LWT - Food Science and Technology 58 (2014) 470–477

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

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

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ARTICLE INFO

Article history:
Received 10 October 2013
Received in revised form
20 March 2014
Accepted 10 April 2014
Available online 25 April 2014

Keywords:
Active packaging
Carvacrol
Thymol
Migration
Diffusion

ABSTRACT

The migration of antioxidant (AO) agents, carvacrol and thymol, from polypropylene (PP) packaging films containing the studied compounds at 80 g/kg separately and an equimolar mixture of them into food simulants was investigated. Fast and reliable analytical procedures were developed and validated for the analysis of the studied AOs in food simulants. For aqueous food simulants, solid phase extraction followed by GC–MS analysis was performed. Fatty food simulants were directly analysed by GC–MS and HPLC-UV for isooctane and ethanol 950 mL/L, respectively. The release of AOs from the films was dependent on the type of food stimulant and AO incorporated. In particular, high levels of migration were obtained for both AOs into isooctane, showing thymol higher migration. The release kinetics of AOs from PP films showed a Fichian behaviour with diffusion coefficients ranging from 1 to 2 × 10^{-14} m^2/s; except for the diffusion into isooctane where 4-6 higher values were obtained. The antioxidant activity of migration extracts was confirmed by the DPPH method, showing thymol a higher antioxidant capacity especially into isooctane with a 42.2% of inhibition. The obtained results suggest that carvacrol and thymol show a potential use as AOs for active packaging for extending the shelf-life of food products.

1. Introduction

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

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

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

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http://dx.doi.org/10.1016/j.lwt.2014.04.019
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2009). Also, concentration and/or isolation of analytes into a suitable solvent may be performed prior to chromatographic analysis by the use of sample preparation and purification techniques such as solid phase extraction (SPE) in order to improve detection and quantification (Burman, Albertsson, & Höglund, 2005; Ridgway, Lalljie, & Smith, 2007).

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

2. Experimental

2.1. Chemicals

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

2.2. Films preparation

PP active films were obtained by melt blending at 190 °C for 6 min at 50 rpm followed by compression moulding at 190 °C in a hot press, according to a method previously developed (Ramos et al., 2012). Three active formulations were obtained: PP containing 80 g/kg of thymol (PPT8) or carvacrol (PPC8); and PP with an equimolar mixture of both additives at 80 g/kg (PPTC8) to study a possible additive effect of both compounds. An additional sample without any active compound was also prepared as control (PP0). The film thickness was measured using a micrometre (Mitutoyo, Japan) at five random positions. The average thickness of PP films incorporated with thymol (PPT8), carvacrol (PPC8) and both additives (PPTC8) was found to be 185 ± 3, 192 ± 6 and 190 ± 4 μm, respectively. The final appearance of the films was completely transparent and homogenous.

2.3. Migration study

2.3.1. Release tests

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

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

2.3.2. Migration kinetics

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

The migration process is described by the kinetic of the diffusion of the migrant in the film and it is expressed by the diffusion coefficient, D (m^2/s) (Manzanarez-López et al., 2011). Considering the case of limited packaging, limited food, where migration occurs from a limited volume packaging film into a well-mixed limited volume of food, the diffusion coefficients of AOs can be determined by using a release kinetic model based in the Fick's second law (Equation (1)). In this case, the food sample initially does not contain any migrant, and as migration occurs, the concentration of migrant in the food increases from zero $C_{F,0}$ to its equilibrium value $C_{F,\infty}$. Equation (1) is the most rigorous general model for describing the migration controlled by Fickian diffusion in a packaging film (Chung, Papadakis, & Yam, 2002; Crank, 1975):

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

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

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

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

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

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

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

2.4. Analysis of released antioxidants into food simulants

The amount of released AOs into aqueous food simulants was analysed by GC–MS with a previous extraction and concentration step by SPE on an octadecyl cartridge (C18, 500 mg, 6 mL) (Teknokroma, Barcelona, Spain). A Büchi V-700 vacuum system (Flawil, Switzerland) and a vacuum manifold from Teknokroma (Barcelona, Spain) were used for SPE sample processing. The cartridge was previously conditioned with 4 mL methanol and 4 mL distilled water at 5 mL/min. Then, the extract was loaded and elution of the AOs was carried out with 4 mL dichloromethane (1 mL/min). On the other hand, extracts obtained from isooctane and ethanol 950 mL/L were directly analysed by GC–MS and HPLC-UV, respectively.

