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► **To cite this version:**

Mercedes Ana Peltzer, Jorge Wagner, Alfonso Jiménez. Migration study of carvacrol as natural antioxidant in High Density Polyethylene for active packaging. *Food Additives and Contaminants*, 2009, 26 (06), pp.938-946. 10.1080/02652030802712681 . hal-00577358

**HAL Id: hal-00577358**

**<https://hal.science/hal-00577358>**

Submitted on 17 Mar 2011

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Journal:	<i>Food Additives and Contaminants</i>
Manuscript ID:	TFAC-2008-251.R1
Manuscript Type:	Original Research Paper
Date Submitted by the Author:	12-Dec-2008
Complete List of Authors:	Peltzer, Mercedes; University of Alicante, Department of Analytical Chemistry, Nutrition & Food Sciences Wagner, Jorge; University of Quilmes, Department of Science and Technology Jiménez, Alfonso; University of Alicante, Department of Analytical Chemistry, Nutrition & Food Sciences
Methods/Techniques:	Chromatography - GC/MS, HPLC
Additives/Contaminants:	Antioxidants, Food contact materials, Migration, Packaging - active
Food Types:	Olive oil, Water

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## Migration study of carvacrol as a natural antioxidant in high density polyethylene for active packaging

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### Abstract

The migration behaviour of low molecular weight compounds from food packaging materials is one of the key issues in assessing the possibility of use in such applications. The aim of this work was to study the migration of carvacrol (1% and 2% w/w) when added to high density polyethylene. All materials were exposed to the food simulants olive oil and distilled water separately at 40°C and 25°C. Three significant variables influencing the migration process were considered: incubation temperatures, initial concentration of antioxidant and type of simulant (oil and aqueous). The amount of carvacrol migrating to olive oil was significantly higher than in water and this was because of the higher solubility of this antioxidant in oil. Experimental results agreed reasonably well with those obtained by the application of a simple model derived from Fick's Second Law. Carvacrol could therefore be used in active packaging formulations as its release from the polymer matrix can be controlled.

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6 **Keywords:** migration, active packaging, carvacrol, high density polyethylene  
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## 10 **Introduction**

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16 Food packaging is intended to protect food from environmental influences, such as  
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18 microbial or chemical degradation, air (oxygen), radiation or water. The use of plastic  
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20 materials compared to glass and metal containers for food packaging shows many  
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22 advantages: polymer-based packaging systems are more flexible, show reduced weight and  
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24 less energy is required for their production and processing. However, plastic materials also  
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26 show some drawbacks in their use in contact with food. For instance, they are generally less  
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28 heat resistant, more permeable with respect to gases and water, and diffusion of low  
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30 molecular weight compounds, such as monomers or additives, into foodstuff can take place.  
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32 The increased use of plastic packaging materials and their possible interaction with food  
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34 have created the need for more research in this field (Sendón-García *et al.* 2006, Perez-  
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36 Bonilla *et al.* 2007). It is essential to have suitable analytical methods to determine  
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38 substances able to migrate from packaging materials to different foodstuff at low  
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40 concentrations.  
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50 Traditional antioxidants used in polymers are often synthetic compounds, mainly phenol  
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52 derivatives, and in some cases they may change organoleptic food quality and even  
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54 promote toxicity in the case of their migration (Garde *et al.* 1998). Nevertheless, the use of  
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56 some synthetic antioxidants in polymeric materials is questionable from the toxicity and  
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3 environmental point of view. Side effects of some synthetic antioxidants frequently used in  
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5 food processing, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole  
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7 (BHA) have been documented and this has stimulated the substitution of synthetic  
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9 antioxidants by natural ones (Till *et al.* 1982, Schwoppe *et al.* 1987, Tawfik and  
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11 Huyghebaert 1999, Perez-Bonilla *et al.* 2007, Cruz *et al.* 2008). Natural antioxidants are  
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13 rising as a valid alternative as they are non-toxic compounds (Strandberg and Albertsson  
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15 2006) with lower risk of being hazardous to foodstuff.  
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23 Natural products have served as an important source of drugs since ancient times. In recent  
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25 years, a renewed interest in obtaining biologically active compounds from natural sources  
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27 has been observed. Plants are rich source of natural antioxidants and some of them, such as  
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29 tocopherols (vitamin E), ascorbic acid (vitamin C) and carotenoids are substances of major  
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31 significance in human physiology as well as in food industry (Mallegol *et al.* 2001, Suffield  
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33 *et al.* 2004, Oral *et al.* 2005, Dimitrios 2006, Perez-Bonilla *et al.* 2007). Oregano was  
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35 reported to possess strong antioxidant activity, better than BHA (Pezo *et al.* 2007).  
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37 Compounds responsible of the antioxidant activity of oregano were among other  
38  
39 polyphenols, carvacrol and tymol (Ladrón de Guevara *et al.* 2002, Pezo *et al.* 2007) and no  
40  
41 harmful effects should potentially arise from possible interactions between the additives  
42  
43 and foodstuff (Cheah and Abu Hasim, 2000). The health benefits of vegetables are mostly  
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45 due to the antioxidant compounds in phytochemicals. These natural compounds can be also  
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47 considered valid alternatives to synthetic antioxidants for the stabilization of polymers used  
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49 for food packaging. However, phenols obtained from plants have not been completely  
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51 studied because of the complexity of their chemical nature and the sometimes complicated  
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3 procedures to get an accurate determination.  
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9 Active packaging, including the concept of release of packaging components to a foodstuff,  
10 has received considerable attention in recent years (Dongen *et al.* 2007). In particular, the  
11 terms active and intelligent packaging were first introduced by Regulation 2004/1935/EC  
12 of the European Parliament and Council (Regulation (EC) No 1935/2004). This regulation  
13 covers the definition of active packaging and creates a legal framework for further  
14 development of active packaging legislation. This regulation is also related to Directive  
15 89/107/EC concerning food additives which classifies additives in different categories such  
16 as flavorings, colorants, stabilizers, depending on their effects on food (Council Directive  
17 89/107/EEC). Natural extracts, such as essential oils and their constituents, such as  
18 carvacrol, are categorized as flavorings by European Decision 2002/113/EC (Commission  
19 Decision 2002/113/EC). Essential oils and their constituents are categorized as ‘Generally  
20 Recognized as Safe’ (GRAS) by the U.S. Food and Drug Administration (Lopez *et al.*  
21 2007).  
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41 In this framework, the formulation of active packaging systems with controlled migration  
42 of natural antioxidants to foodstuff may result in prolonged shelf-life and preservation of  
43 quality of the product (Bounatirou *et al.* 2007, Nakatani 1997, Ben Arfa *et al.* 2006).  
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47 Therefore, the incorporation of carvacrol in materials intended to be in contact with  
48 foodstuff is an attractive option for consumers and also for packaging manufacturers.  
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55 The aim of this work was to study of the release of carvacrol from high density  
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3 polyethylene (HDPE) as a function of time by determination of the concentration of this  
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5 compound in two simulants and two different temperatures. The final goal is to be to  
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7 control the release of carvacrol to prepare an active packaging system.  
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## 10 11 12 **Materials and methods**

