m_t/m_\infty < 0.6$ with respect to $t^{1/2}$ demonstrates that the data are well
336 described by the diffusion model given in equation (3) for the short-term migration of the
337 AM agent. In the long-term migration of carvacrol from the moulded starch-based films, the
338 linearity of $\ln(1 - m_t/m_\infty)$ versus time (for the long-term migration ($m_t/m_\infty > 0.6$), according to
339 equations 4 and 5) is also very good with correlation coefficients of $r^2 = 0.991$, 0.963 and
340 0.985 for 15, 25 and 35°C respectively.
341

342 Figure 4 shows the short-term and long-term analyses of the migration of carvacrol from the
343 coated films. The respective behaviour of these systems is similar to those shown in Figure 3
344 with good linear correlations in both time regimes. The results obtained for carvacrol and
345 thymol systems confirm that the rate of AM agent release increases with temperature in the
346 range of 15 to 35°C as could have been anticipated and are in agreement with the migration
347 pattern found by Mistry (2006) for LDPE films incorporated with linalool or carvacrol. The
348 complete numerical results of the analyses depicted in Figures 3 and 4 are also included in
349 Table 1 for direct comparison.
350

351 The plot of $\ln(1 - m_t/m_\infty)$ versus time for the migration of carvacrol at 25°C from the heat
352 pressed starch-based film into isooctane yielded a straight line ($r^2 = 0.963$) as shown in
353 Figure 3(b). From the slope of this line the diffusion coefficient, D, was determined. The

354 diffusion coefficients determined from the gradients of similar regression lines shown in
355 Figures 3(b) and 4(b) are presented in Table 1 for all studied systems. The results listed in
356 Table 1 confirm that the diffusion coefficients of carvacrol, thymol and linalool in the heat
357 pressed as well as in the MC-HPMC coated films increased with increasing temperature.
358
359 The effect of temperature on the diffusion coefficient for the migration of carvacrol into
360 isooctane is plotted in Figure 5 according to the Arrhenius relationship given in equation (6).
361 Similar plots (not shown) were obtained for the two other AM agents. From the slopes of
362 these plots values of the activation energy for the diffusion process, $E_{a,}$ were calculated**.** The
363 activation represents the sensitivity of the diffusion coefficient to temperature (Chung,
364 Papadakis & Yam, 2001).
365
366 The activation energies for the migration of carvacrol, thymol and linalool from the heat
367 pressed systems were found to be: 26.2, 33.6 and 25.5 kJ mol^{-1} and for the MC-HMPC coated
368 systems: 31.3, 29.9 and 22.5 kJ mol^{-1} respectively. It can be seen that there is a clear
369 difference between the E_a values of the AM agents in the heat pressed films compared with
370 the MC-HPMC coated films. In the heat pressed films, thymol and linalool exhibited higher
371 E_a values than in the coated films whereas the reverse was found for carvacrol. These
372 observations presumably reflect the differences in the molecular interactions and hydrogen
373 bonding that exists amongst the different AM agents and the polymeric matrices. The
374 observed differences may also stem from the different concentrations of AM agents in the
375 moulded starch-based films and in the MC-HPMC coatings. According to Cho et al. (2005), a
376 high concentration of AM agent in a polymer matrix may reduce the activation energy for
377 diffusion due to lower molecular movements.
378

4 Conclusions

A first-order model satisfactorily described the kinetics of the overall release of carvacrol, thymol and linalool from heat pressed and MC-HPMC coated starch-based films. The results suggest that carvacrol, thymol and linalool incorporated into starch-based films or a MC-HPMC coating is readily released into isooctane. The short-term and long-term diffusion models also adequately describe the migration of these AM agents. The results further suggest that an increase in temperature has a significant effect on the migration of each of the AM agents from either the moulded starch-based films or the MC-HPMC coatings into isooctane. The diffusion coefficients and the rate constants determined from the diffusion model and the overall kinetics analyses increased with an increase in the temperature. An Arrhenius relationship was found to adequately describe the relationship between the diffusion coefficient and temperature for all systems studied. This enabled the activation energy for diffusion of the AM agents to be determined. The high efficiencies of release of carvacrol, thymol and linalool from starch-based films point to the great potential of these systems in AM packaging of food products to extend their shelf life and reduce the risk of food-borne illness associated with microbial contamination. Further studies are underway in our laboratory to determine the efficiency of these AM films during storage.