Stock (4000 mg/kg) and working solutions of each AO were prepared in the appropriate solvent (dichloromethane, isooctane or

ethanol 950 mL/L) depending on the food simulant and chromatographic technique used and stored in a freezer. Carvacrol and thymol quantification was performed using external calibration in triplicate.

2.4.1. GC–MS Analysis

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

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

2.4.2. HPLC-UV Analysis

A Shimadzu LC-20A liquid chromatograph equipped with UV detector and a LiChrospher 100 RP18 column (250 mm × 5 mm × 5 μm, Agilent Technologies) was used. The mobile phase consisted of acetonitrile: distilled water, 40:60 (v:v) at 1 mL/min 20 μL of sample were injected. Detection of carvacrol and thymol was performed at 274 nm with retention times of 18.9 and 21.0 min, respectively.

2.4.3. Determination of antioxidant activity

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

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

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

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

2.5. Statistical analysis

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

3. Results and discussion

3.1. Validation of the developed methods

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

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

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

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

3.2. Release of AOs into food simulants

Both AOs were readily released into aqueous and fatty food simulants from all PP films (Table 3). Similar behaviour was

Table 1
Main analytical parameters obtained for the studied AOs using the optimized methods ($n = 3$).

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 $S_{y/x}$.

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)

observed for thymol and carvacrol migration under the same migration conditions. However, thymol showed higher migration tendencies than carvacrol for distilled water. Also, the amount of active additives released into fatty food simulants was higher than those obtained for the aqueous ones. In particular, the highest migration levels were obtained into isooctane at 20 °C during 2 days compared with the rest of simulants where 40 °C and 10 days were used. This phenomenon might result from the higher affinity of the non-polar PP to the also non-polar isooctane than to the highly polarity of ethanol 950 mL/L or other polar food simulants used, therefore showing diffusion behaviour near to extraction rather than migration, ultimately leading to high migration values.

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

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

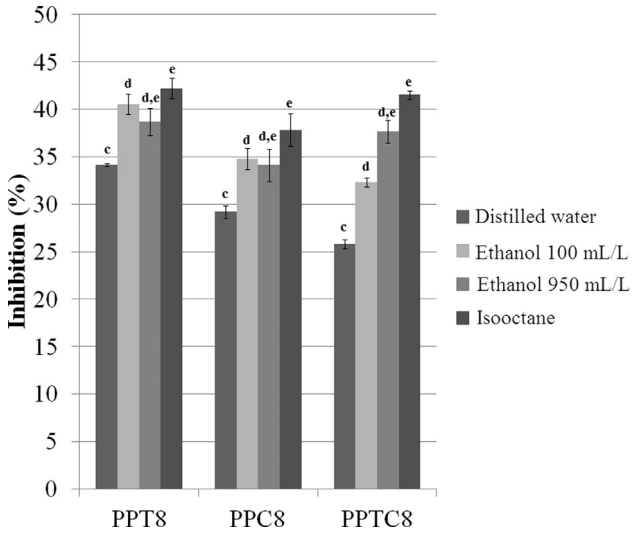

Fig. 1. Radical scavenging activity measured by DPPH, expressed as percent of inhibition, obtained for migration extracts from the three formulations studied (isooctane: 20 °C, 2 days; rest of simulants: 40 °C, 10 days) (m ± SD, $n = 3$). Different letters represent significant difference at $P < 0.05$.