### 13 14 *Materials and sample preparation*

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16 High-density polyethylene, HDPE (ALCUDIA<sup>®</sup> C-200RB, Repsol-YPF, Spain) was used  
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18 as polymer matrix. Each sample consisted of 2 kg (approximately) of HDPE mixed with  
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20 two concentrations of carvacrol (98%, Sigma-Aldrich, Spain) 1wt% and 2wt%, in separated  
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22 samples. In addition, a control sample (pure HDPE) without the addition of any antioxidant  
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24 was prepared. Carvacrol was dissolved in absolute ethanol (Merck KgaA, Germany) before  
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26 their addition to the polymer.  
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35 Polymer and antioxidant were blended in an orbital mixer during 20 min at  $23 \pm 2^\circ\text{C}$ . Then,  
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37 each mixture was processed in a twin-screw extruder operated at 25 rpm and 22 rpm at  
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39 feeding (MD 30 Bausano, Italy) with seven temperature zones. These temperatures were set  
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41 at 120/130/150/150/155/160/160°C. After the extrusion process, samples were pelletized  
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43 and compression moulded (Robima, S.A., Valencia, Spain) in order to obtain 307cm<sup>2</sup>  
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45 sheets to carry out migration tests. Samples were moulded by compression at 150°C and  
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47 0.45 kPa during 15 min.  
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### 53 54 *Migration study*

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56 Specific migration tests were performed according to the European Standard EN 13130 part  
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3 1. The release of carvacrol from the polymer was studied in two simulants: distilled water  
4 and virgin olive oil at two different temperatures: 25 and 40°C. Incubation was always  
5 carried out in a water bath at controlled temperature ( $\pm 0.1^\circ\text{C}$ ) (Mettler WB 22, 22 litres).  
6  
7 Temperature and incubation times were changed in order to simulate actual storing  
8 conditions for foodstuff. Sheets of  $5\text{ cm}^2$  area and 4 mm thickness were immersed  
9 completely in 10 mL of simulant in their both sides and incubated at the chosen  
10 temperature for 30 days. Extracts of simulant in contact with HDPE stabilized with  
11 carvacrol were taken at 2, 5, 10, 20 and 30 days of incubation in order to follow the release  
12 of additive with time.  
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#### 24 25 26 27 *GC-MS*

