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1 **Release and antioxidant activity of carvacrol and thymol from polypropylene active**
2 **packaging films**

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12

13 **ABSTRACT**

14 The migration of antioxidant (AO) agents, carvacrol and thymol, from polypropylene (PP)
15 packaging films containing the studied compounds at 80 g/kg separately and an equimolar
16 mixture of them into food simulants was investigated. Fast and reliable analytical procedures
17 were developed and validated for the analysis of the studied AOs in food simulants. For
18 aqueous food simulants, solid phase extraction followed by GC-MS analysis was performed.
19 Fatty food simulants were directly analyzed by GC-MS and HPLC-UV for isooctane and
20 ethanol 950 mL/L, respectively. The release of AOs from the films was dependent on the type
21 of food stimulant and AO incorporated. In particular, high levels of migration were obtained for
22 both AOs into isooctane, showing thymol higher migration. The release kinetics of AOs from
23 PP films showed a Fichian behaviour with diffusion coefficients ranging from 1-2$\times10^{-14}$ m^2/s;
24 except for the diffusion into isooctane where 4-6 higher values were obtained. The antioxidant
25 activity of migration extracts was confirmed by the DPPH method, showing thymol a higher
26 antioxidant capacity especially into isooctane with a 42.2 % of inhibition. The obtained results
27 suggest that carvacrol and thymol show a potential use as AOs for active packaging for
28 extending the shelf-life of food products.

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29 **Keywords:** Active packaging; Carvacrol; Thymol; Migration; Diffusion

30

31 1. Introduction

32 Antioxidant active food packaging is a growing alternative to common procedures to
33 protect sensitive oxidation of food such as the addition of antioxidants directly in food samples
34 in combination with vacuum or modified atmosphere (Lopez-de-Dicastillo et al., 2011). These
35 systems are usually based on materials in which some additives showing antioxidant properties
36 are added directly into the polymer matrix. These additives can play a double role: a food
37 protection by their controlled release, in particular avoiding fat and pigment components
38 oxidation (Del Nobile et al., 2009); and to protect the polymer from degradation during
39 processing. In fact, the addition of antioxidants to polyolefins is a common practice during film
40 manufacturing (Siró et al., 2006; Tovar, Salafranca, Sanchez, & Nerin, 2005).

41 The new trends in using natural additives have produced a clear increase in the number of
42 studies based on natural active compounds, such as α-tocopherol (Barbosa-Pereira et al., 2013),
43 aromatic plant extracts (Dopico-García et al., 2011; Lopez-de-Dicastillo et al., 2011) and
44 polyphenols from natural oils (Park et al., 2012; Peltzer, Wagner, & Jiménez, 2009). The
45 principal constituents of oregano essential oil, thymol and carvacrol, exhibit a high antioxidant
46 activity as it has been reported (Tomaino et al., 2005); and they are generally recognized as safe
47 (possess "GRAS" status) and as flavouring substances according to European Commission
48 Decision 2002/113/EC. Their antioxidant activity can be evaluated by using diverse methods,
49 such as DPPH (2,2'-diphenyl-1-picrylhydrazyl), which is usually used due to its simple, rapid,
50 sensitive, and reproducible procedure (Ozcelik, Lee, & Min, 2003).

51 The release rate of AOs from the packaging material can be evaluated by using migration
52 studies, which are usually performed using food simulants and conditions specified in European
53 food packaging regulations (EC, 2011; Kuorwel, Cran, Sonneveld, Miltz, & Bigger, 2013).
54 Migration is the result of diffusion, dissolution and equilibrium processes involving the mass
55 transfer of low molecular mass compounds initially present in the package into a food sample or
56 food simulant; and it is often described by Fick's second law (Manzanarez-López, Soto-Valdez,

57 Auras, & Peralta, 2011). Chromatographic methods are usually used for identification and
58 quantification of migrated compounds (Salafranca, Pezo, & Nerín, 2009). Also, concentration
59 and/or isolation of analytes into a suitable solvent may be performed prior to chromatographic
60 analysis by the use of sample preparation and purification techniques such as solid phase
61 extraction (SPE) in order to improve detection and quantification (Burman, Albertsson, &
62 Höglund, 2005; Ridgway, Lalljie, & Smith, 2007).

63 In a previous study, the effect of the addition of carvacrol and thymol at different
64 concentrations to PP films on the thermal, structural, mechanical and functional properties was
65 studied as well as the evaluation of the antimicrobial activity against two typical food born
66 bacteria, *E. coli and S aureus*, (Ramos, Jiménez, Peltzer, & Garrigós, 2012). It was concluded
67 that the addition of both compounds at 80 g/kg showed some potential to be used as active
68 additives in PP formulations; showing thymol higher inhibition against the studied bacteria,
69 leading to higher antimicrobial activity. The aim of this study was to evaluate the release of
70 these compounds from PP films into different aqueous and fatty food simulants; including a
71 kinetics diffusion study and the evaluation of the antioxidant efficiency by the DPPH method.

72

73 **2. Experimental**

74 **2.1. Chemicals**

75 All reagents used were of analytical or chromatographic grade and were purchased from
76 Panreac (Barcelona, Spain). Standards of carvacrol (≥ 98 %), thymol (99.5 %), and 2,2-
77 diphenyl-1-picrylhydrazyl (DPPH, 95 %) were acquired from Sigma–Aldrich Inc. (St. Louis,
78 MO). Ultrapure water was obtained from a Millipore Milli-Q system (Millipore, Bedford, MA,
79 USA). Polypropylene PP ECOLEN HZ10K pellets (Hellenic Petroleum, Greece) was kindly
80 supplied by Ashland Chemical Hispania (Barcelona, Spain).

81

82 **2.2. Films preparation**

83 PP active films were obtained by melt blending at 190 ºC for 6 min at 50 rpm followed by
84 compression moulding at 190 ºC in a hot press, according to a method previously developed

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85 (Ramos et al., 2012). Three active formulations were obtained: PP containing 80 g/kg of thymol
86 (PPT8) or carvacrol (PPC8); and PP with an equimolar mixture of both additives at 80 g/kg
87 (PPTC8) to study a possible additive effect of both compounds. An additional sample without
88 any active compound was also prepared as control (PP0). The film thickness was measured
89 using a micrometer (Mitutoyo, Japan) at five random positions. The average thickness of PP
90 films incorporated with thymol (PPT8), carvacrol (PPC8) and both additives (PPTC8) was
91 found to be 185 ± 3, 192 ± 6 and 190 ± 4 µm, respectively. The final appearance of the films
92 was completely transparent and homogenous.

93

94 **2.3. Migration study**

95 **2.3.1. Release tests**

96 The release of AOs from PP films was performed into five food simulants according to
97 European Standard EN 13130-2005 (UNE-EN, 2005): distilled water (A), acetic acid 30 g/L
98 (B), and ethanol 100 mL/L (C) were used as aqueous food simulants; whereas ethanol 950
99 mL/L and isooctane were employed as fatty food simulants.

100 Migration studies were conducted in triplicate at 40 ºC for 10 days in an oven (J.P. Selecta,
101 Barcelona, Spain), except for isooctane studies which were performed at 20 ºC and 50% relative
102 humidity for 2 days in a climatic chamber (Dycometal, Barcelona, Spain). Double-sided, total
103 immersion migration tests were performed with 12 cm^2 of films and 20 mL of each simulant
104 (area-to-volume ratio around 6 dm^2/L). A blank test for each simulant was also carried out.

