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Polymer Degradation and Stability

DOI:
10.1016/j.polymdegradstab.2016.05.030

Published: 01/08/2016

Version created as part of publication process; publisher's layout; not normally made publicly available

Dyfyniad o'r fersiwn a gyhoeddwyd / Citation for published version (APA):
Doudin, K., Al-Malaika, S., Sheena, H. H., Tverezovskiy, V., & Fowler, P. (2016). New genre of antioxidants from renewable natural resources: Synthesis and characterisation of rosemary plant-derived antioxidants and their performance in polyolefins. *Polymer Degradation and Stability*, *130*(August), 126-134. https://doi.org/10.1016/j.polymdegradstab.2016.05.030

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PII: S0141-3910(16)30160-4

DOI: 10.1016/j.polymdegradstab.2016.05.030

Reference: PDST 7977

To appear in: *Polymer Degradation and Stability*

Received Date: 21 January 2014

Revised Date: 20 May 2016

Accepted Date: 29 May 2016

Please cite this article as: Doudin K, Al-Malaika S, Sheena HH, Tverezovskiy V, Fowler P, New genre of antioxidants from renewable natural resources: Synthesis and characterisation of rosemary plant-derived antioxidants and their performance in polyolefins, *Polymer Degradation and Stability* (2016), doi: 10.1016/j.polymdegradstab.2016.05.030.

New Genre of Antioxidants from Renewable Natural Resources: Synthesis and Characterisation of Rosemary Plant-derived Antioxidants and their Performance in Polyolefins

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ABSTRACT

Several ester derivatives of rosmarinic acid (rosmarinates) were synthesised, characterised (1D and 2D NMR, UV and FTIR spectroscopy) and tested for their potential use as antioxidants derived from a renewable natural resource. The intrinsic free radical scavenging activity of the rosmarinates was assessed, initially using a modified DPPH (2, 2-diphenyl-1-picrylhydrazyl radical) method, and found to be higher than that of commercial synthetic hindered phenol antioxidants Irganox 1076 and Irganox 1010. The thermal stabilising performance of the rosmarinates in polyethylene (PE) and polypropylene (PP) was subsequently examined and compared to that of samples prepared similarly but in the presence of Irganox 1076 (in PE) and Irganox 1010 (in PP) which are typically used for polyolefin stabilisation in industrial practice. The melt stability and the long-term thermo-oxidative stability (LTTS) of processed polymers containing the antioxidants were assessed by measuring the melt flow index (MFI), melt viscosity, oxidation induction time (OIT) and long-term (accelerated) thermal ageing performance. The results show that both the melt and the thermo-oxidative stabilisation afforded by the rosmarinates, and in particular the stearyl derivative, in both PE and PP, are superior to those of Irganox 1076 and Irganox 1010, hence their potential as effective sustainable bio-based antioxidants for polymers.

The rosmarinic acid used for the synthesis of the rosmarinates esters in this study was obtained from commercial rosemary extracts (AquaROX80). Furthermore, a large number of different strains of UK-grown rosemary plants (Rosmarinum officinalis) were also extracted and analysed in order to examine their antioxidant content. It was found that the carnosic and the rosmarinic acids, and to a much lesser extent the carnosol, constituted the main antioxidant components of the UK-plants, with the two acids being present at a ratio of 3:1, respectively.

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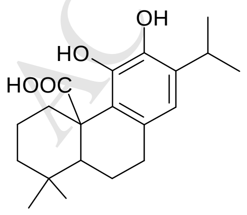
Keywords: Esters of rosmarinic acid, rosemary-based antioxidants, antioxidant performance, polyolefins

1. INTRODUCTION

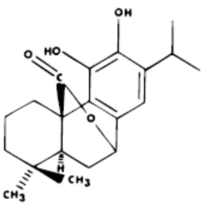
The potential replacement, partially or fully, of synthetic additives for polymers by bio-based alternatives derived from indigenous renewable non-food crop resources offers potentially a market opportunity for a green supply of raw materials for different industrial and health products. The main challenge for a bio-based sustainable development is primarily an economic one, hence the imperative of adopting an integrated approach to establish a profitable commercially viable utilisation strategy for harnessing the benefits of the entire biomass crop. To achieve this, much depends on a proactive involvement of the farming community in crop production while addressing the ever more stringent environmental and pollution laws.

Several plant extracts known to contain different polyphenols have been investigated as potential natural antioxidants for polymers. Extracts of green tea and black tea were examined as possible source of natural antioxidants for polypropylene (PP) and found to have good antioxidant activity, based on a DPPH (2,2-diphenyl-1-picrylhydrazyl radical) test [1]. By-products from industrial processing of crops containing polyphenols and tannins derived from red and white grape processing for wine production, and carotenoid-containing waste from processing of tomatoes, were used as antioxidants for PP [2, 3]. The grape seed extracts were shown to be suitable as long-term antioxidants while the carotenoids and tocopherols present in the tomato extracts were effective processing stabilisers for the polymer [2]. Another by-product antioxidant obtained from olive processing, hydroxytyrosol (3,4-dihydroxy-phenylethanol), was found to offer good melt stability to PP [4]. Different flavonoids such as chrysin, hesperidin, naringin and quercetin were also considered as possible natural phenolic antioxidants and UV- stabilisers for PP [5]. Quercetin was found to be effective in stabilising PP against thermal oxidation and UV radiation, and against oxidation in PE melt. Curcumin, the principal natural phenol curcuminoid of turmeric, was used also as a processing stabiliser in LLDPE and was shown to be more efficient than Irganox 1010 [7]. Further, the stabilising performance of vitamin E, both in its most bioactive form α-tocopherol [8-11] and as a mixture of the α, β, γ-tocopherols [12] were examined as natural antioxidants for protecting PE and PP against oxidation during high melt processing temperatures, and in-service, and was found to afford strong antioxidancy in both polymers, much higher than those obtained with the synthetic hindered phenols Irganox 1076 and Irganox 1010, and particularly, in the polymer melt.

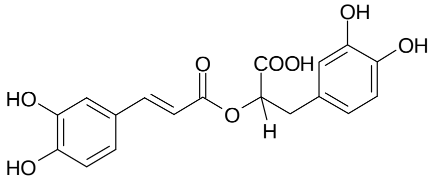
Herbs and spices from the Labiatae family, particularly rosemary (Rosmarinum officinalis), a plant that is native to the Mediterranean basin but is now successfully grown in the UK and worldwide, contain also a variety of bioactive polyphenolic antioxidant compounds, most abundant of which are the carnosic acid (**CA, I**), the carnosol (**C-OH, II**) and the rosmarinic acid (**RA, III**) [13, 14].



CA (I)



C-OH (II)



RA (III)

In spite of the large volume of literature available on the assessment of the antioxidant activity of rosemary extracts in a variety of unsaturated food lipids and food model compounds [15-17] , to our knowledge, there is scant information in the open literature on the stabilising performance of the main rosemary-antioxidants in polyolefins [18-20], and the antioxidant role of the ester derivatives of rosmarinic acid in polyolefins under melt processing and long-term thermo-oxidative conditions. The aim of this work was, therefore, to first synthesise (and characterise) a number of new ester derivatives (rosmarinates) of rosmarinic acid, RA, (the RA used for the synthesis was obtained from a commercial rosemary extract), and then to investigate the antioxidant efficacy of the synthesised rosmarinates in polyethylene (PE) and polypropylene (PP) and to compare their overall performance with that of the commercial hindered phenol antioxidants used normally for the stabilisation of polyolefins, i.e. Irganox 1076 and Irganox 1010 (synthetic), and vitamin E (bio-based).