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29 Gas chromatography coupled to mass spectrometry (GC-MS) was used for identification  
30 and quantitative analysis of carvacrol in olive oil. Samples were previously diluted in  
31 cyclohexane (1:10) before the injection to the chromatograph and further introduced (1  $\mu\text{L}$ )  
32 into an Agilent (HP/Agilent Technologies 6890N) GC equipped with a TRB-5MS capillary  
33 column (30m x 0.25 mm i.d, 0.25 $\mu\text{m}$  film thickness, 95% dimethyl- 5% diphenyl  
34 polysiloxane, Teknokroma, Spain). Helium was used as carrier gas with flow rate 1 mL  
35  $\text{min}^{-1}$  in splitless injection method. The injector temperature was 280°C. Temperature oven  
36 conditions were: initial temperature 60°C, holding at this temperature for 2 min, with  
37 further temperature increase to 300°C at 10°C  $\text{min}^{-1}$ , held isothermally for 15 min. The  
38 mass spectrometer (Agilent Technologies 5973N) was operated in ionization mode with  
39 impact electron (70eV) and selected ion monitoring (SIM) mode.  
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3 Figure 1a shows the GC-MS chromatogram of a carvacrol standard ( $2\text{mg kg}^{-1}$ ). Only one  
4 peak with a retention time of 11.2 minutes was observed indicating the excellent specificity  
5 of the method for the determination of this analyte. Figure 1b shows the Mass spectrum of  
6 this peak. The ions monitored for carvacrol were:  $m/z$  77,  $m/z$  91,  $m/z$  107,  $m/z$  135,  $m/z$  150  
7 as they were characteristic of this compound according to previous measurements carried  
8 out in scan mode. We could observe that  $m/z$  135 ion was the most abundant, corresponding  
9 to the loss of a methyl group from the carvacrol molecule. In consequence, the  
10 quantification in all samples studied by GC-MS was carried out by using this ion.  
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22 (Figure 1)  
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27 Calibration curve was obtained for carvacrol by injection of known amounts of this  
28 compound dissolved in cyclohexane. Samples and calibration standards were spiked with  
29  $0.5\text{ mg kg}^{-1}$  of BHT (2,6-di-*tert*-butyl-4-methylphenol) as internal standard (IS). The ions  
30 monitored for BHT were  $m/z$  205 and  $m/z$  220, and the quantification was carried out with  
31  $m/z$  205 as it was the most abundant ion for this compound. Three replicates were measured  
32 for each standard. Carvacrol was quantified by assessing their peak area ratio and the area  
33 of the peak corresponding to IS ( $[\text{carvacrol}]/[\text{IS}]$ ).  
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#### 46 *HPLC-UV*

47 Aqueous extracts of carvacrol were directly analyzed by HPLC-UV in a liquid  
48 chromatograph Agilent 1100 Series equipped with a C18 column (25 cm x 0.46 cm x 5  
49  $\mu\text{m}$ ). The injection volume was 40  $\mu\text{L}$ . The mobile phase consisted of a methanol:water  
50 (60:40) mixture with 0.1% of formic acid, at a flow rate of  $1\text{ mL min}^{-1}$ . Detection was  
51 performed by UV spectroscopy at 274 nm. Calibration curves were obtained for carvacrol  
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3 by injection of known amounts of this compound in methanol:water (50:50) solutions.  
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6 Three replicates were measured for each standard.  
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## 10 **Results and Discussion**

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12 Un-weighted linear regression analysis was employed for the construction of the calibration  
13 curves. The calibration curve of carvacrol in cyclohexane by GC-MS as well as the  
14 calibration curve of carvacrol in methanol:water (50:50) by HPLC-UV were obtained and  
15 linear regressions with  $R^2 > 0.999$  were determined, as could be observed in Table 1, where  
16 calibration parameters for both techniques are presented. The internal standard method was  
17 used to perform GC-MS calibration curve.  
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29 (Table 1)  
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34 It was possible to use the calibration curve with cyclohexane to quantify carvacrol in olive  
35 oil samples because no interferences from the oil matrix were observed in the  
36 chromatograms. A statistical test was performed in order to determine if the results  
37 obtained with the two series of standards were different: series 1 corresponds to carvacrol  
38 directly dissolved in cyclohexane while series 2 corresponds to carvacrol dissolved in olive  
39 oil and then in cyclohexane (1:10). Series 2 represents the same treatment used in olive oil  
40 samples. Both series were plotted on the two axes of a regression graph (Figure 2). We  
41 found that there were not statistical differences between results obtained with both series of  
42 standards (at 95% probability) as  $r$  was close to +1, the confidence interval in the intercept,  
43  $a$ , included zero, and the confidence interval for the slope,  $b$ , included 1 (Miller J. N. 1991).  
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58 In conclusion, the use of cyclohexane solutions for carvacrol determination was selected as  
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3 experimental conditions were more favorable in practice.  
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8 Figure 3a shows the GC-MS chromatogram of carvacrol 2wt% migrated in olive oil at 25°C  
9 after ten days of incubation. The mass spectrum of the peak at 11.2 minutes is shown in  
10 figure 3(b) and it is exactly the same than the spectrum obtained for the standard (figure  
11 3(b)), so this peak was identified as carvacrol.  
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20 (Figure 3)  
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24 Migration models based on Fick's second law have been extensively used for the  
25 assessment of migration of additives and contaminants from food packaging films (Reid *et*  
26 *al.* 1980, Sharma *et al.* 1990, Begley 1997, Chung *et al.* 2002). The main equation obtained  
27 from simplifications and considering the partition term negligible is as follows:  
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$$\frac{M_{f,t}}{M_{p,o}} = \frac{2}{\delta} \cdot \left( \frac{D \cdot t}{\pi} \right)^{1/2} \quad (1)$$