105

106 **2.3.2. Migration kinetics**

107 In order to study the release of carvacrol and thymol during a suitable period of time (15
108 days), a kinetic study was performed using acetic acid 30 g/L, ethanol 100 mL/L, ethanol 950
109 mL/L and isooctane as food simulants, at the same temperature conditions described in section
110 2.3.1. Extract samples were taken at 2, 6, 12, 24, 48 hours and 5, 10 and 15 days in triplicate.

111 The migration process is described by the kinetic of the diffusion of the migrant in the film
112 and it is expressed by the diffusion coefficient, D (m^2/s) (Manzanarez-López et al., 2011).

113 Considering the case of limited packaging, limited food, where migration occurs from a limited
114 volume packaging film into a well-mixed limited volume of food, the diffusion coefficients of
115 AOs can be determined by using a release kinetic model based in the Fick's second law
116 (Equation 1). In this case, the food sample initially does not contain any migrant, and as
117 migration occurs, the concentration of migrant in the food increases from zero $C_{F,0}$ to its
118 equilibrium value $C_{F,\infty}$. Equation (1) is the most rigorous general model for describing the
119 migration controlled by Fickian diffusion in a packaging film (Crank, 1975; Chung, Papadakis,
120 & Yam, 2002):

121 $$\frac{M_{F,t}}{M_{F,\infty}} = 1 - \sum_{n=1}^{\infty} \frac{2\alpha(1+\alpha)}{1+\alpha+\alpha^2 q_n^2} \exp\left[\frac{-Dq_n^2 t}{L_P^2}\right] \quad (1)$$

122 where $M_{F,t}$ and $M_{F,\infty}$ are the total amount of diffusing substance (AO) released by the film at
123 time t and after infinite time, respectively; L_P is the film thickness; q_n are the non-zero
124 positive roots of $\tan q_n = -\alpha \cdot q_n$; and α is the partition expressed as:

125 $$\alpha = (K_{FP} V_F) / V_p \quad (2)$$

126 where V_F and V_P are the volumes of the simulant and the polymer, respectively; and K_{FP} is the
127 partition coefficient of the active compound between the simulant and the polymer.

128 A simplified migration model derived from Equation (1) was proposed by Chung et al.
129 useful for linear regression analysis (Chung et al., 2002):

130 $$\left[\frac{1}{\pi} - \frac{1}{\alpha} \cdot \frac{M_{F,t}}{M_{P,0}}\right]^{0.5} = -\frac{D^{0.5}}{\alpha \cdot L_P} \cdot t^{0.5} + \frac{1}{\pi^{0.5}} \quad (3)$$

131 where $M_{P,0}$ is the initial amount of migrant in the packaging film (for a complete migration
132 $M_{P,0} = M_{F,\infty}$). Thus, the diffusion coefficient can be directly computed from the fitting of
133 Equation (3) to experimental migration data.

134

135 **2.4. Analysis of released antioxidants into food simulants**

136 The amount of released AOs into aqueous food simulants was analyzed by GC-MS with a
137 previous extraction and concentration step by SPE on an octadecyl cartridge (C18, 500 mg, 6

138 mL) (Teknokroma, Barcelona, Spain). A Büchi V-700 vacuum system (Flawil, Switzerland) and
139 a vacuum manifold from Teknokroma (Barcelona, Spain) were used for SPE sample processing.
140 The cartridge was previously conditioned with 4 mL methanol and 4 mL distilled water at 5
141 mL/min. Then, the extract was loaded and elution of the AOs was carried out with 4 mL
142 dichloromethane (1 mL/min). On the other hand, extracts obtained from isooctane and ethanol
143 950 mL/L were directly analyzed by GC-MS and HPLC-UV, respectively.

144 Stock (4000 mg/kg) and working solutions of each AO were prepared in the appropriate
145 solvent (dichloromethane, isooctane or ethanol 950 mL/L) depending on the food simulant and
146 chromatographic technique used and stored in a freezer. Carvacrol and thymol quantification
147 was performed using external calibration in triplicate.

148

149 **2.4.1. GC-MS analysis**

150 A Perkin Elmer TurboMass Gold GC-MS (Boston, MA, USA) operating in electronic
151 impact ionisation mode (70 eV) with a SPB-5 capillary column (30 m × 0.25 mm × 0.25 μm;
152 Supelco, Bellefonte, PA) was used. The column temperature was programmed from 60 ºC (1
153 min) to 120 ºC (1 min) at 10 ºC/min and to 150 ºC at 2 ºC/min (2 min). Helium was used as
154 carrier gas at 1 mL/min. Ion source and GC–MS transfer line temperatures were 250 and 270
155 ºC, respectively. Injector temperature was 270 ºC and 1 μL of extracts were injected (split mode
156 1:100).

157 Identification of thymol and carvacrol were performed in full scan mode (m/z 30–550) by a
158 combination of NIST mass spectral library and retention times of standard compounds.
159 Quantification of AOs was performed by using selected ion monitoring (SIM) mode focused on
160 m/z 91, 135 and 150. Retention times obtained for thymol and carvacrol were 10.7 and 11.0
161 min, respectively.

162

163 **2.4.2. HPLC-UV analysis**

164 A Shimadzu LC-20A liquid chromatograph equipped with UV detector and a LiChrospher
165 100 RP18 column (250 mm × 5 mm × 5 μm, Agilent Technologies) was used. The mobile phase

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166 consisted of acetonitrile: distilled water, 40:60 (v:v) at 1 mL/min. 20 µL of sample were
167 injected. Detection of carvacrol and thymol was performed at 274 nm with retention times of
168 18.9 and 21.0 min, respectively.
169

170 **2.4.3. Determination of antioxidant activity**

171 The antioxidant activity of AOs released into food simulants was analyzed in terms of
172 radical scavenging ability, using the stable radical DPPH method as proposed by Byun et al.
173 (Byun, Kim, & Whiteside, 2010) with some modifications. An aliquot of 100 µl of each
174 simulant extract was mixed with 3.9 mL of a methanolic solution of DPPH (23 mg/L) in a
175 capped cuvette. The mixture was shaken quickly at room temperature and the absorbance of the
176 solution was measured immediately at 517 nm every 1 min until the absorbance value was
177 stabilized (200 min), by using a Biomate-3 UV-VIS spectrophotometer (Thermospectronic,
178 USA). All analyses were performed in duplicate.

179 The ability to scavenge the stable radical DPPH was calculated as percent of inhibition (*I*
180 %) using the following Equation (4):

181 $$I(\%) = [(A_{Control}-A_{Sample})/A_{Control}]\cdot 100 \quad (4)$$

182 where $A_{Control}$ and A_{Sample} are the absorbances of the control at $t = 0$ min (using methanol instead
183 of sample) and of the tested sample at $t = 200$ min, respectively.
184

185 **2.5. Statistical analysis**

186 One way analysis of variance (ANOVA) was applied on DPPH experimental data with the
187 aid of the statistical program "Statgraphics Centurion program v.16.1.18 (StatPoint, Inc.,
188 Warrenton, USA)" and significant differences among sample data were recorded at $P < 0.05$
189 according to Tukey´s post hoc test (Barbara G. Tabachnick, 2013).
190

191 **3. Results and Discussion**

192 **3.1. Validation of the developed methods.**

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193 The analytical methods developed in this study were validated by assessing the main
194 analytical characteristics: linearity, precision (repeatability), detection (LOD) and quantification
195 (LOQ) limits and accuracy (recovery test).

196 Linear ranges were calculated with five calibration points, each one in triplicate (0.15-2.10
197 mg/kg in dichloromethane for SPE-GC-MS method and aqueous food simulants; 0.15-4.00
198 mg/kg for isooctane and 950 mL/L ethanol (v/v) for direct GC-MS and HPLC-UV analyses,
199 respectively). The calculated calibration curves gave an acceptable level of linearity for AOs
200 and studied methods with determination coefficients (R^2) ranging between 0.9963-0.9989, as
201 shown in Table 1.