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493

494 **Figure Captions**

495

496 **Figure 1**. Plot of the mass fraction m_t/m_∞ of carvacrol released into isooctane versus t at
497 (●) 15, (○) 25 and (Δ) 35°C from: (a) heat pressed and (b) MC-HPMC coated
498 starch-based film.

499

500 **Figure 2**. Plots of $\ln(1 - m_t/m_\infty)$ versus t for the migration of carvacrol into isooctane at (●)
501 15, (○) 25 and (Δ) 35°C from: (a) heat pressed and (b) MC-HPMC coated
502 starch-based film.

503

504 **Figure 3**. Plots of: (a) m_t/m_∞ versus $t^{1/2}$ and (b) $\ln(1 - m_t/m_\infty)$ versus t for the migration of
505 carvacrol from heat pressed starch-based film into isooctane at 25°C.

506

507 **Figure 4**. Plots of: (a) m_t/m_∞ versus $t^{1/2}$ and (b) $\ln(1 - m_t/m_\infty)$ versus t for the migration of
508 carvacrol from MC-HPMC coatings on starch-based films into isooctane at
509 25°C.

510

511 **Figure 5**. Arrhenius plots of $\ln(D)$ versus $1/T$ for the release of carvacrol into isooctane
512 from: (a) the heat pressed and (b) MC-HPMC coated starch-based films

513

Table 1: Kinetic and the diffusion analyses for the release of carvacrol, thymol and linalool from: (a) heat pressed and (b) MC-HMPC coated starch-based film into isooctane at 15, 25 and 35°C.

AM Agent	**Temperature** /°C	**Kinetic Analysis**		**Diffusion Analysis**	
		$v_0 \times 10^{-4}$ /g s^{-1}	$k_1 \times 10^{-4}$ /s^{-1}	$D \times 10^{-13}$ /m^2 s^{-1}	$k_2 \times 10^{-4}$ /s^{-1}
(a) Release from Heat Pressed Starch-Based Film					
Linalool	15	0.1	1.8	9.5	1.1
	25	0.2	2.3	13.0	1.7
	35	0.4	3.2	19.0	2.4
Carvacrol	15	0.2	2.0	6.3	1.2
	25	0.3	2.7	7.9	1.9
	35	0.5	3.6	12.9	3.0
Thymol	15	0.1	1.8	12.0	0.9
	25	0.2	2.4	21.1	1.7
	35	0.6	3.5	29.7	2.8
(b) Release from MC-HMPC Coating of Starch-Based Film					
Linalool	15	0.3	2.8	5.1	2.5
	25	0.4	3.1	6.3	2.5
	35	0.7	3.6	9.4	4.8
Carvacrol	15	1.2	1.5	2.2	1.5
	25	1.8	2.8	3.9	1.8
	35	3.6	3.8	8.7	2.4
Thymol	15	0.8	1.7	2.7	1.6
	25	1.2	2.2	3.0	2.1
	35	3.1	3.4	6.1	2.8