2. EXPERIMENTAL

2.1 Materials

Unstabilised Ziegler–catalysed linear low density polyethylene (LLDPE), ex. ExxonMobil Chemical Company, used has a density of 0.92g/cm^3, peak melting temperature of 120°C and a melt flow rate (MFR) of 1 g/10 min (at 190°C/2.16 kg). Isotactic polypropylene homopolymer (PP), Moplen HF500N (Lyondell Basell) used has a melt flow rate of 12 g/10 min (at 230°C/2.16 kg). Low density polyethylene (LDPE), Lupolen 2420H (Lyondell Basell) used has a density of 0.92 g/cm^3 and MFR of 1.9 g/10 min (at 190^{o}C/2.16 kg). The commercial hindered phenol antioxidants Irganox 1076, Irganox 1010 and vitamin E (alpha-tocopherol) were kindly donated by Ciba Specialty Chemicals (now BASF). Low odour, low taste rosemary aqueous extract (AquaROX 80) which contain mainly rosmarinic acid (minimum 93.4%) was purchased from Vitiva d.d., Slovenia, and used for the synthesis of all the ester derivatives reported here. The active ingredients (CA and RA) extracted from UK-grown rosemary leaves during the course of this collaborative project were used only for the quantification of the amount of these active ingredients in UK-grown rosemary plants. DMAP (4-dimethylaminopyridine) and DCC (N, N'-dicyclohexylcarbodiimide), ex. Aldrich, were used as catalysts. All solvents, either deuterated for the NMR measurements (ex. Goss Scientific) or of HPLC grade (Fisher), were used without further purification.

2.2 Synthesis of Rosmarinic Acid Esters (Rosmarinates)

Aliphatic alcohols (1.5 mol) and rosmarinic acid, AquaRox 80, (216 g, 0.6mol) were dissolved in (THF) tetrahydrofuran (1.6 L). A solution of (DMPA) 4-Dimethylaminopyridine (1.46 g, 12 mmol) in THF (50 ml) was added dropwise. A solution of (DCC) *N,N'*-Dicyclohexylcarbodiimide (148.5 g, 0.72 mol) in THF (300 ml) was then added dropwise over 1 h at 20-25^{o}C. The reaction mixture was stirred over 4 days at room temperature. Acetic acid (9 ml, 0.15 mol) was added and after 2 h, the precipitate formed was filtered through a 5 mm layer of Celite and washed with THF (200 ml). The solvent was evaporated and the crude product was purified on silica gel eluting with petroleum ether. Brown material was decolourised with charcoal in methanol to obtain rosmarinates produced from different aliphatic alcohols: methyl (**RA-Me**), n-butyl (**RA-But**), ethyl-hexyl (**RA-EH**), n-octyl (**RA-Oct**) and stearyl (**RA-Str**). All the esters were highly viscous yellowish products which solidified in the fridge with melting points at around room temperature; yields were 70% (except for the octyl rosmarinates which gave a yield of 68%).

2.3 Characterisation of Rosmarinates

The synthesised rosmarinate derivatives, and the commercial rosemary extract containing rosmarinic (**AquaROX 80**) acid were characterised by spectroscopic techniques. NMR experiments were performed on a Bruker Avance-300 spectrometer at ambient temperature using tetramethylsilane (TMS) as internal standard. 1D and 2D NMR spectra were recorded using a 5 mm normal dual detection probe and operating at controlled temperatures. The NMR samples were prepared by dissolving 20 mg material in 0.7 ml $CDCl_3$ or DMSO-d6 solvents which contained 0.05% TMS. For **^{1}H NMR,** the spectra were recorded at 300.13 MHz using a high-resolution dual (^{1}H ^{13}C) gradients probe. Spectra were recorded using the zg30 pulse program with 32 scans and referenced to an internal TMS standard at 0.0 ppm**.** 2-Dimensional ^{1}H-^{13}C

Heteronuclear Multiple Bond Coherence (**HMBC**) spectra, which correlate ^{1}H and ^{13}C chemical shifts through multiple-bond heteronuclear scalar coupling ($^nJ_{CH}$, n = 2 or 3) were also recorded. The cross peaks in the ^{1}H-^{13}C HMBC spectra shows the chemical shifts of ^{1}H on one axis (horizontal) which correlated to ^{13}C (on the vertical axis) that belongs to H and C atoms which are separated by two or three chemical bonds. The HMBC pulse sequence utilized ^{1}H to ^{13}C polarisation transfer and inverse (^{1}H) detection for optimum sensitivity [22]. The ^{13}C chemical shifts were determined from **PENDANT** ^{13}C NMR spectra obtained at 75 MHz for carbon. The pendant pulse program was used with waltz16 decoupling during acquisition with 2048 scans and phased for CH_3/CH positive and quaternary carbons and CH_2 negative with internal reference TMS at 0.0 ppm.

FTIR measurements were performed on a Perkin Elmer (PE) Spectrum One over the range of 4000-400 cm^{-1}; spectral collection was taken over 16 scans with resolution of 4 cm^{-1}. Electronic absorption spectra of the antioxidants were recorded at room temperature on Cary 5000i (Agilent) UV-vis spectrophotometer at a scanning rate of 600 nm/min using 1mg/ml solutions in methanol in 1 mm quartz cell. Thermogravimetric analyses (TGA) were performed using Pyris 1 (PE) at different heating rates under a dynamic environment (nitrogen or air at 60 cc/min).

2.4 Polymer Processing and Sample Preparation

Melt processing of LLDPE and PP in the presence and absence of different amounts of antioxidants was carried out batch-wise in a closed mixer of a torque rheometer (Brabender) at temperatures of 180°C and 210°C for PE and PP, respectively, for 5 minutes with a rotor speed of 60 rpm, followed by quench cooling in water, drying and storing in the dark at room temperature until required for further analysis. Thin film specimens (100 μm thick) were prepared by compression moulding at 165^o C and 175^o C under full pressure (10 MPa). LDPE was processed at a collaborating partner laboratory on a lab-scale twin-screw extruder (Haake TW100) in the presence of 1000 ppm antioxidants at a die temperature of 210^o C (screw temperature profile from feed to die was 190, 200, 210 ^{o}C) and screw speed of 35 rpm. The extrudates (through a single rod capillary die) were cooled in a water bath, pelletised and subjected to a further five multi-pass extrusions under the same conditions, with sampling after each extrusion pass, followed by sheet extrusion into thin films (75 μm thick); the samples were stored in the dark until required for further analysis.

2.5 Polymer characterisation

2.5.1 Rheological Measurements for Polymer Melt Stability

The melt stability of the samples was measured using two different rheological methodologies. The first relied on observing differences in the Melt flow index (MFI) of the different antioxidant polymer compositions performed under the same conditions: 190^{o}C (for PE) and 230^{o}C (for PP), standard die (2.095 mm) and a standard load of 2.16 kg according to ASTM standard D1238-95. The second approach was based on measuring the relative viscosity from changes in the pressure measured across a capillary slit-die in a **C**losed **L**oop **R**ecycle **T**est (**CLRT**) protocol developed in-house, comprising real-time rheological measurements of a polymer (with or without additives) undergoing repeated multi-pass extrusion cycles, carried out at 190 ^{o}C and screw speed of 100 rpm, in a Thermo Scientific HAAKE^{TM} Mini Lab II Micro Compounder. The conical twin screw HAAKE extruder is fitted with co-rotating screws, a by-pass for recirculation of the melt, and an in-built backflow channel with a slit-capillary die for in-line rheological measurements.

2.5.2 Determination of Long-Term Thermo-oxidative Stability and Oxidative Induction Time

Long-Term Thermo-oxidative Stability (LTTS) of polymer film samples was determined following extended periods of thermo-oxidative ageing at 115^{o}C and 125°C (tests done in duplicates) in multi-chamber single cell air-circulating (Wallace) oven and determining the time-to-embrittlement, EMT, at which time the polymer film was found to fail on gentle flexing through 180° between thumb and forefinger.