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).

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

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 °C, 10 days.
[b] 20 °C, 2 days.

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

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

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

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

3.4. Kinetics of AOs migration from active films

Information about diffusion coefficients through packaging materials is, in general, very useful to evaluate the performance of new active packaging materials, as the critical point in antioxidant performance is the kinetics of release of the AO agent from the packaging. In order to have a deeper knowledge of the migration mechanism of the target AOs from PP films, kinetic experiments were carried out by using four different food simulants during 15 days. For this study, only formulations containing the studied AOs at 80 g/kg separately were considered.

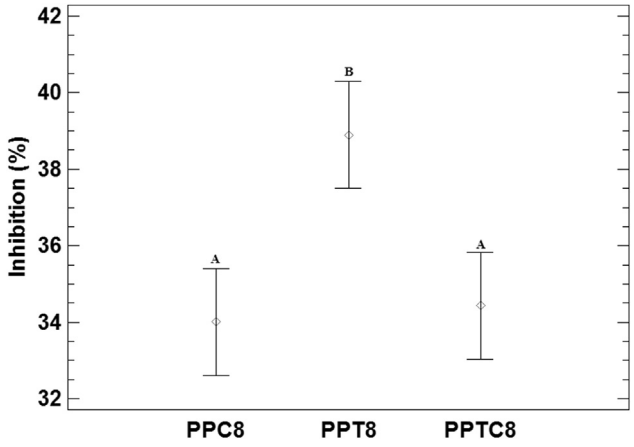

Fig. 2. Plot representing mean DPPH inhibition values (%) for the formulations PPT8, PPC8 and PPTC8. Different letter represent significant differences at $P < 0.05$.

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

In a first approach, the higher amount of migrated analytes occurs to isooctane (see α values in Table 4). Furthermore, for this simulant, at $t > 120$ h, the equilibrium is not well defined and, for example, for PPT8 almost all previously encapsulated thymol is released in isooctane. This can be argued in two different ways: either by experimental or physical grounds. Starting with the former, it seems that the quantification of AOs in isooctane, for longer times, lead to scatter values and, consequently, the somewhat relevant increase of the amount of AOs released after 15 days cannot be relevant from statistics point of view. Another possible justification is based on the behaviour of the release of active compounds from polyolefin films immersed in food simulants by the "swelling-controlled" model (Suppakul et al., 2011). According to this model, a simulant penetrates first into the polymer matrix and dissolves the active agents thereby enabling their subsequent release. Indeed, it is expected that a simulant uptake will cause polymer swelling. The migration of AOs is thus expected to increase with an increase in the simulant penetration into the PP film, reaching a plateau when the matrix is saturated with the simulant. However, many interactions take place during the migration of species from polymers into liquids. Moreover, it has pointed out that a time-dependent relaxation process could occur as a result of the swelling that takes place during the diffusion of the liquid into the polymer. As a consequence, release rates change continuously and the accurate mathematical analysis of the migration is difficult. The penetration of simulant molecules facilitates further penetration by the plasticization of the polymer matrix, until a plateau is reached. As pointed out before, for isooctane an increase of migration after reaching the equilibrium was observed for both studied AOs at 360 h. This could be due to a combination of temperature and a longer time in which the PP films were penetrated by isooctane producing the increase on the release. Also, it can be speculated about the sorption of isooctane by the PP matrix and a consequent creation of void spaces favouring the migration of the phenolic compounds (Manzanarez-López et al., 2011).