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41 where  $M_{f,t}$  is the amount of migrant in the food at time  $t$ ;  $M_{p,o}$  is the initial amount of  
42 migrant in the packaging film;  $D$  is the diffusion coefficient ( $\text{cm}^2 \text{s}^{-1}$ );  $\delta$  is the thickness of  
43 the packaging film (cm).  
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51 This equation could lead to reliable values of diffusion coefficients in the case of complete  
52 migration where partition between solutions and resistance to mass transfer are negligible  
53 (Chung *et al.* 2002). Anyway, this model could result in considerable errors in the  
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3 determination of diffusion coefficients in the case of significant partitioning as it  
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5 underestimates the value of diffusion coefficient. When partitioning occurs the model is no  
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7 longer the same, and it yields different diffusion coefficients for the same migration rate.  
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9 Nevertheless, these assumptions could be acceptable for comparison purposes, as the main  
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11 goal of this work would be to assess the release of carvacrol to foodstuff at two different  
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13 carvacrol concentrations. This model is simple and it is often used to provide estimations of  
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15 the diffusion coefficient based on the available data, knowing that some errors must be  
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17 assumed. It could be used for short migration times or when  $\delta$  is large, as it was previously  
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19 described (Chung *et al.* 2002).  
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27 The use of multiple data points and linear regression analysis, as used in this work, can  
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29 provide a reliable estimation of  $D$  (Chung *et al.* 2002). Figures 3 and 4 show the fitting of  
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31 migration data with equation 1 for carvacrol 1wt% and carvacrol 2wt%, respectively,  
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33 during 30 days of migration for both food simulants at 25°C and 40°C. The fitting between  
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35 experimental points (dots) and calculated values (lines) is reasonably good, in particular at  
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37 the lower temperature and distilled water as simulant. Diffusion coefficients could be  
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39 further calculated.  
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46 (Figure 4)

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48 (Figure 5)

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53 The underestimation of the diffusion factor could be a problem when evaluating the  
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55 Specific Migration Limits (SML) for a particular analyte in the food contact material. This  
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3 is not critical in the case of carvacrol, as this compound has not SML recognized in the  
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5 legislation, and the main goal of this study is the evaluation of the possible use of carvacrol  
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7 in active packaging systems.  
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12 Another simple approach for the estimation of diffusion coefficients is based on the  
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14 estimation of  $D$  correlated with the relative molar mass  $M_r$  of the migrant and the absolute  
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16 temperature  $T$ , based on empirical data. This is called the “worst case approach” and also  
17  
18 defines a matrix specific parameter  $A_p$ . This approach was proposed by Piringer and others  
19  
20 (Baner *et al.* 1995, 1996; Brandsch *et al.* 2000; Piringer, 1994; Piringer & Baner, 2000)  
21  
22 who developed this model to predict migration values. They proposed the following  
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24 equation:  
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$$D = 10^4 \exp\left(A_p' - 0,1351M_r^{2/3} + 0,003M_r - \frac{\tau + 10454}{T}\right) \quad (2)$$

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34 Where  $A_p'$  and  $\tau$  are polymer specific parameters,  $M_r$  is the molar mass of the migrant and  
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36  $T$  is the absolute temperature (K). Equation 2 was proposed for determination of additives  
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38 in different polymers, including polyethylenes, polypropylenes and polyamides. For HDPE  
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40 the value of  $A_p'$  ( $T < 100^\circ\text{C}$ ) is 14.5;  $\tau$  is 1577.  
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47 Some different variables should be considered in migration studies, in order to determine  
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49 the actual release of chemicals to foodstuff. Tables 2 and 3 show the amount of carvacrol  
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51 migrated in olive oil and distilled water respectively for all the experimental conditions  
52  
53 (different times and temperatures). A clear dependence on the release of the analyte from  
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55 the material bulk with the initial concentration of migrant could be observed. The amount  
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57 of carvacrol migrated in both simulants was higher in samples with carvacrol 2wt%. As  
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3 expected the release of the analyte was higher at 40°C since migration is directly dependent  
4 on temperature according to the Arrhenius equation (Koros and Hopfenberg 1979, Linsen  
5 *et al.* 1992).

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10 (Table 2)

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12 (Table 3)

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15 Diffusion coefficients calculated by fitting the experimental data for carvacrol with those  
16 obtained after application of equation (1) can be observed in Table 4 while values  
17 calculated with the worst-case approach according to equation (2) are shown in Table 5. As  
18 was previously mentioned,  $D$  increased with incubation temperature in both simulants and  
19 for both carvacrol concentrations. Therefore, higher temperatures resulted in an increase in  
20 the release of the antioxidant due to the activation of both diffusion and desorption  
21 processes at higher temperatures (Haider and Karlsson, 2000).