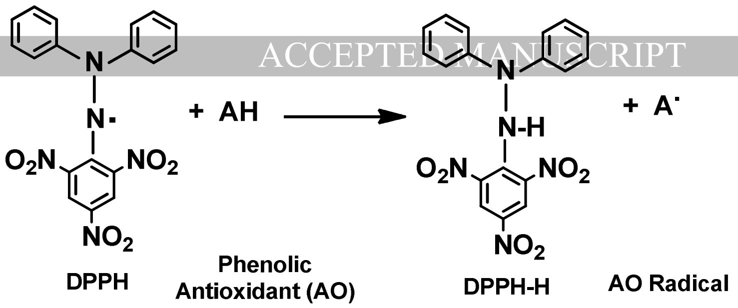
Oxidative Induction Time (OIT) of PE and PP melts was measured under a stream of oxygen (flow rate of 40ml/min) at an elevated temperature using DSC 823 (Mettler Toledo) according to ASTM-D3895-03. Triplicate samples (2-5 mg) were heated at 25°C/min to 190°C under nitrogen flow before switching to 100% oxygen.

2.5.3 Determination of Solubility and Diffusion Coefficients of Antioxidants in PE

Experiments for the determination of Diffusion coefficients of antioxidants were carried out on an FTIR spectrometer (PE) fitted with a zinc-selenide (ZnSe) Horizontal Attenuated Total Reflectance (HATR) attachment (Specac) and an external heater for temperature control. A diffusion assembly cell was built around the HATR base plate. An antioxidant-free LDPE film (50 μm thick) was first laid flat on the exposed surface of the ZnSe crystal. An antioxidant saturated LDPE plaque (produced by compression moulding) was subsequently placed on top of the film and held in place by a steel plate to ensure good contact between the plaque, film and the crystal in the assembly. Monitoring of the FTIR signature of the antioxidant-free LDPE film was undertake throughout the experiment which was conducted under nitrogen. Time resolved infrared absorbance spectra (using the ester carbonyl absorption peak around 1720 cm^{-1} for the rosmarinates with a polymer reference peak at 2019 cm^{-1}) were recorded and later used to generate the diffusion coefficients in accordance with Fick's Law [21]. The solubility of the antioxidants in LDPE was determined using the stack method [22] at different temperatures (stack assemblies placed in vacuum oven) by analysing the concentration profile of the antioxidant that diffuses through the successive layers of a stack of very thin films of PE, until equilibrium concentration is attained at the middle of the stack, using their characteristic FTIR and UV-absorption peaks (measured on both FTIR and UV-Vis spectrophotometers). All runs were carried out in duplicates with regular change in the orientation of the stack assembly to minimise the effects of gravity on migration of antioxidants.

2.6 Free Radical Activity of Hindered Phenol Antioxidants

The free radical scavenging activity of the antioxidants were determined using a slightly modified **DPPH** (2, 2-diphenyl-1-picrylhydrazyl) method described in the literature [23]. The DPPH test is typically used to determine the free radical scavenging activity of antioxidants measured normally close to room temperature (~37°C). The method was modified (using DMSO instead of ethanol) to enable tests to be carried out at higher temperatures in an attempt to be as close as possible to the elevated temperatures typically used in the assessment of LTTS performance of antioxidants in polyolefins. Instead of using an ethanolic solution of DPPH/antioxidant which limits the test close to room temperature, dimethyl sulfoxide, DMSO, was used. For antioxidants that showed low solubility in DMSO, a stock solution was first prepared in ethanol which was then diluted with DMSO to achieve the low concentration needed for the test. The DPPH solution (67 μM) was added to the antioxidant solution (7.5 μM) and kept for 30 min at 90 °C, after which the decrease in the DPPH absorbance at 516 nm, due to hydrogen atom abstraction by the DPPH radical, see **eqn.1**, was measured against a blank/control (containing the same amount of the DPPH reagent and DMSO but with no antioxidants); each test was performed in triplicates. The DPPH scavenging capacity was estimated from the difference in absorbance (A) with and without the antioxidants and expressed as percentage of DPPH scavenging activity and calculated as shown in **eqn. 2**.



(1)

$$\% \text{ DPPH Scavenging Activity} = (\mathbf{A}_{\text{control}} - \mathbf{A}_{\text{test}}) \times 100 / \mathbf{A}_{\text{control}} \qquad \textbf{(2)}$$

3 RESULTS AND DISCUSSION

3.1. Quantification of Carnosic Acid, Rosmarinic Acid and Carnosol in UK-Grown Rosemary

Literature studies on quantification of the amount of phenolic compounds in extracts of rosemary leaves have shown that the most abundant compounds were carnosic acid (**CA**) and rosmarinic acid (**RA**) followed by carnosol (**CA-OH**); the concentrations of the three compounds were found to be 12.18, 2.15 and 0.53 mg g^{-1} fresh weight biomass [24] and these were estimated to account for >90% of the antioxidant activity of the plant [25,26]. Commercial rosemary extract containing rosmarinic acid (93.4% pure) was used as the starting material for the synthesis of the rosmarinates esters investigated and reported here. However, as part of a large collaborative investigation of rosemary antioxidants (this paper forms part of this study), over 300 samples of UK-Grown rosemary plants were examined to find out the suitability of UK-grown rosemary plants for future use as a source of natural antioxidants. Extracts from harvests of three years (2007 - 2009) were then analysed (by HPLC) and quantified for their content of rosmarinic acid (**RA**), carnosic acid (**CA**) and carnosol (**CA-OH**) with many samples analysed in triplicates. Approximately 6.5% of the dry weight of the rosemary leaves was found to comprise the three phenolic antioxidants with their overall proportions in the same order as that found in other rosemary plants reported in the literature, i.e. CA> RA >> CA-OH. However, the yield ratio of the three antioxidants was shown to be affected by environmental influences and was particularly dependent on the harvest, hence maximum yield of the rosmarinic acid can be obtained at the appropriate harvest timing. An example of the component yield of two harvests of the same line in two years is shown below (full details of this study and the experimental methodology used will be reported elsewhere):

Amount in dried leaf of: **CA: RA: CA-OH** (Apr 2008):	**50.7: 41.9: 6.7** mg g^{-1}
Amount in dried leaf of: **CA: RA: CA-OH** (Oct 2007):	**82.1: 22.2: 11.9** mg g^{-1}

3.2. Characterisation of the Synthesised Rosmarinates

The synthesised rosemary-derived antioxidants were characterised using different spectroscopic techniques. The 1D-^{1}H-NMR spectra for the rosmarinic acid esters are similar to that of the parent acid except for the appearance of additional peaks characteristics of the ester groups, see for example **Figure 1A** for the stearyl ester, **RA-Str;** the ester peaks are labelled α, β, *R* and ω, the other peaks are similar to the corresponding ones for rosmarinic acid, **Figure 1B**, with slight shifts due to the esterification and the use of a different NMR solvent. Minor peaks attributed to the unreacted starting alcohol (e.g. stearyl alcohol in RA-Str spectrum) are also detected, see **Figure 1A** peak at 3.7ppm. Two-dimensional 2D-^{1}H-^{13}C Heteronuclear Multiple Bond Coherence (**HMBC**) NMR experiments for the rosmarinates were also carried out to show both the 1D - ^{1}H and ^{13}C NMR - and the 2D- correlations in one spectrum. The 2D-HMBC NMR spectrum of the **RA-Str** is shown here as an example, **Figure 2A** reveals a multiple bond correlation between the α-CH_2 of the ester group and the ester carbonyl group as indicated by the blue arrows**,** similar NMR characteristics and correlations were observed for all the other rosmarinates (spectra not shown) confirming the correct structure of the synthesised antioxidants. FTIR spectra of the rosmarinates (not shown) showed two clear carbonyl absorption peaks corresponding to a α,β-unsaturated ester (e.g. for **RA-Str** at 1684 cm^{-1}) and to a saturated ester (**RA-St** at 1725 cm^{-1}).