202 **Table 1.**

203 Repeatability was evaluated by analyzing three replicates of standard solutions processed
204 the same day. All methods showed similar results for relative standard deviation (RSD) values
205 which were lower than 10 %. LOD and LOQ values were determined by using regression
206 parameters from the calibration curves ($3\ S_{y/x}/a$ and $10\ S_{y/x}/a$, respectively; where $S_{y/x}$ is the
207 standard deviation of the residues and *a* is the slope). As it can be seen in Table 1, lower values
208 for LOD and LOQ were obtained for thymol by the HPLC-UV method. On the other hand,
209 carvacrol showed lower values for these parameters considering the SPE-GC-MS method. As a
210 result, the LODs and LOQs values obtained for AOs ranged between 0.16-0.22 mg/kg and
211 between 0.50-0.74 mg/kg, respectively.

212 Recovery tests for the SPE-GC-MS method were accomplished, in triplicate, in order to
213 evaluate accuracy, by spiking aqueous food simulants with known amounts of each AO at three
214 concentration levels (0.03, 0.27 and 2.60 mg). A working solution containing both AOs (4000
215 mg/kg) in methanol was used. Satisfactory results were obtained for mean recoveries at all
216 levels tested (Table 2), ranging from 86.7-108.2 % with RSD values between 2.1-11.0 %. In
217 conclusion, the results obtained for methods validation were considered acceptable for the
218 determination of carvacrol and thymol migration in aqueous and fatty food simulants.

219 **Table 2.**

220

221 **3.2. Release of AOs into food simulants.**

222 Both AOs were readily released into aqueous and fatty food simulants from all PP films
223 (Table 3). Similar behaviour was observed for thymol and carvacrol migration under the same
224 migration conditions. However, thymol showed higher migration tendencies than carvacrol for
225 distilled water. Also, the amount of active additives released into fatty food simulants was
226 higher than those obtained for the aqueous ones. In particular, the highest migration levels were
227 obtained into isooctane at 20 ºC during 2 days compared with the rest of simulants where 40 ºC
228 and 10 days were used. This phenomenon might result from the higher affinity of the non-polar
229 PP to the also non-polar isooctane than to the highly polarity of ethanol 950 mL/L or other polar
230 food simulants used, therefore showing diffusion behaviour near to extraction rather than
231 migration, ultimately leading to high migration values.

232 **Table 3.**

233 The higher migration observed into fatty food simulants could be also attributed to two
234 factors: the higher solubility of migrated AOs into these solvents and the phenomenon of
235 swelling of the polymer matrix when the films come into contact with simulants (Suppakul,
236 Sonneveld, Bigger, & Miltz, 2011). Tehrany et al. (Tehrany, Mouawad, & Desobry, 2007)
237 indicated that migrant polarity can be a predominant controlling factor and that a simulant with
238 similar high polarity could have a great effect on sorption. In this sense, partitioning depends on
239 the polarity and solubility of the migrant in the food simulant. In our case, the higher release of
240 carvacrol and thymol into 950 mL/L ethanol rather than 100 mL/L ethanol showed the influence
241 of simulant polarity and AO solubility. Also, it can be assumed that certain amount of simulant
242 will penetrate into the matrix, enhancing the mobility of the target AOs inside the polymer
243 chains, which could promote migration. This behaviour has also been suggested in previous
244 studies for the migration of some AOs from polyolefins into fatty food simulants (Haider &
245 Karlsson, 2000; Kuorwel et al., 2013; Peltzer et al., 2009; Tovar et al., 2005).

246 Regarding aqueous food simulants, migration of AOs was also observed although the
247 solubility of both compounds in aqueous solutions is low; with migration values increasing by
248 using acetic acid 30 g/L and ethanol 100 mL/L. The migration of AOs into these simulants

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might be due mainly to two factors: the hydrophilic character of these additives described in literature (Peltzer et al., 2009); and the small size of these compounds, and thus faster diffusion, since the diffusion rate is governed by the mobility of the additives which is determined by the size and geometry of the diffusing compound (Haider & Karlsson, 2000; Reynier, Dole, Humbel, & Figenbaum, 2001). This behaviour was also observed for Mastromatteo et al. who demonstrated that the release of thymol from a swelling homogeneous polymeric network could be viewed as a result of the water diffusion from the outer water solution into the polymeric matrix, the macromolecular matrix relaxation and the diffusion of the active compound from the swollen polymeric network into the outer water solution (Mastromatteo, Barbuzzi, Conte, & Del Nobile, 2009). Also, it has to be considered the difference in polarity between the polar migrated substances and the non-polar polymer.

3.3. Antioxidant activity of migration extracts.

The antioxidant activity of carvacrol and thymol has been reported in previous studies, although the mechanism of such activity is not fully understood (Yanishlieva, Marinova, Gordon, & Raneva, 1999). The antioxidant activity depends not only on the compound structure but also on many other factors, such as concentration, temperature, light, simulant type and physical state of the system (e.g. pH).

Fig. 1

The antioxidant capacities of the obtained extracts were evaluated by DPPH radical assay (Fig. 1). No DPPH inhibition was observed in acetic acid 30 g/L, possibly due to the pH of this simulant. The absorbance of DPPH could decrease by light exposure, oxygen content, pH, and solvent type (Ozcelik et al., 2003). Regarding pH, it has been reported that generally, the increase of hydrogen ion concentration leads to the decrease of the reaction rate of chromogen radical scavenging, whereas under basic conditions proton dissociation of polyphenolics would enhance the reducing capacity of compounds (Pyrzynska & Pekal, 2013). On the other hand, all the other extracts presented an appreciable antioxidant activity, showing a significant inhibition of the DPPH radical. A higher antioxidant capacity was observed for thymol extracts, with the

277 highest inhibition obtained into isooctane (42.2 ± 1.1 %). The ANOVA results also showed that
278 independently of the variations introduced by the use of the different simulants, the formulation
279 with 80 g/kg of thymol was significantly different from the other ones regarding antioxidant
280 capacity (Fig. 2).

281 **Fig. 2.**

282 These results showed that thymol antioxidant activity was superior to that of carvacrol,
283 possibly due to greater steric hindrace of the thymol phenolic group; as different authors have
284 concluded when considering the mechanism of action of these compounds in the DPPH assay
285 (Wu, Luo, & Wang, 2012; Yanishlieva et al., 1999). Other compounds having a hydroxyl group
286 sterically hindered, such as BHT, have been also reported to possess high antioxidant activity
287 (Mastelic et al., 2008).

288 The DPPH inhibition values were correlated with the AOs amount released from the films
289 (Table 3). The highest amount of released additives was observed into fatty food simulants;
290 although no significant differences between isooctane and ethanol 950 mL/L were observed
291 with different formulations at $P < 0.05$ (Fig. 1). The obtained results indicate that a considerable
292 quantity of the AOs remain in the polymer matrix and consequently could act as active agents in
293 these materials. In this sense, the obtained PP films could be used as AO films for food
294 packaging applications in order to extend the shelf-life of food products, retarding oxidation
295 processes. In addition, it has been reported that these additives could be also used to protect the
296 polymer against oxidative degradation during processing and further use (Ramos et al., 2012).

297 Finally, the study of the combined activity of carvacrol and thymol in the same film at 40
298 g/kg of each compound (PPTC8) (Fig. 1) showed some additive effect between them as similar
299 results were obtained for samples with 80 g/kg of each compound (PPC8 and PPT8) separately.
300 This effect was more evident into fatty food simulants.