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34 Other important parameters to be considered in the migration of a compound through a  
35 polymer matrix could be the intrinsic properties of the migrant itself, such as polarity and  
36 solubility. Both parameters influenced the migration rate due to interactions between  
37 polymer, migrant and food simulant (Helmroth *et al.* 2002). The nature (oil or aqueous) of  
38 food simulants in contact with the polymer had also a large influence in antioxidants  
39 migration (Sharma *et al.* 1990, Miltz *et al.* 1998). The amount of carvacrol that migrated to  
40 olive oil was clearly higher because of the high solubility of this antioxidant in oil. It is also  
41 necessary to consider that sorption phenomena could occur in oil environment, with some  
42 simulant penetrating in the polymer matrix and increasing the possibility of movement of  
43 polymer additives and consequently favouring their migration (Haider and Karlsson 2000).  
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As it is known the migration rate depends on the size and shape of the migrant and on the

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3 number of voids and gaps in the polymer structure (Helmroth *et al.* 2002). The absorption  
4 of certain amount of simulant to the polymer matrix could cause an increase in the gap size  
5 and consequently an increase in migration could be expected.  
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12 Carvacrol also migrated in distilled water, but in a lower amount than in olive oil (Tables 2  
13 and 3). As carvacrol is a lipophilic molecule (Nostro *et al.* 2007) the diffusion and further  
14 migration of carvacrol through the polymer in contact with olive oil is a natural  
15 consequence of the analyte characteristics. Nevertheless, we also observed some migration  
16 of carvacrol to water and this could be mainly attributed to the small size of the molecule  
17 (molar mass, 150 g mol<sup>-1</sup>) that could move easily through the polymer structure. However,  
18 carvacrol has been reported to possess a relative hydrophilicity (Griffin *et al.* 1999) and this  
19 factor could also contribute to the slight migration of this analyte in water.  
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34 On the other hand, if we think in carvacrol as polymer stabilizer, we should be aware of the  
35 high migration tendency of this compound, which could result in some loss in stability of  
36 the own polymer. Some published work (Linssen *et al.* 1998) demonstrated that  
37 antioxidants added to a polymer could lose their protective effect in long-term basis by a  
38 phenomenon called “physical loss”, which is controlled by diffusion of the additive through  
39 the polymer.  
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## 50 **Conclusions**

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52 Carvacrol is present in a natural extract and its main source (oregano) is used as flavoring  
53 spice, and is totally harmless to the human health. In addition, their phenolic chemical  
54 structure makes it a very promising natural additive for HDPE used in food packaging. The  
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3 addition of carvacrol to HDPE could be interesting in terms of active packaging, as the  
4 release of this compound from the polymer matrix can be controlled. Results showed that  
5 carvacrol migrated in oil and aqueous simulants. Therefore we could think about this  
6 compound as an additive for active packaging and a wide range of foodstuff, not only as an  
7 antioxidant but also by its antimicrobial properties. The use of this packaging system for  
8 any particular food will depend on the compatibility between foodstuff and the antioxidant,  
9 suitability of the polymer matrix and the foreseen shelf-life. Higher migration in oil  
10 simulant is also a positive feature for the protection of foodstuff with high fat content, since  
11 they need a higher protection from oxidation. In addition, it was observed that higher  
12 temperatures lead to an increase of migration. Concentrations of carvacrol found in this  
13 study are high enough to ensure the antioxidant activity of the material. Some authors  
14 (Vardar-Ünlü *et al.* 2003) claimed that antioxidant activity of pure carvacrol was adequate  
15 to protect foodstuff at concentrations around  $5 \mu\text{L mL}^{-1}$ .  
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36 A main drawback for the use of essential oils in food packaging applications is their  
37 organoleptical alteration of the foodstuff due to the release of high odor chemicals. A  
38 sensory evaluation of oregano essential oils (including carvacrol) suggested that  
39 concentrations lower than  $20 \mu\text{g g}^{-1}$  would be acceptable to consumers (Chi *et al.* 2006).  
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46 Anyway, a specific sensorial evaluation for these samples will be carried out in the future.  
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### 50 **Acknowledgements**

51  
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53 The authors wish to thank the Department of Civil and Environmental Engineering,  
54 University of Perugia in Terni (Prof. José M. Kenny and Dr. Antonio Iannoni), the  
55 Department of Materials Science and Engineering (Polytechnic University of Valencia) for  
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3 their support in the samples preparation and testing and Dr. M. Carmen Garrigós for useful  
4  
5 discussion. Also authors thank Repsol-YPF for supplying the material. Mercedes Peltzer is  
6  
7 granted by Alban Programme (European Union) No (E04D031633AR).  
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Table 1 – Carvacrol calibration parameters<sup>a</sup>.