TGA thermograms of three of the synthesised rosmarinic acid derivatives (butyl, octyl and stearyl rosmarinates) are shown in **Figure 2B.** This shows clearly that the rosmarinates are stable at normal polyolefin processing temperatures (180-240^{o}C). The stearyl derivative has the highest stability under air (also under N$_{2,}$ not shown here) with its main decomposition temperature occurring at 335 ^{o}C compared to 315 ^{o}C for octyl and 285/328 ^{o}C for the butyl derivatives; the stearyl rosmarinates contains small amount of unreacted stearyl alcohol (shown earlier in its NMR spectrum, see **Figure 1A**) that would most certainly be responsible for the small observed TGA peak with a T$_d$ of 210^{o}C (its BP=210 ^{o}C). Similarly, the small TGA peak at 118 ^{o}C for the butyl rosmarinates must be due to unreacted butyl alcohol (it's BP=117 ^{o}C).

3.3 Physical Characteristics and Radical Scavenging Activity of the Rosmarinate Antioxidants

Successful stabilisation of polymers requires the use of antioxidants that possess both high inherent chemical activity to enable them to interfer with the oxidation chemistry of the polymer by inhibiting or retarding the oxidative degradation process, and physical permanance and resistance to loss from the polymer (by evaporation, blooming and leaching). Solubility and diffusion are, therefore, important parameters and are closely related processes which are often measured when assessing the migration behaviour of antioxidants in contact with a specific medium, e.g. food [22, 27-29]. In view of the importance of the physical retention of antioxidants in polymers, the solubility and diffusion coefficient of one of the rosemarinates, the stearyl rosmarinate (**RA-Str**), were measured in LDPE. Data presented in **Table 1** show that the diffusion coefficients of the **RA-Str** at all measured temperatures are considerably lower than those for Irganox 1076. Likewise, the diffusion coefficient of the methyl and octyl rosmarinates were found, likewise, to be ~~l~~ower than that of Irganox 1076. The slower diffusion of the rosmarinate is almost certainly due to its more rigid structure due to the presence of the two benzene rings compared to the more flexible structure of Irganox 1076. Although the solubility of the rosmarinates were found to be generally lower than that of Irganox 1076 in the same polymer, for example, the solubility of **RA-Str** in LDPE at 85^{o}C was 1.0 w/w% compared to 3.5 w/w% for Irganox 1076, the overall the physical characteristics of the rosmarinate indicate clearly that the new rosmarinate antioxidants have also the added advantage of good retention in the polymer.

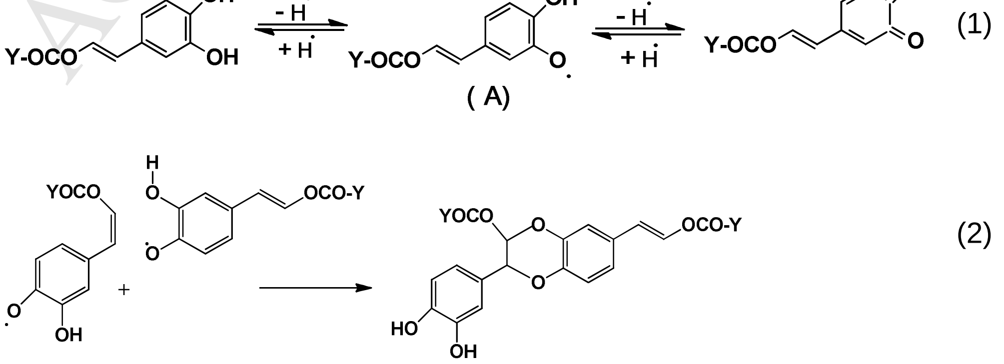
The free radical scavenging activity of the rosmarinate antioxidants was also investigated using DPPH assay test at 90^{o}C [23]. **Figure 3** shows that the DPPH scavenging activity of the synthesised rosmarinates (each has four –OH grups) is significantly higher than that of the commercial hindered phenols Irganox 1010 (containing also four –OH groups) and Irganox 1076 (bearing one –OH group); the stearyl ester (**RA-Str)** gave the highest DPPH activity.

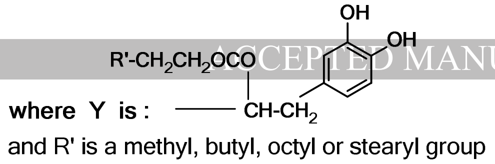
3.4 Effect of the Rosmarinate Antioxidants on the Melt and Long Term Thermal Stability of Polyolefins

The melt stabilising activity of the rosemarinate antioxidants in PE (LDPE processed in an extruder and LLDPE processed in an internal mixer) and PP (processed in an internal mixer) was investigated by measuring their melt flow index (MFI). LLDPE containing rosemarinate antioxidants (RA-Me, RA-But, RA-Oct, RA-Str) examined at three different concentrations (300, 500 and 1000 ppm) showed an excellent melt stabilising performance at all concentrations with almost no change to the MFI value of the base LLDPE polymer, see **Figure 4,** compared to a drop of 40% in the MFI value of a similarly processed samples stabilised with Irganox 1076 at the lower concentration of 300 ppm. To further examine the extent of melt stabilising activity of the rosemary antioxidants, a Closed Loop Recycle Testing (CLRT) methodology (see section 2.5.1) was adopted for ranking their performance in both LDPE and PP, see **Figure 5**. The superior performance of the stearyl rosmarinate (RA-Str) in both LDPE and PP is clearly discernible from the much lower extent of change in the relative viscosity (and the torque, not shown here) of the polymer with processing time compared to the large drop observed in the presence of Irganox 1010, Irganox 1076 and vitamin E. This again illustrates the extremely high efficacy of the rosmarinates in polyolefins under melt processing conditions of high temperatures and shear. These results are in full accord with both the excellent polymer melt flow results obtained and the superior free radical scavenging activity observed in solution (from DPPH test) with the stearyl rosmarinate antioxidant compared to that of the commercial hindered phenol antioxidants.

Evaluation of the effect of the rosemary antioxidants (at 1000 ppm) on the long-term thermal stability of LLDPE in solid polymer films (from measurement of embrittlement time, EMT, during thermo-oxidative ageing) at two elevated temperatures of 115 and 125°C has shown that the stearyl rosmarinates outperforms all the other derivatives, as well as that of Irganox 1076 and vitamin E examined under the same conditions, see **Figure 6**. The Oxidation Induction Time (OIT) for PE and PP samples containing antioxidants was also examined (according to ASTM D3895-03) at 190°C and the results show that, in LDPE after three individual multi-pass extrusions (passes: P1, P3 and P5), and in LLDPE after processing in an internal mixer, the stearyl rosmarinate (RA-Str) outperforms (at all concentrations tested) both Irganox 1010 and 1076, and affords higher performance than the corresponding low molecular weight rosmarinates RA-Me, RA-But, RA-EH, RA-Oct, see **Figures 7A** and **B**. In polypropylene, however, the results show that both RA-Oct and RA-But give longer OITs than the Stearyl rosmarinate, **Figure 7C**. The reason as to why the OIT of the PP polymer is lower for the Stearyl rosmarinate compared to that observed with the butyl and octyl derivatives is not yet clear. However, the superiority of the Stearyl rosmarinate antioxidant under the OIT tests conducted under different processing conditions of PE (both LLDPE and LDPE) are quite clear illustrating a much higher efficacy than its other low molecular weight analogues, as well as the commercial benchmark hindered phenol antioxidants tested under the same conditions. It is quite interesting to find that there is a very good linear relationship between the thermo-oxidative ageing results (LTTS in solid polymer films at temperatures up to 125°C) and the OIT results at 190°C in the polymer melt for all the synthesised derivatives of rosmarinic acid in PE and PP, **Figure 8**.