301

302 **3.4. Kinetics of AOs migration from active films.**

303 Information about diffusion coefficients through packaging materials is, in general, very
304 useful to evaluate the performance of new active packaging materials, as the critical point in

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305 antioxidant performance is the kinetics of release of the AO agent from the packaging. In order
306 to have a deeper knowledge of the migration mechanism of the target AOs from PP films,
307 kinetic experiments were carried out by using four different food simulants during 15 days. For
308 this study, only formulations containing the studied AOs at 80 g/kg separately were considered.

309 Fig. 3 and Fig. 4 show the release of thymol (a) and carvacrol (b) from PPT8 and PPC8
310 films as a function of time, respectively. Similar behaviour was observed for both compounds,
311 being rapidly released from their respective films into the studied food simulants, with an
312 expected increase in their release with increasing time and reaching equilibrium after
313 approximately 120 h.

314 **Fig. 3 and Fig. 4**

315 In a first approach, the higher amount of migrated analytes occurs to isooctane (see α
316 values in Table 4). Furthermore, for this simulant, at $t > 120$ h, the equilibrium is not well
317 defined and, for example, for PPT8 almost all previously encapsulated thymol is released in
318 isooctane. This can be argued in two different ways: either by experimental or physical grounds.
319 Starting with the former, it seems that the quantification of AOs in isooctane, for longer times,
320 lead to scatter values and, consequently, the somewhat relevant increase of the amount of AOs
321 released after 15 days cannot be relevant from statistics point of view. Another possible
322 justification is based on the behaviour of the release of active compounds from polyolefin films
323 immersed in food simulants by the "swelling-controlled" model (Suppakul et al., 2011).
324 According to this model, a simulant penetrates first into the polymer matrix and dissolves the
325 active agents thereby enabling their subsequent release. Indeed, it is expected that a simulant
326 uptake will cause polymer swelling. The migration of AOs is thus expected to increase with an
327 increase in the simulant penetration into the PP film, reaching a plateau when the matrix is
328 saturated with the simulant. However, many interactions take place during the migration of
329 species from polymers into liquids. Moreover, it has pointed out that a time-dependent
330 relaxation process could occur as a result of the swelling that takes place during the diffusion of
331 the liquid into the polymer. As a consequence, release rates change continuously and the
332 accurate mathematical analysis of the migration is difficult. The penetration of simulant

333 molecules facilitates further penetration by the plasticization of the polymer matrix, until a
334 plateau is reached. As pointed out before, for isooctane an increase of migration after reaching
335 the equilibrium was observed for both studied AOs at 360 h. This could be due to a combination
336 of temperature and a longer time in which the PP films were penetrated by isooctane producing
337 the increase on the release. Also, it can be speculated about the sorption of isooctane by the PP
338 matrix and a consequent creation of void spaces favouring the migration of the phenolic
339 compounds (Manzanarez-López et al., 2011).

340 **Table 4.**

341 The experimental release data shown in Fig. 3 were further analysed in terms of a diffusion
342 model according to Equation (3). However, the use of this equation in order to compute
343 diffusion coefficients needs the previous knowledge of partition values. According to the
344 previous discussion it can be assumed that the amount of AO release can be estimated as being
345 constant after ca. 120 h, and once Equation (3) can only be applied to $M_{F,t}/M_{P,0} < 0.6$, the use of
346 this equation do not interfere with the hypothetical (not confirmed) effect of the swelling-
347 process in the mass transport by diffusion. In these circumstances the release of AOs from PPT8
348 and PPC8 to different simulations can be modelled by using the following equation:

349 $$M_{F,t} / M_{P,0} = (M_{F,\infty} / M_{P,0}) (1 - e^{-k't}) \quad (5)$$

350 where k' is a constant related with the release rate constant. By fitting Eq. (5) to experimental
351 AOs release data (see solid lines in Figs. 2 and 3), the values of $M_{F,\infty}/M_{P,0}$ can be obtained and,
352 finally apparent partition coefficients (α_{ap}) values can be calculated through the equation:

353 $$M_{F\infty} / M_{P,0} = \alpha_{ap} / (1+\alpha_{ap}) \quad (6)$$

354 Both parameters, $M_{F,\infty} / M_{P,0}$ and α are reported in Table 4.

355 It should be stressed that the choice of Eq. (5) has been done once, for the border and limit
356 conditions of the experiments reported in this work, it describes a first order kinetic process
357 (Reis, Guilherme, Rubira, & Muniz, 2007). In general, the fitting determination coefficients are
358 higher than 0.96, with the exception of isooctane-containing systems where determination
359 coefficients of 0.819 (PPC8) and 0.713 (PPT8) were obtained.

360 The results obtained for thymol and carvacrol are shown in Fig. 5A and Fig. 5B,

361 respectively. As it can be seen, the linearity of $\left[\frac{1}{\pi}-\frac{1}{\alpha}\cdot\frac{M_{F,t}}{M_{F,0}}\right]^{0.5}$ versus $t^{0.5}$ was very good for

362 both AOs and all simulants tested with determination coefficient values (R^2) ranging from

363 0.961-0.995 for thymol and 0.983-0.992 for carvacrol, suggesting that experimental release data

364 is well described by the proposed diffusion model for short-range times.

365 **Fig. 5A and 5B**

366 The analysis of diffusion coefficients (*D*) (Table 4) shows that the diffusion process in

367 different simulants are independent on the AOs, with *D* values ranging from 1×10^{-14} to 2×10^{-14}

368 m^2/s. This behaviour was expected if considering that carvacrol and thymol are isomers having

369 similar molecular weights and polarity (Licciardello, Muratore, Mercea, Tosa, & Nerin, 2013).

370 The exception occurs for the diffusion of AOs into isooctane. In fact, *D* values for thymol and

371 carvacrol are 4 and 6 times higher for this simulant than the average values for remaining ones.

372 This is, however, in line with the discussion carried out in the previous section and with results

373 reported in section 3.2 as well. It is also worth noticing that the magnitude of *D* values found for

374 these films are one order of magnitude lower than those obtained for similar AOs and films

375 (Suppakul, Sonneveld, Bigger, & Miltz, 2011), suggesting that these films can provide a long

376 term release, or higher retention inside films, of AOs.

377

378 **Conclusions**

379 The release study of carvacrol and thymol from PP films into aqueous and fatty food

380 simulants was accomplished. Analytical methods for the determination of the target compounds

381 in the studied food simulants were successfully developed and validated. Release of AOs from

382 PP films showed some differences depending on the food simulant type used; being isooctane

383 the most exhaustive one resulting in high levels of migration. In addition, positive results were

384 obtained for all migration extracts by the antioxidant activity study performed by the DPPH

385 method, showing thymol a higher antioxidant capacity. Finally, the results obtained for the

386 migration kinetics study showed that carvacrol and thymol incorporated into PP films at 80 g/kg

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387 were readily released into different food simulants, being these additives still remaining in the
388 polymer after 15 days. In this sense, the high efficiencies of release of these compounds from
389 PP films point to the great potential of these systems in antioxidant packaging of different food
390 products to extend their shelf life and avoid the direct addition of additives to food formulations.