Method	<i>b</i> (slope)	<i>SDb</i> <sup>b</sup>	<i>a</i> (intercept)	<i>SDa</i> <sup>b</sup>	<i>r</i> <sup>2</sup>
HPLC-UV	30.5	0.4	-50.0	29.7	0.999
GC-MS	1.7	0.1	0.0	0.1	0.999

<sup>a</sup>Calibration curve:  $y=bx+a$ ; <sup>b</sup>*SD*: Standard Deviations.

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Table 2 – Migration of carvacrol in olive oil at 25°C and 40°C.

Olive oil		Carvacrol 1%		Carvacrol 2%	
T (°C)	Time	mg kg <sup>-1</sup>	mg dm <sup>-2</sup>	mg kg <sup>-1</sup>	mg dm <sup>-2</sup>
25	2 days	2.8 ± 0.3	0.3 ± 0.1	8.3 ± 0.1	0.72 ± 0.01
	5 days	6.9 ± 2.5	0.6 ± 0.2	17 ± 1	1.4 ± 0.1
	10 days	13 ± 1	0.6 ± 0.8	35 ± 6	3.0 ± 0.5

	20 days	$16 \pm 1$	$1.4 \pm 0.1$	$56 \pm 9$	$4.7 \pm 0.6$
	30 days	$25 \pm 2$	$2.1 \pm 0.1$	$91 \pm 10$	$7.9 \pm 0.5$
40	2 days	$17 \pm 2$	$1.5 \pm 0.2$	$52 \pm 14$	$4.4 \pm 1.4$
	5 days	$44 \pm 8$	$3.8 \pm 0.7$	$90 \pm 23$	$7.4 \pm 2.3$
	10 days	$76 \pm 1$	$6.8 \pm 0.0$	$242 \pm 69$	$15 \pm 6$
	20 days	$131 \pm 8$	$11 \pm 1$	$302 \pm 60$	$25 \pm 5$
	30 days	$275 \pm 6$	$28 \pm 6$	$560 \pm 34$	$47 \pm 2$

Table 3 – Migration of carvacrol in distilled water at 25°C and 40°C.

Distilled water		Carvacrol 1%		Carvacrol 2%	
T (°C)	Time	mg kg <sup>-1</sup>	mg dm <sup>-2</sup>	mg kg <sup>-1</sup>	mg dm <sup>-2</sup>
25	2 days	$0.9 \pm 0.3$	$0.08 \pm 0.03$	$2.0 \pm 0.2$	$0.2 \pm 0.0$
	5 days	$1.2 \pm 0.1$	$0.1 \pm 0.0$	$5.3 \pm 0.7$	$0.5 \pm 0.1$

	10 days	$3.7 \pm 0.1$	$0.4 \pm 0.0$	$11 \pm 1$	$1.1 \pm 0.1$
	20 days	$5.7 \pm 0.1$	$0.6 \pm 0.1$	$18 \pm 1$	$1.8 \pm 0.0$
	30 days	$8.1 \pm 1.3$	$0.8 \pm 0.1$	$25 \pm 2$	$2.4 \pm 0.1$
40	2 days	$24 \pm 4$	$2.1 \pm 0.1$	$31 \pm 1$	$3.1 \pm 0.1$
	5 days	$45 \pm 2$	$3.5 \pm 0.1$	$92 \pm 21$	$8.5 \pm 1.0$
	10 days	$61 \pm 1$	$4.5 \pm 0.5$	$123 \pm 1$	$11 \pm 0$
	20 days	$80 \pm 4$	$8.9 \pm 3.0$	$186 \pm 2$	$14 \pm 3$
	30 days	$97 \pm 4$	$7.8 \pm 1.5$	$245 \pm 3$	$19 \pm 3$

Table 4 – Diffusion coefficient calculated by using Equation (1).

Sample	Temp (°C)	Simulant	D (cm <sup>2</sup> s <sup>-1</sup> )
Carvacrol 1wt%	40	Olive oil	$(2.03 \pm 0.30) 10^{-10}$
Carvacrol 1wt%	25	Olive oil	$(6.36 \pm 0.86) 10^{-12}$

Carvacrol 1wt%	40	Water	$(5.01 \pm 0.32) 10^{-11}$
Carvacrol 1wt%	25	Water	$(8.13 \pm 1.72) 10^{-13}$
Carvacrol 2wt%	40	Olive oil	$(2.37 \pm 0.54) 10^{-10}$
Carvacrol 2wt%	25	Olive oil	$(2.08 \pm 0.56) 10^{-11}$
Carvacrol 2wt%	40	Water	$(5.13 \pm 0.44) 10^{-11}$
Carvacrol 2wt%	25	Water	$(2.38 \pm 0.46) 10^{-12}$

Table 5 – Diffusion coefficient by Equation (2).

Sample	Temp (°C)	D (cm <sup>2</sup> s <sup>-1</sup> )
Carvacrol	40	$1.39 10^{-9}$

Carvacrol	25	$2.01 \cdot 10^{-10}$
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### Figure Captions

Figure 1 – a) Chromatogram of a carvacrol standard. b) Mass spectrum of the peak at 11.2 min.