The experimental release data shown in Fig. 3 were further analysed in terms of a diffusion model according to Equation (3). However, the use of this equation in order to compute diffusion coefficients needs the previous knowledge of partition values. According to the previous discussion it can be assumed that the amount of AO release can be estimated as being 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 this equation do not interfere with the hypothetical (not confirmed) effect of the swelling-process in the mass transport by diffusion. In these circumstances the release of AOs from PPT8 and PPC8 to different simulations can be modelled by using the following equation:

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

where k' is a constant related with the release rate constant. By fitting Eq. (5) to experimental AOs release data (see solid lines in

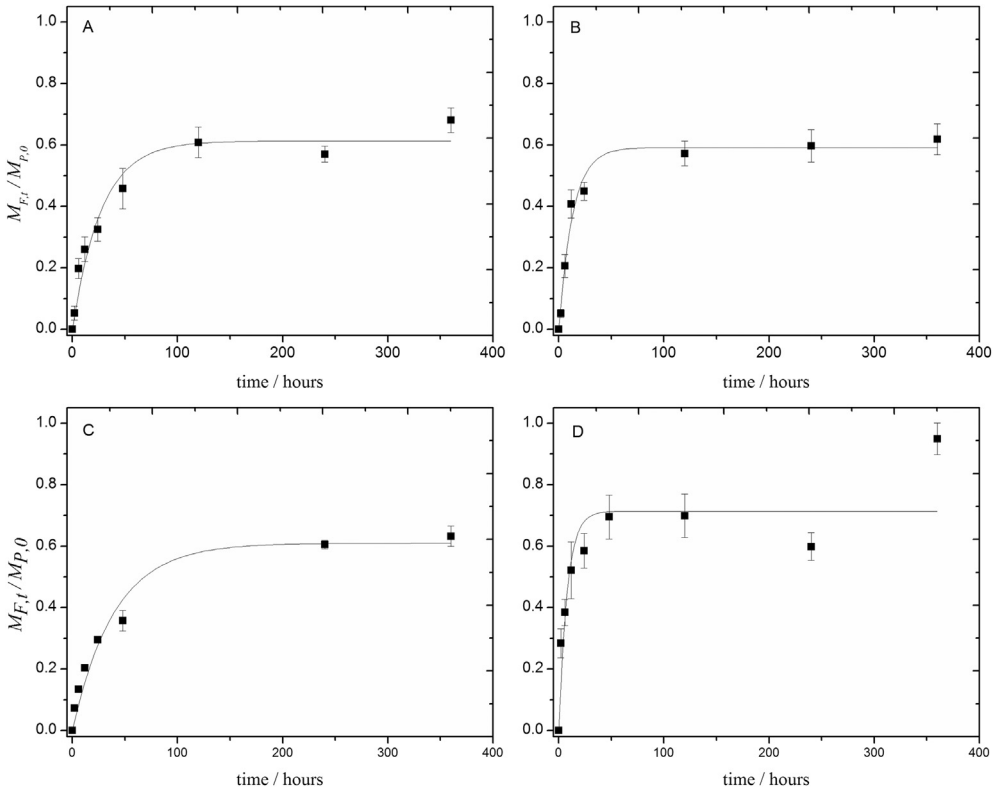

Fig. 3. Release of thymol from PPT8 into different food simulants over 15 days. (A) Ethanol 100 mL/L, 40 °C; (B) acetic acid, 40 °C; (C) Ethanol 950 mL/L, 40 °C; and (D) isooctane, 20 °C. Solid lines were obtained by fitting Eq. (4) to experimental data. For further details see Section 3.4.