It is well known that the antioxidant activity (which is closely related to the free radical scavenging activity) of phenolic compounds is controlled by both the number of –OH groups on the benzene ring and the substitutions on the ring that stabilises the phenoxyl radical when formed and that play a major role in the ranking of the antioxidant activity of these molecules [30-32]. The superior antioxidant activity of the rosmarinates is, at least in part, due to the structure of rosmarinic acid where the presence of two *o*-hydroxy groups would give rise to additional resonance stabilisation for the generated phenoxyl radical, and more importantly, could lead to the formation of an *o*-quinone which are known to act as highly effective chain breaking acceptor antioxidants [30]. There is also the possibility of re-generating the parent rosmarinic acid esters in the polymer through a redox-type reaction, i.e. by conversion of the quinone carbonyls back to the hydroxyl groups, see **Scheme 1, reaction 1**). The ortho-hydroxy groups may also be re-generated via dimerisation reactions, e.g. through the dimerisation of the semi-quinone radical **A**$^{\cdot}$ [32,33], see **Scheme 1, reaction 2**





Scheme 1: Proposed chain breaking antioxidant activity of the rosmarinate esters

Further, oxidative transformation of the rosemary antioxidants at the high polymer processing temperatures may result in other products that have strong antioxidant properties; the TGA results (**Figure 2B** for the rosmarinates) support this suggestion. In addition to the observed excellent chemical (chain breaking-donor) activity in the absence of oxidising substrate (based on the DPPH test), see **Figure 3**, these new rosmarinate antioxidants show also high resistance to physical loss from the polymer due to their very low diffusion coefficients (lower than that of Irganox 1076), see **Table 1**, with the ester derivatives being more soluble than the parent rosmarinic acid in the polymer, though they are still less soluble than Irganox 1076.

4 Conclusion

Rosemary plants and the extracts of the herb have been known since ancient times for their antioxidancy, hence the long tradition of their use for medicinal benefits and in food preservation. However, 'mother nature' has given this plant a powerful defence mechanism to survive its harsh native Mediterranean environment whereby the active ingredients act to protect the plant under ambient conditions. Antioxidants from rosemary extracts may not be expected to show, therefore, the same level of antioxidant potency in polymers when subjected to very high processing temperatures during the polymer manufacturing process.

The results presented here, however, clearly indicate that the rosemary-derived phenolic compounds, especially the newly synthesised lipid-soluble esters of rosmarinic acid, are extremely efficient polymer antioxidants when tested under both melt processing and long term thermo-oxidative conditions. In particular, the stearyl rosmarinate show superior radical scavenging ability and powerful melt and thermal stabilising activity in PE and PP under all thermal ageing temperatures and processing conditions used in this study.

Both stearyl and octyl rosmarinates hold tremendous promise as antioxidants derived from a natural and sustainable resource not only for stabilising polymers but also for the stabilisation of many other hydrocarbon substrates (studied in this work but not reported here), e.g. lubricating and diesel oil, cosmetics and health products.

ACKNOWLEDGMENTS

The authors are grateful for the financial support of the DEFRA-LINK Project LK0823. We wish to thank all the project partners for useful discussions, with particular thanks to Dr Adam Maltby for providing the LDPE extruded samples.

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Caption of Figures

Figure 1: ^{1}H NMR spectra for stearyl rosmarinate (RA-Str) in $CDCl_3$, **A**, and rosmarinic acid (RA) in DMSO-d_6, **B.**

Figure 2: 2D ^{1}H-^{13}C HMBC NMR spectrum of stearyl rosmarinate (RA-Str), **A**, and the TGA of the butyl (RA-But), octyl (RA-Oct) and stearyl (RA-Str) rosmarinates, B.

Figure 3: Free radical scavenging activity (using DPPH assay) of the butyl, octyl and stearyl rosmarinates (RA-But, RA-Oct, RA-Str, respectively) and Irganox 1010 (Irg1010) and Irganox 1076 (Irg1076).

Figure 4: MFI of LLDPE in the absence and presence of 300, 500 and 1000 ppm antioxidants: Irganox 1076 (Irg1076), methyl, butyl, octyl and stearyl rosmarinates (RA-Me, RA-But, RA-Oct, RA-Str, respectively)

Figure 5: Change in the melt relative melt viscosity determined from Closed Loop Recycle Tests (CLRT) of PP (processed in internal mixer) and LDPE (processed in mini-Haake extruder) in the absence and presence of 1000 ppm (in PP) and 250 ppm (in LDPE) antioxidants. For LDPE the CLRT test was determined after the first and third multi-pass extrusions.

Figure 6: Embrittlement time (EMT) of LLDPE samples, in the absence and presence of 1000 ppm antioxidants, exposed to accelerated thermo-oxidative ageing in an air circulating single cell oven at 115°C and 125°C.

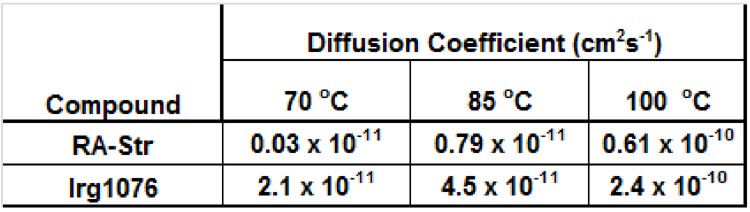
Figure 7: DSC-OIT of LLDPE, **A**, LDPE, **B,** and PP, **C,** containing 1000 ppm antioxidants. The results for LDPE are shown after the first (P1), third (P3) and fifth (P5) multi-extrusion passes.

Figure 8: Correlation between the thermo-oxidative embrittlement time (EMT) and the DSC-OIT of LDPE and PP samples containing 1000 ppm antioxidants