547 **Figure 1**

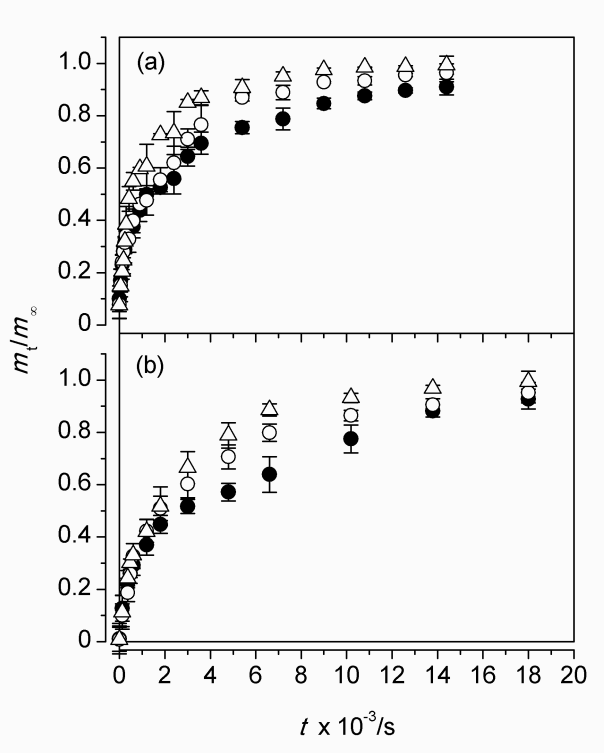

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Figure 2

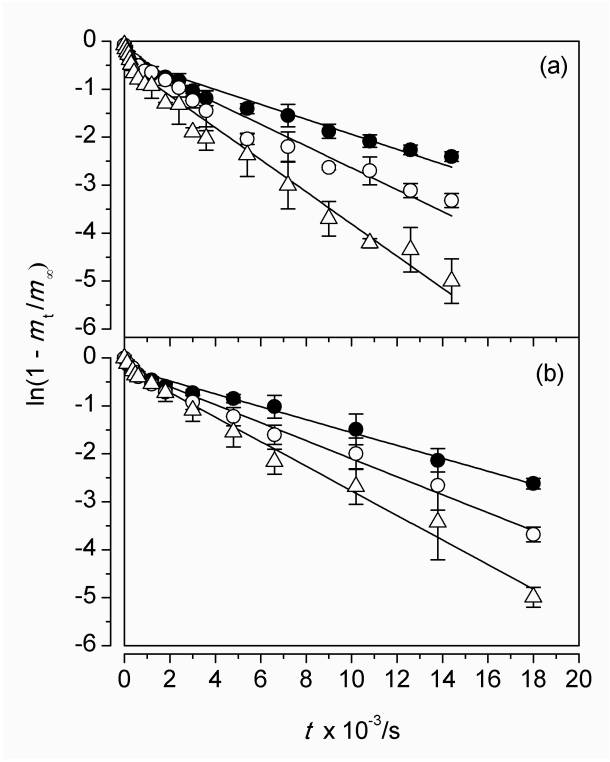
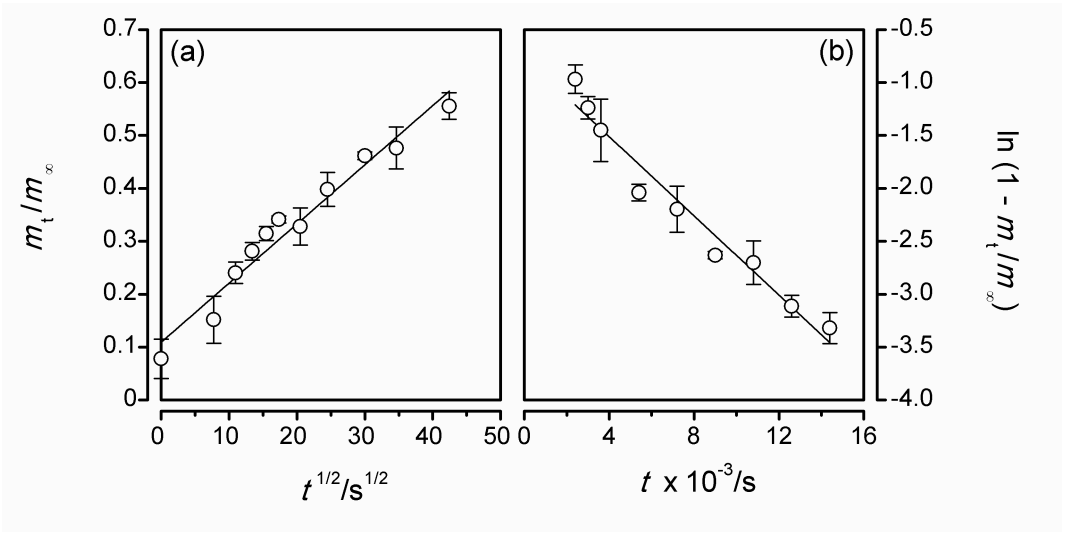
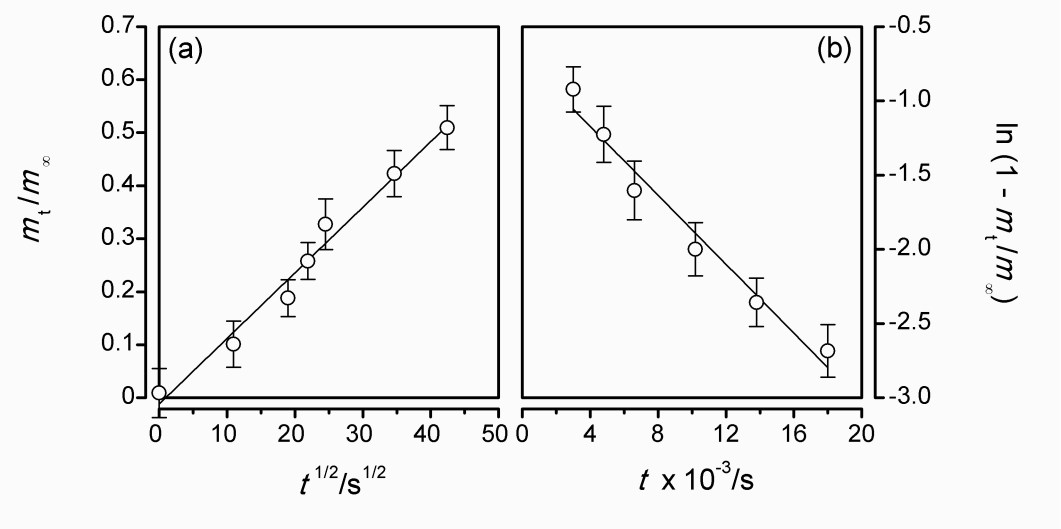