391

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397

398 **References**

399 Barbara G. Tabachnick, L. S. F. (2013). *Using Multivariate Statistics: Pearson New*
400 *International Edition* (6 ed.): Pearson Education.
401 Barbosa-Pereira, L., Cruz, J. M., Sendón, R., Rodríguez Bernaldo de Quirós, A., Ares, A.,
402 Castro-López, M. (2013). Development of antioxidant active films containing tocopherols to
403 extend the shelf life of fish. *Food Control, 31*(1), 236-243.
404 Burman, L., Albertsson, A.-C., & Höglund, A. (2005). Solid-phase microextraction for
405 qualitative and quantitative determination of migrated degradation products of antioxidants
406 in an organic aqueous solution. *Journal of Chromatography A, 1080*(2), 107-116.
407 Byun, Y., Kim, Y. T., & Whiteside, S. (2010). Characterization of an antioxidant polylactic acid
408 (PLA) film prepared with α-tocopherol, BHT and polyethylene glycol using film cast
409 extruder. *Journal of Food Engineering, 100*(2), 239-244.
410 Crank, J. (1975). *The Mathematics of Diffusion* (2nd ed. ed.). Oxford: Oxford University Press.
411 Chung, D., Papadakis, S. E., & Yam, K. L. (2002). Simple models for assessing migration from
412 food-packaging films. *Food Additives and Contaminants, 19*(6), 611-617.

413 Del Nobile, M. A., Conte, A., Buonocore, G. G., Incoronato, A. L., Massaro, A., & Panza, O.
414 (2009). Active packaging by extrusion processing of recyclable and biodegradable
415 polymers. *Journal of Food Engineering, 93*(1), 1-6.

416 Dopico-García, M. S., Castro-López, M. M., López-Vilariño, J. M., González-Rodríguez, M.
417 V., Valentão, P., Andrade, P. B. (2011). Natural extracts as potential source of antioxidants
418 to stabilize polyolefins. *Journal of Applied Polymer Science, 119*(6), 3553-3559.

419 EC. (2011). Commission regulation (EU) no 10/2011 of 14 January 2011 on plastic materials
420 and articles intended to come into contact with food. *Official Journal of the European*
421 *Union*, 2-89.

422 Haider, N., & Karlsson, S. (2000). Kinetics of migration of antioxidants from polyolefins in
423 natural environments as a basis for bioconversion studies. *Biomacromolecules, 1*, 481-487.

424 Kuorwel, K. K., Cran, M. J., Sonneveld, K., Miltz, J., & Bigger, S. W. (2013). Migration of
425 antimicrobial agents from starch-based films into a food simulant. *LWT - Food Science and*
426 *Technology, 50*(2), 432-438.

427 Licciardello, F., Muratore, G., Mercea, P., Tosa, V., & Nerin, C. (2013). Diffusional Behaviour
428 of Essential Oil Components in Active Packaging Polypropylene Films by Multiple
429 Headspace Solid Phase Microextraction–Gas Chromatography. *Packaging Technology and*
430 *Science, 26*(3), 173-185.

431 Lopez-de-Dicastillo, C., Nerin, C., Alfaro, P., Catala, R., Gavara, R., & Hernandez-Munoz, P.
432 (2011). Development of New Antioxidant Active Packaging Films Based on Ethylene Vinyl
433 Alcohol Copolymer (EVOH) and Green Tea Extract. *Journal of Agricultural and Food*
434 *Chemistry, 59*(14), 7832-7840.

435 Manzanarez-López, F., Soto-Valdez, H., Auras, R., & Peralta, E. (2011). Release of [alpha]-
436 Tocopherol from Poly(lactic acid) films, and its effect on the oxidative stability of soybean
437 oil. *Journal of Food Engineering, 104*(4), 508-517.

438 Mastelic, J., Jerkovic, I., Blazevic, I., Poljak-Blazi, M., Borovic, S., Ivancic-Bace, I. (2008).
439 Comparative study on the antioxidant and biological activities of carvacrol, thymol, and
440 eugenol derivatives. *Journal of Agricultural and Food Chemistry, 56*(11), 3989-3996.

441 Mastromatteo, M., Barbuzzi, G., Conte, A., & Del Nobile, M. A. (2009). Controlled release of
442 thymol from zein based film. *Innovative Food Science & Emerging Technologies, 10*(2),
443 222-227.
444 Ozcelik, B., Lee, J. H., & Min, D. B. (2003). Effects of Light, Oxygen, and pH on the
445 Absorbance of 2,2-Diphenyl-1-picrylhydrazyl. *Journal of Food Science, 68*(2), 487-490.
446 Park, H.-Y., Kim, S.-J., Kim, K. M., You, Y.-S., Kim, S. Y., & Han, J. (2012). Development of
447 Antioxidant Packaging Material by Applying Corn-Zein to LLDPE Film in Combination
448 with Phenolic Compounds. *Journal of Food Science, 77*(10), E273-E279.
449 Peltzer, M., Wagner, J., & Jiménez, A. (2009). Migration study of carvacrol as a natural
450 antioxidant in high-density polyethylene for active packaging. *Food Additives &*
451 *Contaminants: Part A: Chemistry, Analysis, Control, Exposure & Risk Assessment, 26*(6),
452 938-946.
453 Pyrzynska, K., & Pekal, A. (2013). Application of free radical diphenylpicrylhydrazyl (DPPH)
454 to estimate the antioxidant capacity of food samples. *Analytical Methods, 5*(17), 4288-4295.
455 Ramos, M., Jiménez, A., Peltzer, M., & Garrigós, M. C. (2012). Characterization and
456 antimicrobial activity studies of polypropylene films with carvacrol and thymol for active
457 packaging. *Journal of Food Engineering, 109*(3), 513-519.
458 Reis, A. V., Guilherme, M. R., Rubira, A. F., & Muniz, E. C. (2007). Mathematical model for
459 the prediction of the overall profile of in vitro solute release from polymer networks.
460 *Journal of Colloid and Interface Science, 310*(1), 128-135.
461 Reynier, A., Dole, P., Humbel, S., & Figenbaum, A. (2001). Diffusion coefficients of additives
462 in polymers. I Correlation with geometric parameters. *Journal of Applied Polymer Science,*
463 *82*, 2422-2433.
464 Ridgway, K., Lalljie, S. P. D., & Smith, R. M. (2007). Sample preparation techniques for the
465 determination of trace residues and contaminants in foods. *Journal of Chromatography A,*
466 *1153*(1-2), 36-53.
467 Salafranca, J., Pezo, D., & Nerín, C. (2009). Assessment of specific migration to aqueous
468 simulants of a new active food packaging containing essential oils by means of an automatic

469 multiple dynamic hollow fibre liquid phase microextraction system. *Journal of*
470 *Chromatography A, 1216*(18), 3731-3739.
471 Siró, I., Fenyvesi, É., Szente, L., De Meulenaer, B., Devlieghere, F., Orgoványi, J. (2006).
472 Release of alpha-tocopherol from antioxidative low-density polyethylene film into fatty food
473 simulant: Influence of complexation in beta-cyclodextrin. *Food Additives and*
474 *Contaminants, 23*(8), 845-853.
475 Suppakul, P., Sonneveld, K., Bigger, S. W., & Miltz, J. (2011). Diffusion of linalool and
476 methylchavicol from polyethylene-based antimicrobial packaging films. *LWT - Food*
477 *Science and Technology, 44*(9), 1888-1893.
478 Tehrany, E. A., Mouawad, C., & Desobry, S. (2007). Determination of partition coefficient of
479 migrants in food simulants by the PRV method. *Food Chemistry, 105*(4), 1571-1577.
480 Tomaino, A., Cimino, F., Zimbalatti, V., Venuti, V., Sulfaro, V., De Pasquale, A. (2005).
481 Influence of heating on antioxidant activity and the chemical composition of some spice
482 essential oils. *Food Chemistry, 89*(4), 549-554.
483 Tovar, L., Salafranca, J., Sanchez, C., & Nerin, C. (2005). Migration studies to assess the safety
484 in use of a new antioxidant active packaging. *Journal of Agricultural and Food Chemistry,*
485 *53*(13), 5270-5275.
486 UNE-EN. (2005). UNE-EN 13130-1:2005. Materials and articles in contact with foodstuffs.
487 Plastics substances subject to limitation. Guide to test methods for the specific migration of
488 substances from plastics to foods and food simulants and the determination of substances in
489 plastics and the selection of conditions of exposure to food simulants.
490 Wu, Y., Luo, Y., & Wang, Q. (2012). Antioxidant and antimicrobial properties of essential oils
491 encapsulated in zein nanoparticles prepared by liquid–liquid dispersion method. *LWT - Food*
492 *Science and Technology, 48*(2), 283-290.
493 Yanishlieva, N. V., Marinova, E. M., Gordon, M. H., & Raneva, V. G. (1999). Antioxidant
494 activity and mechanism of action of thymol and carvacrol in two lipid systems. *Food*
495 *Chemistry, 64*(1), 59-66.