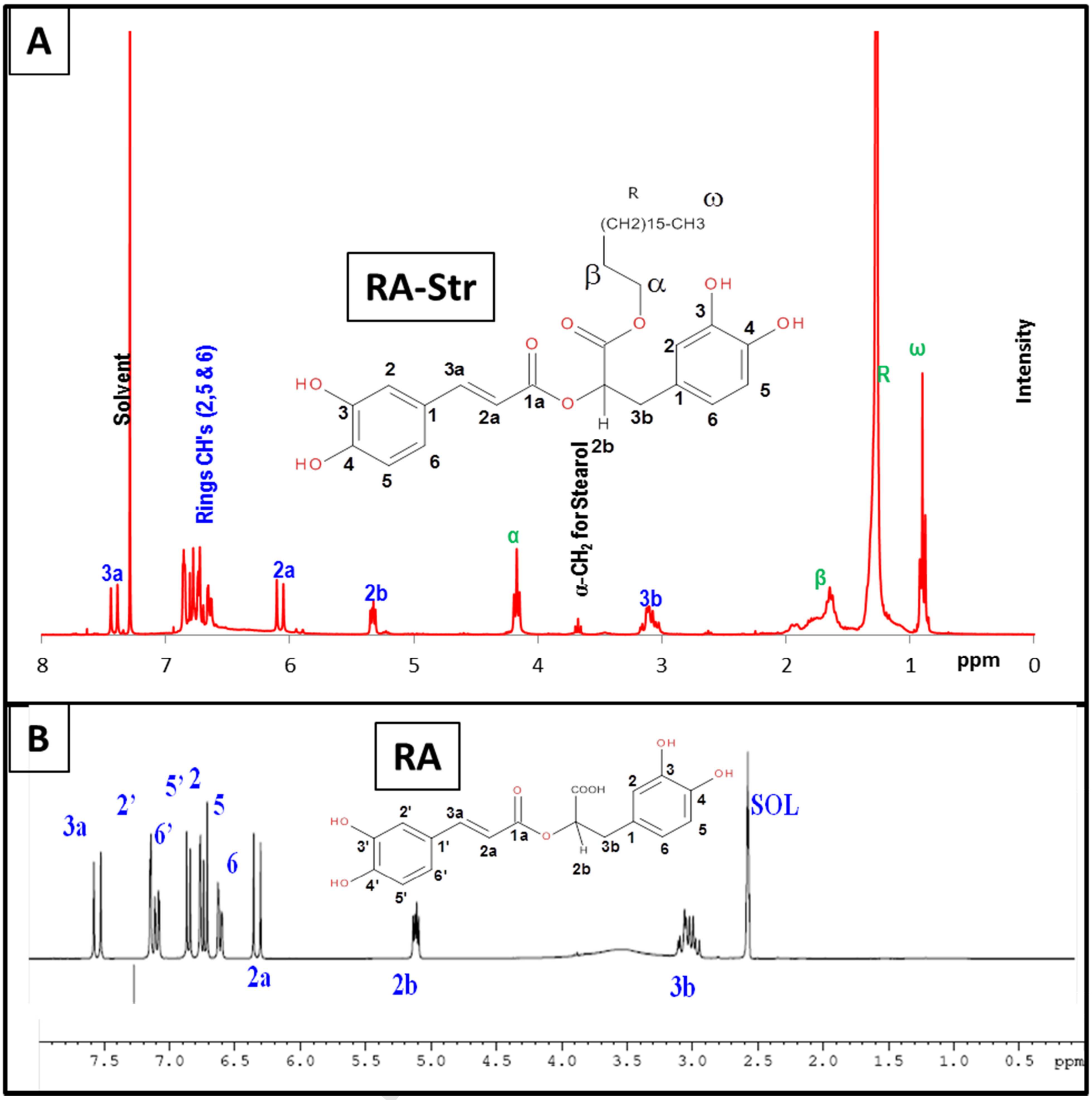
Caption of Tables

Table 1: Diffusion coefficients for stearyl rosmarinate and Irganox 1076 measured at three temperatures

Table 1



Compound	Diffusion Coefficient (cm^2s^{-1})		
	70 °C	85 °C	100 °C
RA-Str	0.03×10^{-11}	0.79×10^{-11}	0.61×10^{-10}
Irg1076	2.1×10^{-11}	4.5×10^{-11}	2.4×10^{-10}


A
RA-Str
R
ω
(CH2)15-CH3
β
α
OH
3
4
OH
O
O
O
2
HO
2
3a
3
1
2a
1a
O
H
3b
2b
1
5
6
HO
4
5
6
Solvent
Rings CH's (2,5 & 6)
3a
2a
2b
α
α-CH₂ for Stearol
3b
β
R
ω
Intensity
8
7
6
5
4
3
2
1
ppm
0
B
RA
OH
3
OH
COOH
2
4
5
6
O
HO
2'
3a
3'
1'
2a
1a
O
H
2b
3b
1
HO
4'
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3a
2'
5' 2
5
6'
6
2a
2b
3b
SOL
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm