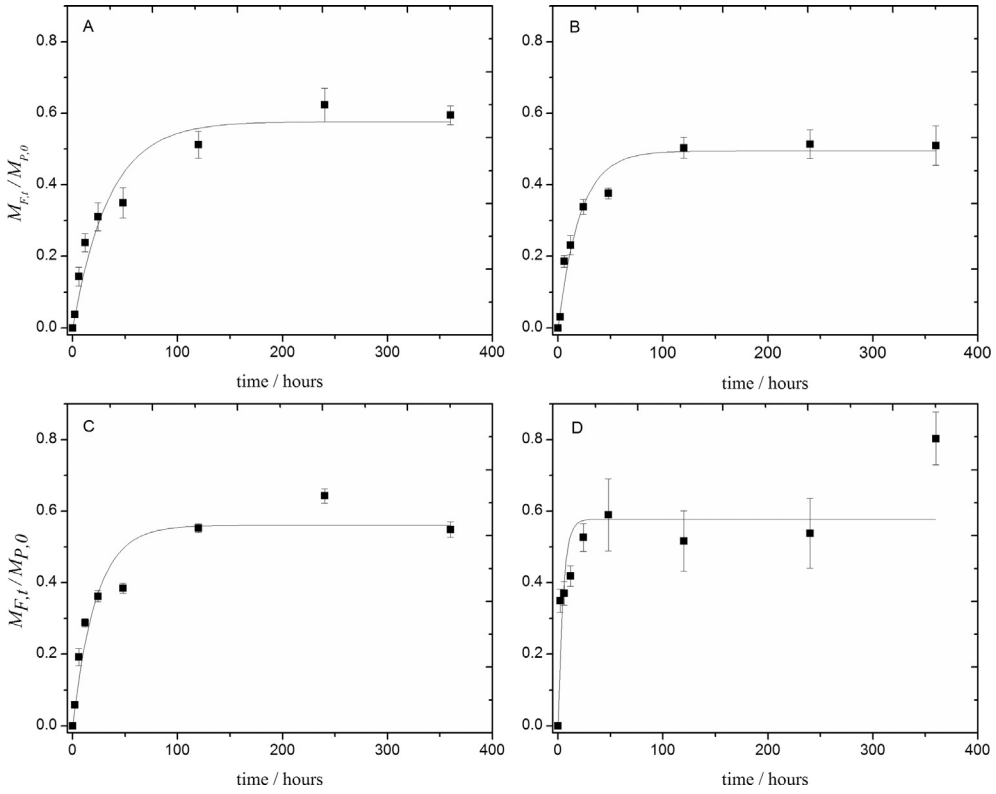

Fig. 4. Release of carvacrol from PPC8 into different food simulants over 15 days. (A) Ethanol 100 mL/L, 40 °C; (B) acetic acid, 40 °C; (C) Ethanol 950 mL/L, 40 °C; and (D) isooctane, 20 °C. Solid lines were obtained by fitting Eq. (4) to experimental data. For further details see Section 3.4.

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 °C.
[b] 20 °C.

Figs. 2 and 3), the values of $M_{F,\infty}/M_{P,0}$ can be obtained and, finally apparent partition coefficients (α_{ap}) values can be calculated through the equation:

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

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

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

The results obtained for thymol and carvacrol are shown in Fig. 5A and B, respectively. As it can be seen, the linearity of $\left[\frac{1}{\pi}-\frac{1}{\alpha}.\frac{M_{F,t}}{M_{F,0}}\right]^{0.5}$ versus $t^{0.5}$ was very good for both AOs and all simulants tested with determination coefficient values (R^2) ranging from 0.961 to 0.995 for thymol and 0.983–0.992 for carvacrol, suggesting that experimental release data is well described by the proposed diffusion model for short-range times.

The analysis of diffusion coefficients (D) (Table 4) shows that the diffusion process in different simulants are independent on the AOs, with D values ranging from 1×10^{-14} to 2×10^{-14} m^2/s. This behaviour was expected if considering that carvacrol and thymol are isomers having similar molecular weights and polarity (Licciardello, Muratore, Mercea, Tosa, & Nerin, 2013). The exception occurs for the diffusion of AOs into isooctane. In fact, D values for thymol and carvacrol are 4 and 6 times higher for this simulant than the average values for remaining ones. This is, however, in line with the discussion carried out in the previous section and with results reported in Section 3.2 as well. It is also worth noticing that the magnitude of D values found for these films are one order of magnitude lower than those obtained for similar AOs and films (Suppakul et al., 2011), suggesting that these films can provide a long term release, or higher retention inside films, of AOs.