496

497 **FIGURE CAPTIONS**

498 **Fig. 1.** Radical scavenging activity measured by DPPH, expressed as percent of inhibition,
499 obtained for migration extracts from the three formulations studied (isooctane: 20 ºC, 2 days;
500 rest of simulants: 40 ºC, 10 days) (m ± SD, n = 3). Different letters represent significant
501 difference at $P < 0.05$.

502 **Fig. 2.** Plot representing mean DPPH inhibition values (%) for the formulations PPT8, PPC8
503 and PPTC8. Different letter represent significant differences at $P < 0.05$.

504 **Fig. 3.** Release of thymol from PPT8 into different food simulants over 15 days. (A) Ethanol
505 100 mL/L, 40 ºC; (B) acetic acid, 40 ºC; (C) Ethanol 950 mL/L, 40 ºC; and (D) isooctane, 20
506 ºC. Solid lines were obtained by fitting Eq. (4) to experimental data. For further details see
507 section 3.4.

508 **Fig. 4.** Release of carvacrol from PPC8 into different food simulants over 15 days. (A) Ethanol
509 100 mL/L, 40 ºC; (B) acetic acid, 40 ºC; (C) Ethanol 950mL/L, 40 ºC; and (D) isooctane, 20 ºC.
510 Solid lines were obtained by fitting Eq. (4) to experimental data. For further details see section
511 3.4.

512 **Fig. 5.** Plots of $\left[\frac{1}{\pi}-\frac{1}{\alpha}\cdot\frac{M_{F,t}}{M_{F,0}}\right]^{0.5}-\frac{1}{\pi^{0.5}}$ versus $t^{0.5}$ for the migration of thymol (A) and
513 carvacrol (B) from PPT8 and PPC8 films, respectively, into different food simulants. Isooctane
514 (◊), 20 ºC; acetic acid (o), 40 ºC; ethanol 100 mL/L (□), 40 ºC; and ethanol 950 mL/L (Δ), 40
515 ºC.

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517 **Table 1.** Main analytical parameters obtained for the studied AOs using the optimized methods

518 (n = 3).

Analyte	Method	Parameter				
		Slope ± SD	Intercept ± SD	Linearity (R^2)[a]	LOD (mg/kg)[b]	LOQ (mg/kg)[c]
Carvacrol	SPE-GC-MS	18416 ± 1907	-3881 ± 2372	0.9968	0.16	0.54
	Direct HPLC-UV	13510 ± 333	1195 ± 82	0.9963	0.20	0.66
	Direct GC-MS	-2241 ±721	15103 ± 320	0.9982	0.22	0.73
Thymol	SPE-GC-MS	19792 ± 1120	-3868 ± 2377	0.9972	0.15	0.50
	Direct HPLC-UV	13936 ± 177	1091 ± 43	0.9989	0.10	0.34
	Direct GC-MS	-1852 ± 701	14568 ± 316	0.9981	0.22	0.74

[a] Number of calibration points = 5. Linear range: 0.15 – 2.10 (SPE-GC-MS); 0.15 – 4.00 (Direct HPLC-UV and GC-MS).

[b] Calculated for 3 $S_{y/x}$.

[c] Calculated for 10 $S_{y/x}$

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Table 2. Mean recoveries (%) and R.S.D. values (%) in parentheses obtained for each AO in aqueous simulants by SPE-GC-MS (n=3).

Analyte	**Simulant**	**Spiking level (mg)**		
		0.03	0.27	2.60
Carvacrol	Distilled water	98.1 (5.4)	94.7 (9.7)	108.2 (2.1)
	Ethanol 100 mL/L	95.2 (4.3)	100.3 (3.3)	89.8 (2.4)
	Acetic acid 30 g/L	99.4 (6.5)	88.0 (3.2)	97.2 (10.9)
Thymol	Distilled water	96.8 (5.8)	101.0 (3.6)	106.1 (2.4)
	Ethanol 100 mL/L	94.1 (3.8)	99.1 (3.4)	88.4 (2.5)
	Acetic acid 30 g/L	94.8 (4.9)	86.7 (3.2)	95.8 (11.0)

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Table 3. Release of AOs (mg/kg simulant) obtained from PP films into aqueous and fatty food simulants under conditions according to European Standard EN 13130-2005 (n = 3, m ± SD).

Analyte	Film	Simulant				
		Water[a]	Ethanol 100 mL/L[a]	Acetic acid 30 g/L[a]	Ethanol 950 mL/L[a]	Isooctane[b]
Carvacrol	PPC8	288 ± 20	718 ± 54	647 ± 47	880 ± 27	921 ± 157
	PPTC8	157 ± 19	285 ± 26	474 ± 44	347 ± 21	633 ± 34
Thymol	PPT8	433 ± 46	656 ± 30	689 ± 61	829 ± 19	1085 ± 112
	PPTC8	162 ± 18	362 ± 53	547 ± 51	367 ± 21	616 ± 49

Migration conditions: [a] 40 ºC, 10 days; [b] 20 ºC, 2 days

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Table 4. Diffusion coefficients ($D \times 10^{-14}$, m^2/s) calculated from equation 3 for the release of AOs from PP films into different food simulants (m ± SD, n = 3).

Analyte (Film)		**Simulant**			
		Ethanol 100 mL/L[a]	Acetic acid 30 g/L [a]	Ethanol 950 mL/L [a]	Isooctane[b]
Carvacrol (PPC8)	α_{ap}	1.38 ± 0.07	0.96± 0.04	1.27 ± 0.07	1.4 ± 0.1
	D	1.20 ± 0.05	1.7 ± 0.1	1.99 ± 0.07	9.4 ± 0.6
Thymol (PPT8)	α_{ap}	1.56 ± 0.1	1.44 ± 0.05	1.56 ± 0.08	2.4 ± 0.2
	D	1.75 ± 0.08	2.51 ± 0.02	1.01 ± 0.03	5.9 ± 0.1