Figure 1

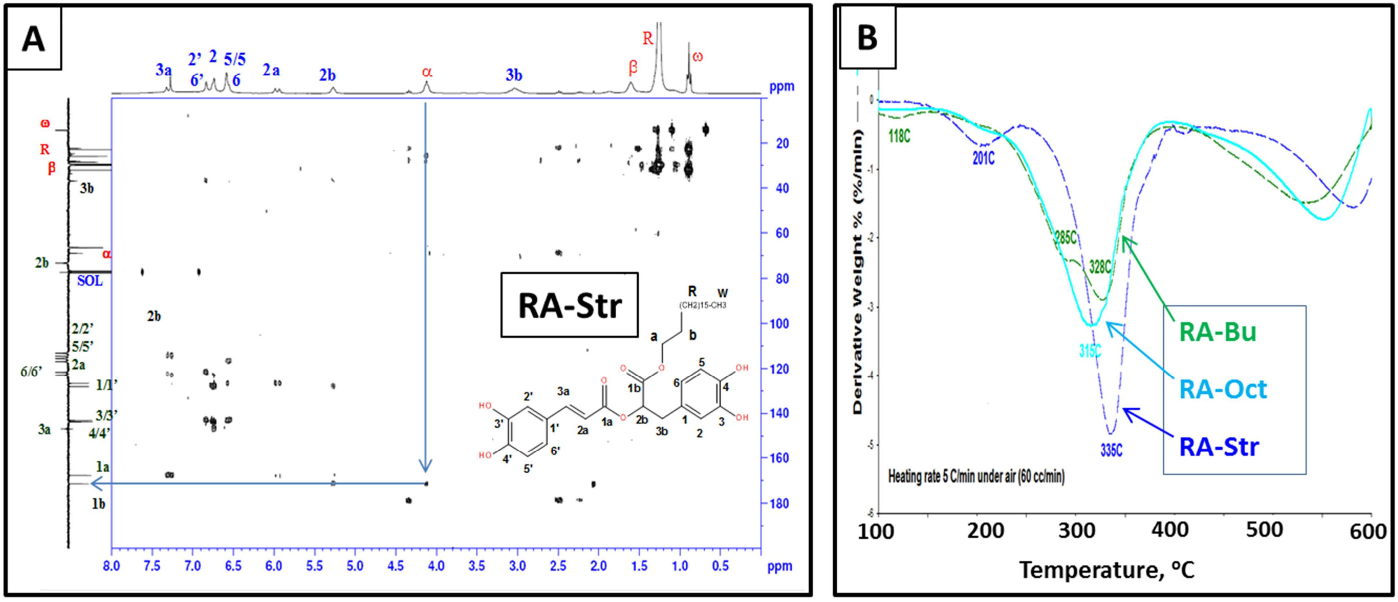

Figure 2

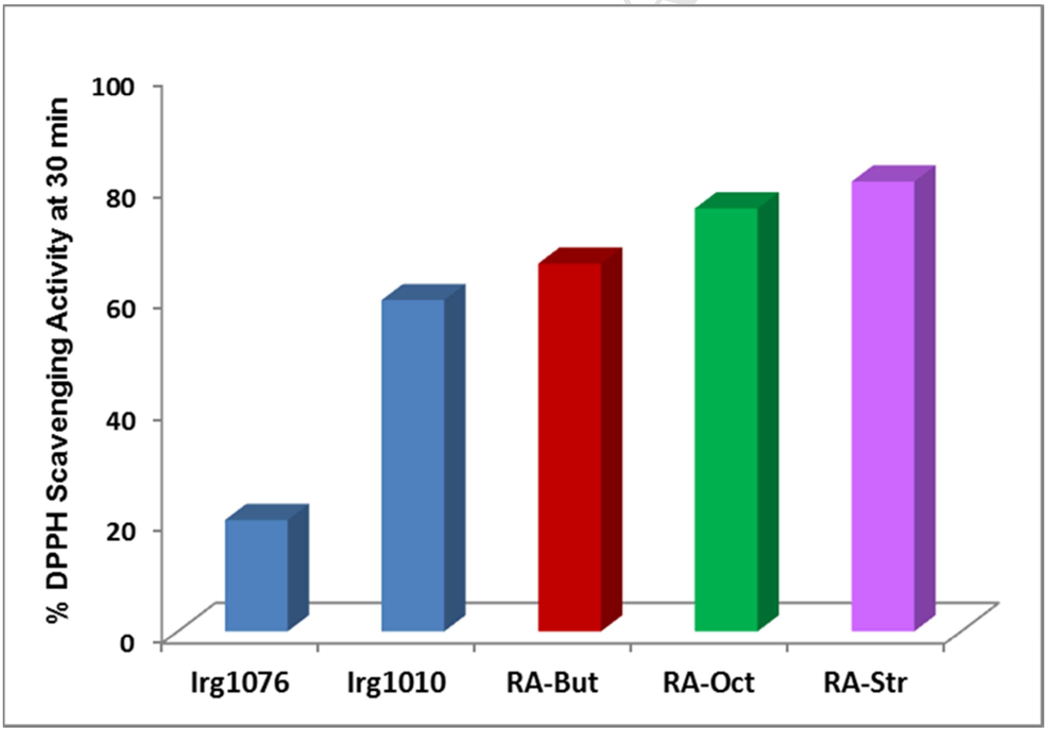

Figure 3

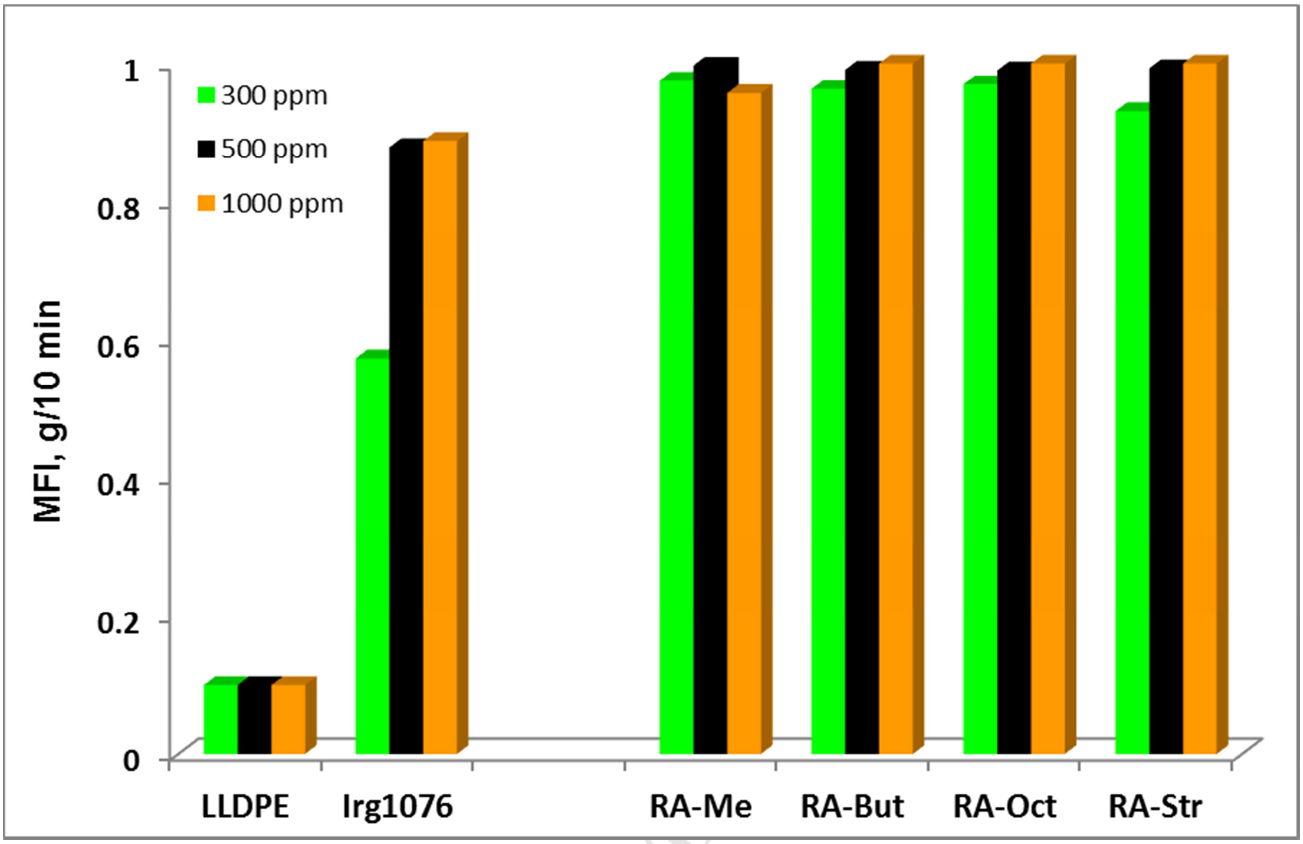

Figure 4

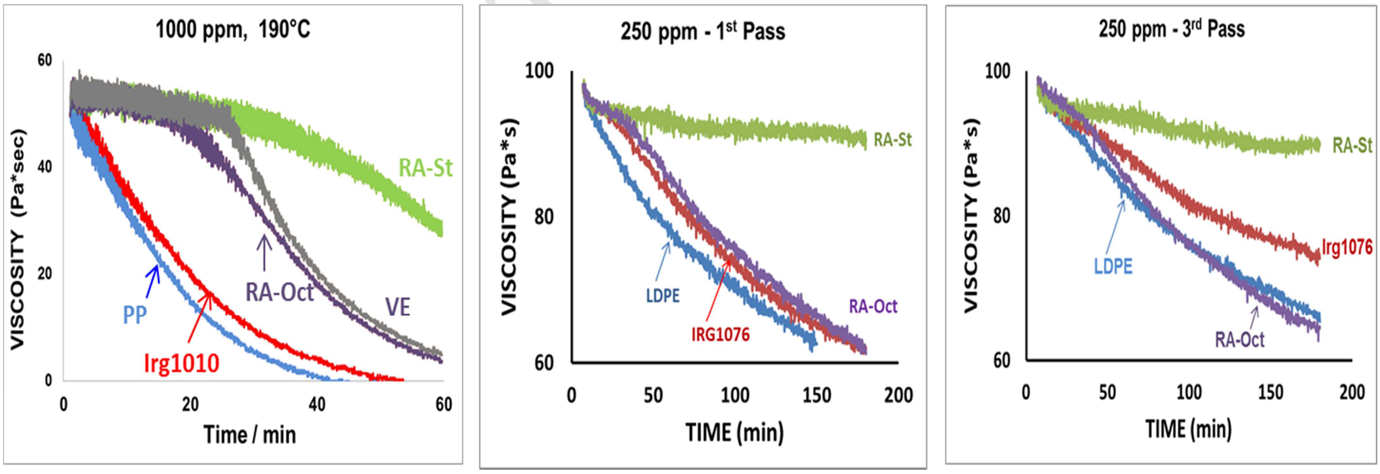

Figure 5

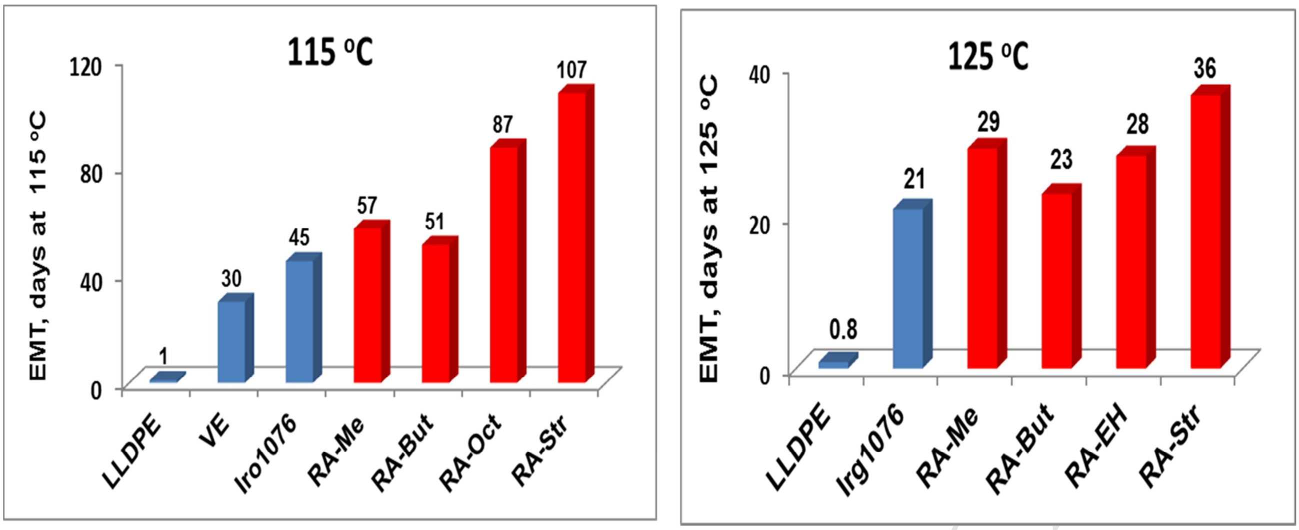

Figure 6

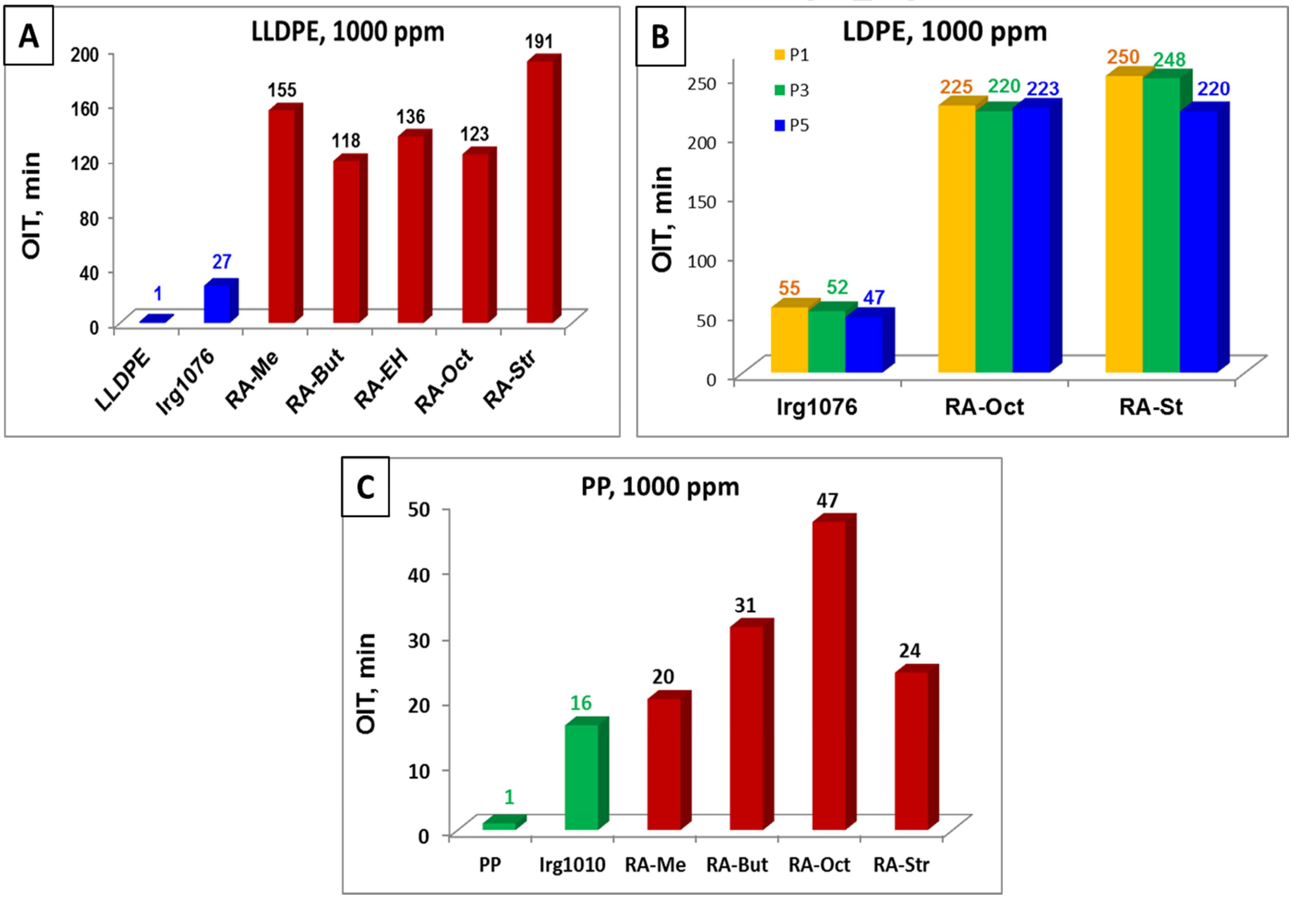

Figure 7