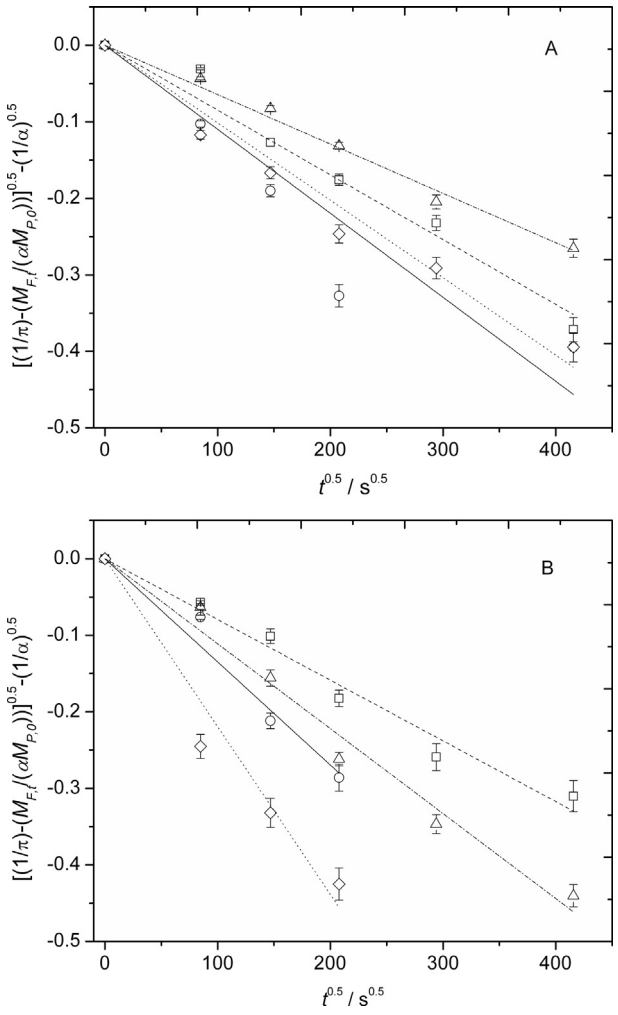

Fig. 5. Plots of $\left[\frac{1}{\pi}-\frac{1}{\alpha}.\frac{M_{F,t}}{M_{F,0}}\right]^{0.5}-\frac{1}{\pi^{0.5}}$ versus $t^{0.5}$ for the migration of thymol (A) and carvacrol (B) from PPT8 and PPC8 films, respectively, into different food simulants. Isooctane (◇), 20 °C; acetic acid (○), 40 °C; ethanol 100 mL/L (□), 40 °C; and ethanol 950 mL/L (△), 40 °C.

4. Conclusions

The release study of carvacrol and thymol from PP films into aqueous and fatty food simulants was accomplished. Analytical methods for the determination of the target compounds in the studied food simulants were successfully developed and validated. Release of AOs from PP films showed some differences depending on the food simulant type used; being isooctane the most exhaustive one resulting in high levels of migration. In addition, positive results were obtained for all migration extracts by the antioxidant activity study performed by the DPPH method, showing thymol a higher antioxidant capacity. Finally, the results obtained for the migration kinetics study showed that carvacrol and thymol incorporated into PP films at 80 g/kg were readily released into different food simulants, being these additives still remaining in the polymer after 15 days. In this sense, the high efficiencies of release of these compounds from PP films point to the great potential of these systems in antioxidant packaging of different food products to extend their shelf life and avoid the direct addition of additives to food formulations.

Acknowledgements

Authors would like to thank Ashland Chemical Hispania for kindly supplying ECOLEN HZ10K PP (Hellenic Petroleum) and

Spanish Ministry of Economy and Competitiveness for financial support (MAT-2011-28648-C02-01). Also, Marina Ramos would like to thank University of Alicante (Spain) for UAFPU2011-48539721S predoctoral research grant.

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