Migration temperature: [a] 40 ºC; [b] 20 ºC

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Fig. 1

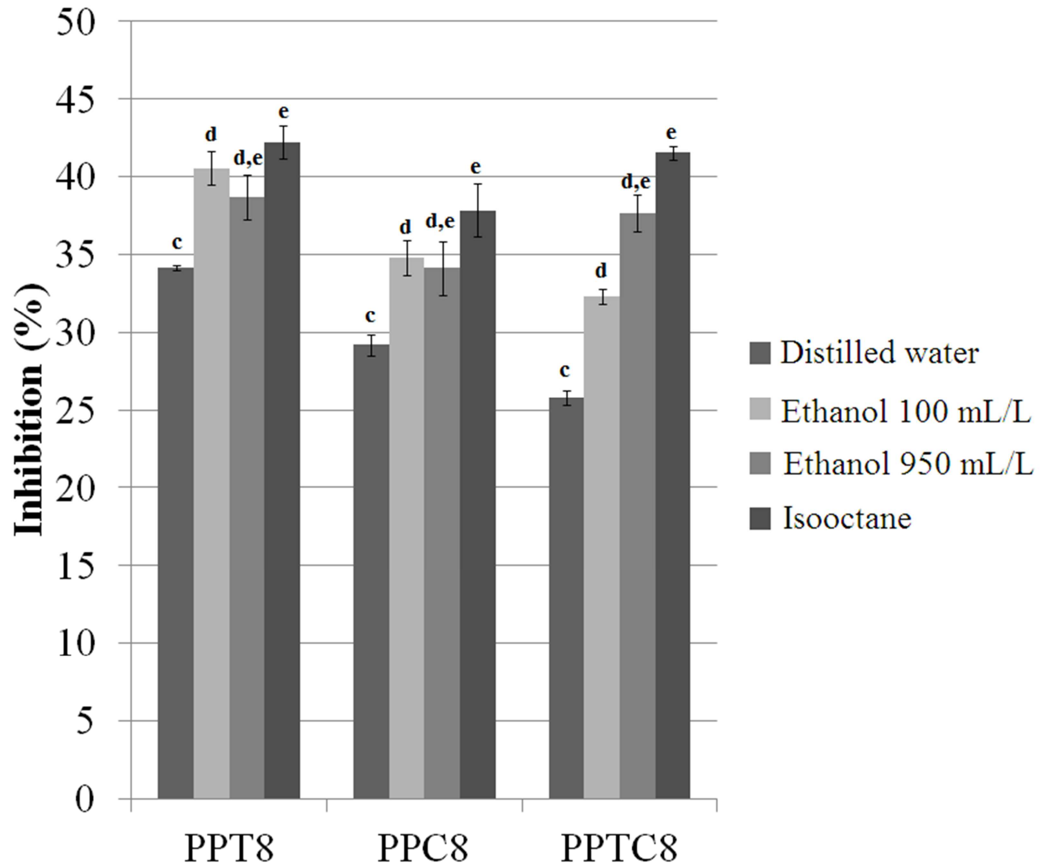

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Fig. 2

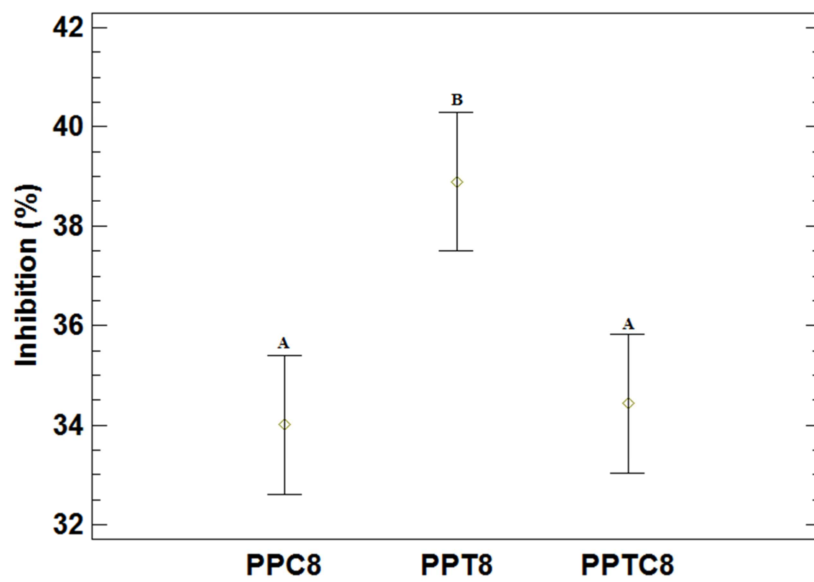

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Fig. 3

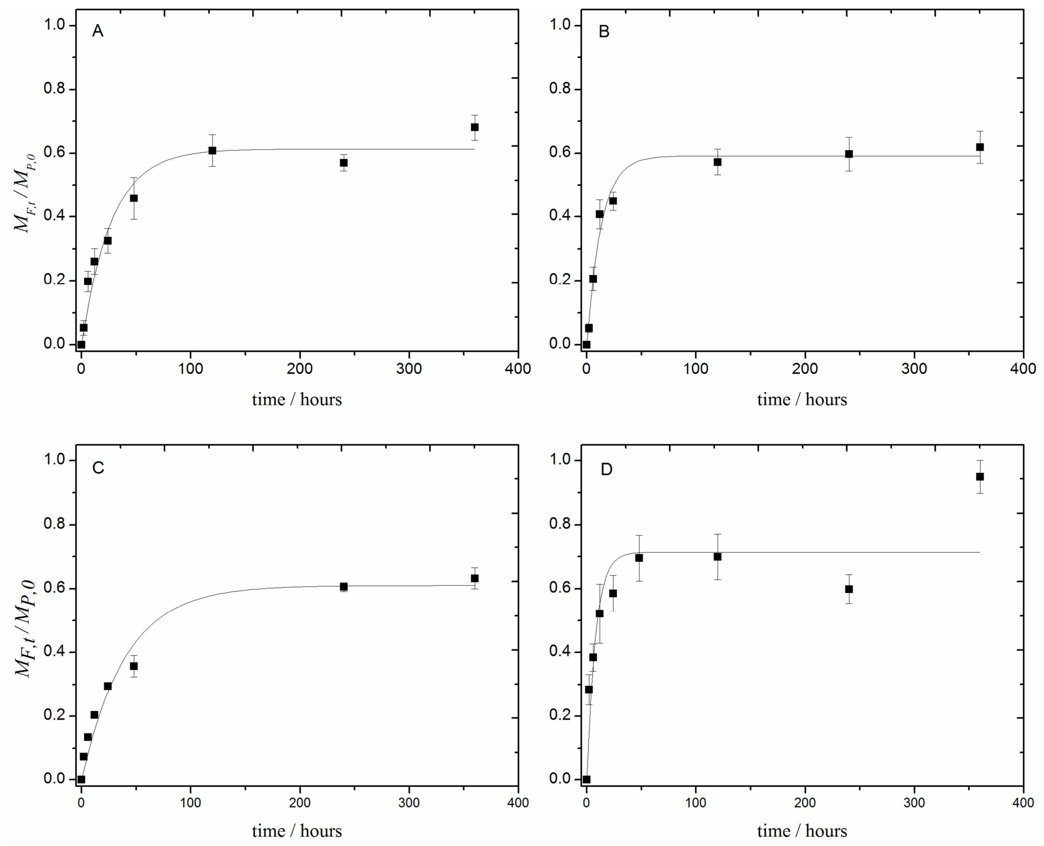

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Fig. 4

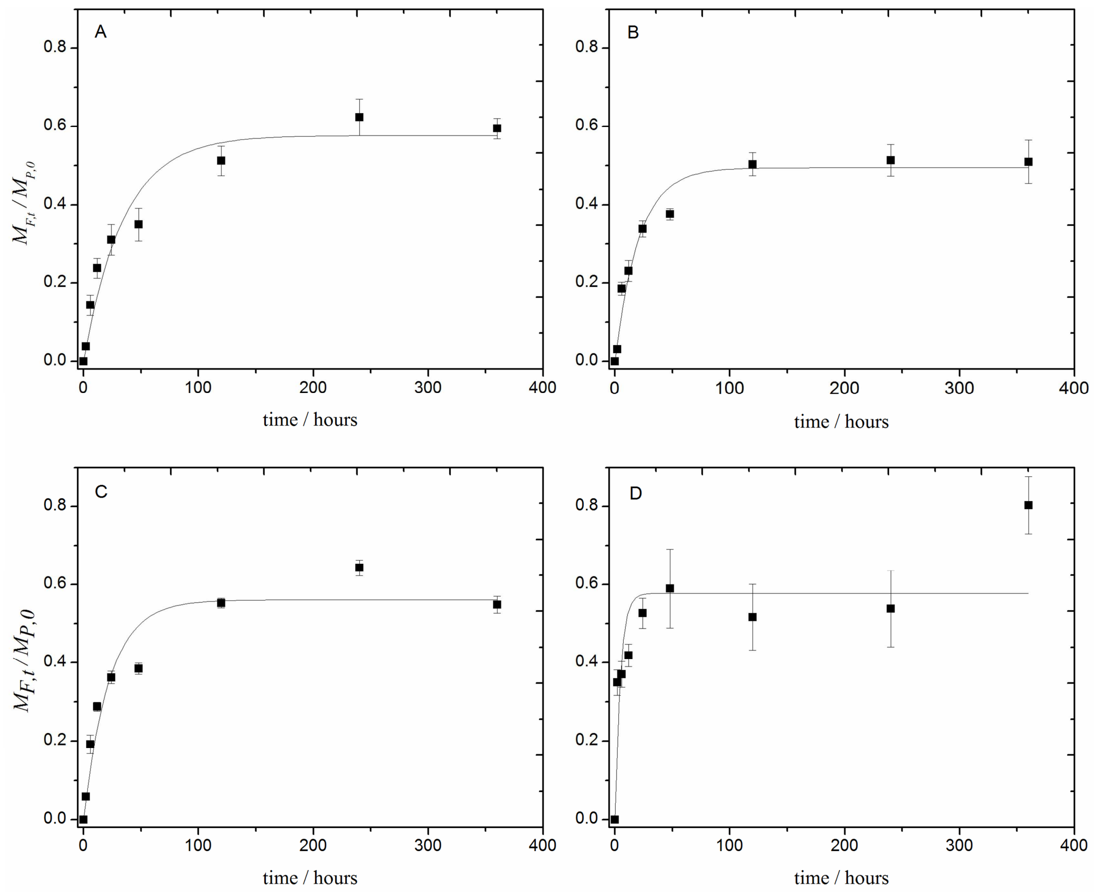

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Fig. 5A

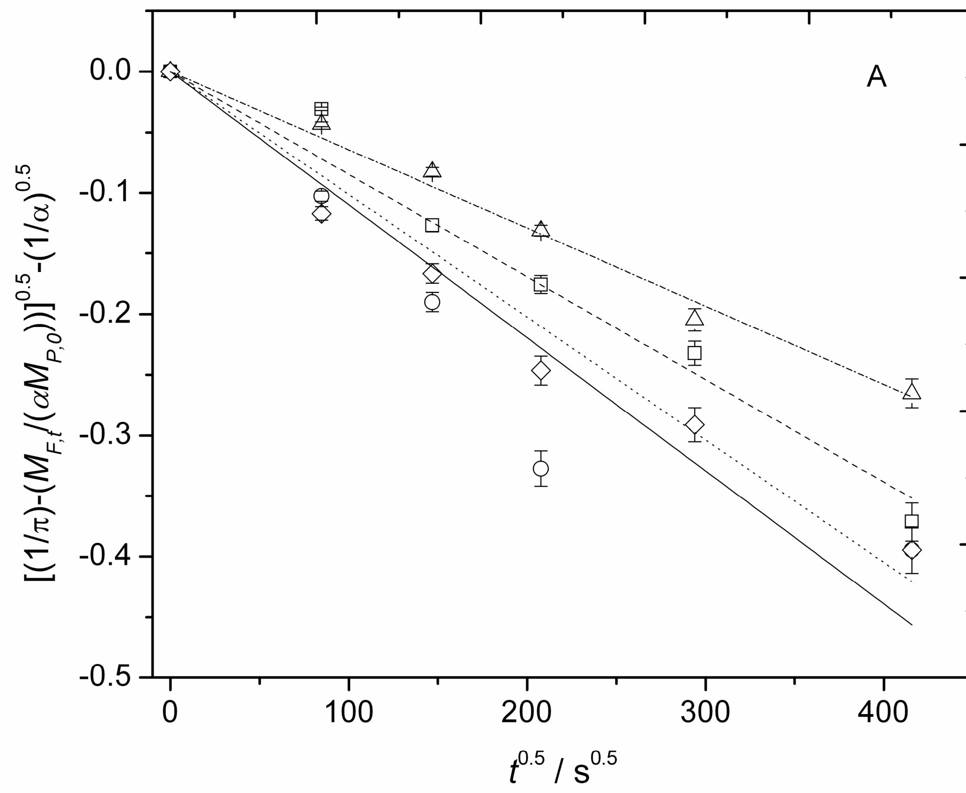