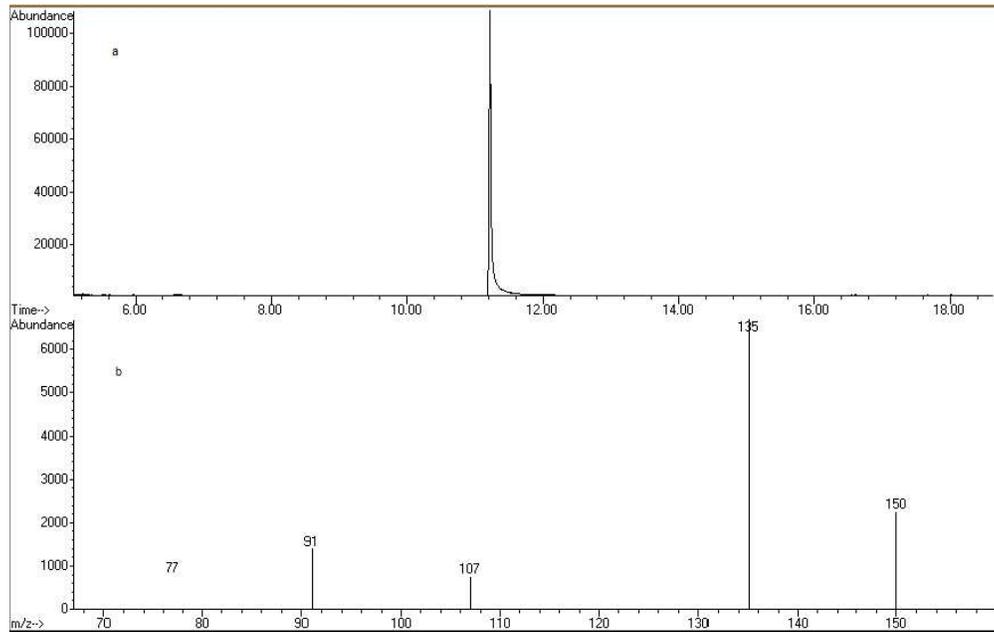
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3 Figure 2 – Regression line for comparison Series 1 and 2. Series 1: carvacrol dissolved in  
4 cyclohexane and series 2: carvacrol in olive oil and dissolved in cyclohexane.  
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8 Figure 3 – a) Chromatogram of carvacrol 2wt% migrated in olive oil at 25°C (10 days of  
9 incubation). b) Mass spectrum of the peak at 11.2 min.  
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12 Figure 4 - Fitting of migration data by equation (1) for carvacrol 1wt% materials.  