Figure 3



555 **Figure 4**



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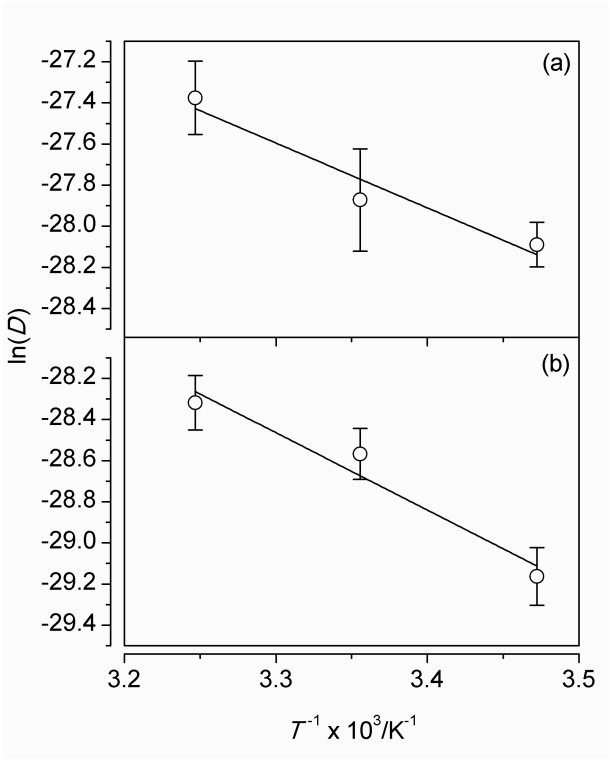
558 **Figure 5**



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