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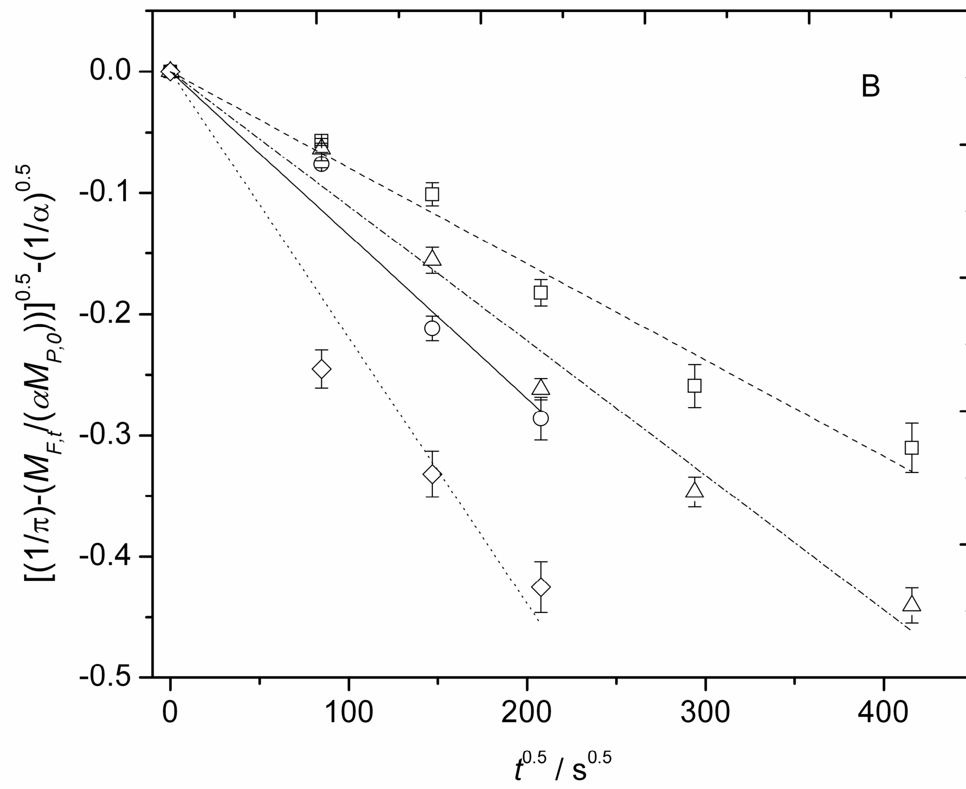
Fig. 5B



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Highlights

- The migration of carvacrol and thymol from PP films into food simulants was studied.

- The release of carvacrol and thymol from PP films showed a Fick's behaviour.

- The antioxidant activity of migration extracts was confirmed by the DPPH method.

- Thymol showed higher migration and antioxidant activity into fatty food simulants.

- Carvacrol and thymol show a potential use as antioxidants for active packaging.

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