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14 Figure 5 - Fitting of migration data by equation (1) for carvacrol 2wt% materials  
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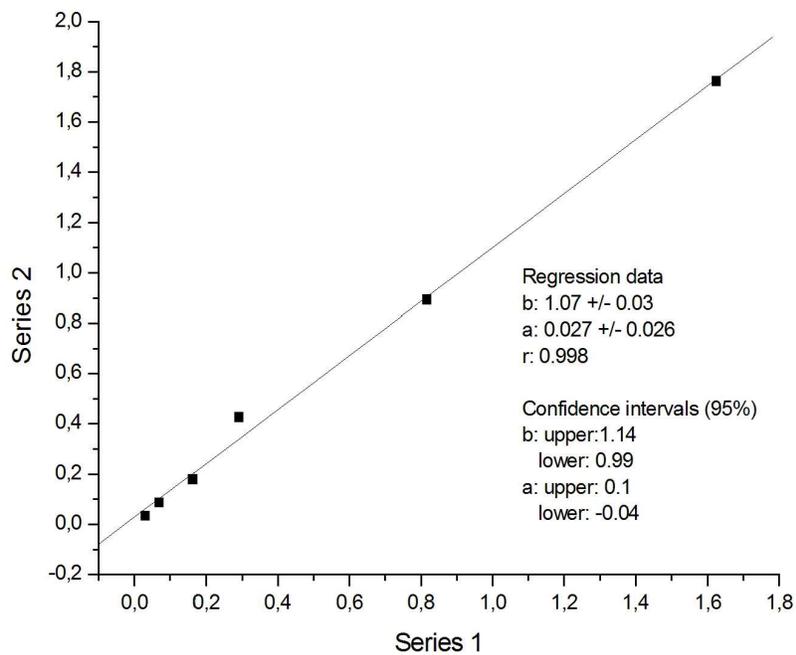
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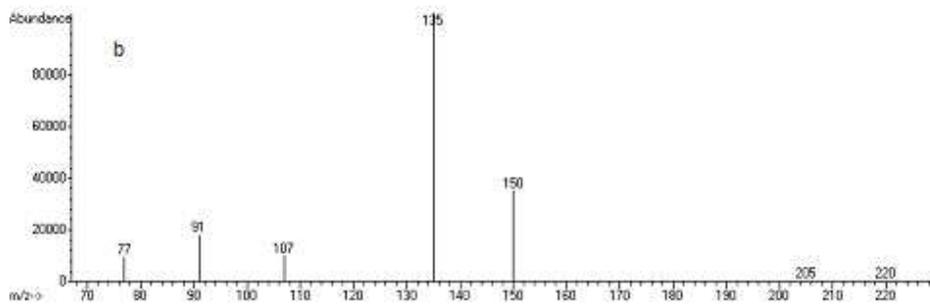
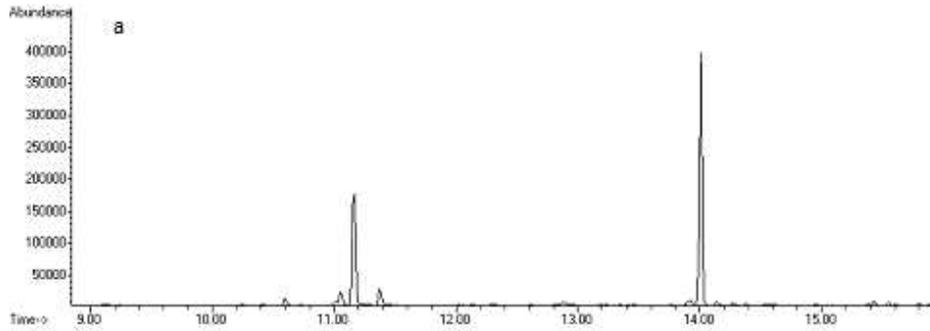
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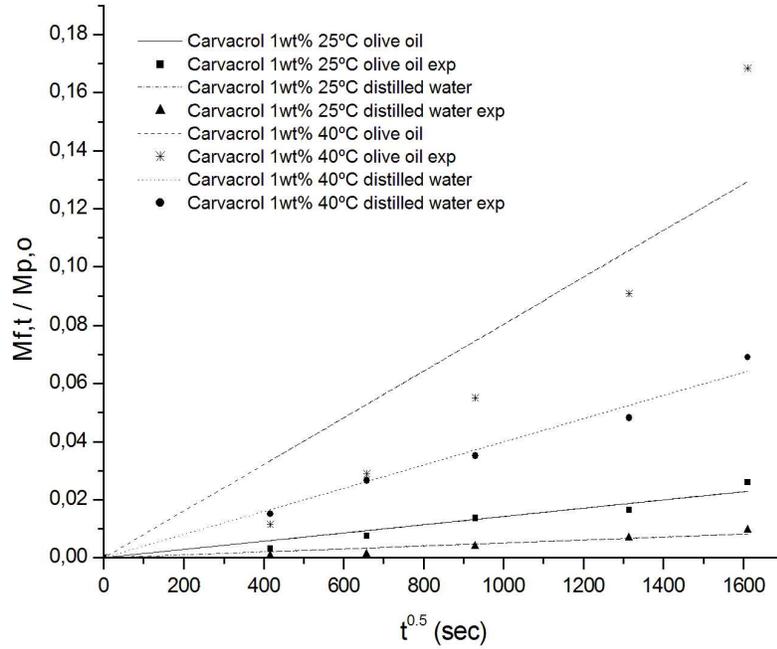
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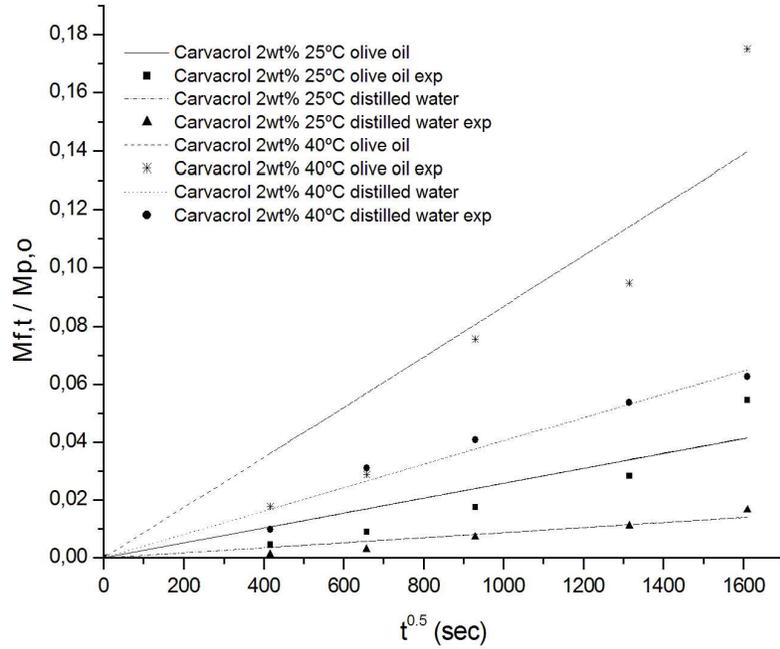
view Only



279x215mm (150 x 150 DPI)

View Only

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279x215mm (150 x 150 DPI)

View Only