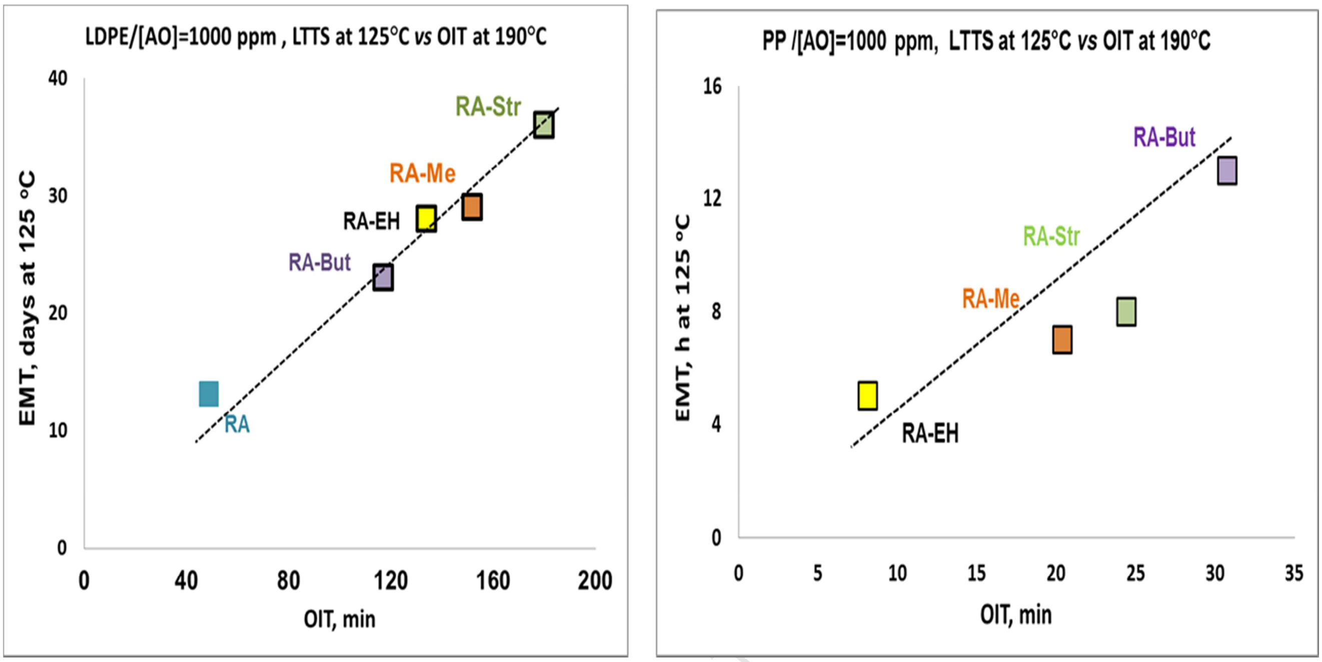
LDPE/[AO]=1000 ppm , LTTS at 125°C vs OIT at 190°C
EMT, days at 125 °C
0
10
20
30
40
0
40
80
120
160
200
OIT, min
RA
RA-But
RA-EH
RA-Me
RA-Str
PP /[AO]=1000 ppm, LTTS at 125°C vs OIT at 190°C
EMT, h at 125 °C
0
4
8
12
16
0
5
10
15
20
25
30
35
OIT, min
RA-EH
RA-Me
RA-Str
RA-But

Figure 8