Research Article

Received: 26 March 2015 Revised: 23 September 2015 Accepted article published: 19 October 2015 Published online in Wiley Online Library: 13 November 2015

(wileyonlinelibrary.com) DOI 10.1002/jsfa.7502

Molecular characterisation of a bio-based active packaging containing *Origanum vulgare* L. essential oil using pyrolysis gas chromatography–mass spectrometry

María Llana-Ruíz-Cabello,[a] Silvia Pichardo,[a] Nicasio T Jiménez-Morillo,[b] José M Bermúdez,[c] Susana Aucejo,[c] Francisco J González-Vila,[b] Ana M Cameán[a] and José A González-Pérez[b*]

Abstract

BACKGROUND: Environmental, economic and safety challenges motivate shift towards safer materials for food packaging. New bioactive packaging techniques, i.e. addition of essential plant oils (EOs), are gaining attention by creating barriers to protect products from spoilage. Analytical pyrolysis gas chromatography–mass spectrometry (Py-GC-MS) was used to fingerprint a bioactive polylactic acid (PLA) with polybutylene succinate (PBS) (950 g kg^{-1}:50 g kg^{-1}) film extruded with variable quantities (0, 20, 50 and 100 g kg^{-1}) of *Origanum vulgare* EO.

RESULTS: Main PLA:PBS pyrolysis products were lactide enantiomers and monomer units from the major PLA fraction and succinic acid anhydride from the PBS fraction. Oregano EO pyrolysis released cymene, terpinene and thymol/carvacrol peaks as diagnostic peaks for EO. In fact, linear correlation coefficients better than 0.950R^2 value ($P < 0.001$) were found between the chromatographic area of the diagnostic peaks and the amount of oregano EO in the bioplastic.

CONCLUSION: The pyrolytic behaviour of a bio-based active package polymer including EO is studied in detail. Identified diagnostic compounds provide a tool to monitor the quantity of EO incorporated into the PLA:PBS polymeric matrix. Analytical pyrolysis is proposed as a rapid technique for the identification and quantification of additives within bio-based plastic matrices.

Keywords: oregano essential oil; polylactic acid; polybutylene succinate; pyrolysis gas chromatography–mass spectrometry; active packaging

INTRODUCTION

Traditionally, materials used for food packaging include a variety of petrochemical-based polymers, such as polyolefins, polyesters, polyamides, etc., because of their high specific strength and durability, ease of processing and their availability at low cost. However, today, environmental, economic and safety concerns have motivated scientists and producers to explore the possibilities of using more environmentally safe biodegradable materials. [1,2] In this sense, polylactic acid (PLA) is one of the most widely used bio-based materials in many applications, including the food packaging industry mainly used for improving the shelf life of perishable products.[3]

Chemically, PLA is an aliphatic polyester made up of lactic acid (2-hydroxypropionic acid) building blocks and is ultimately derived from renewable plant sources, such as starch and sugar.[4] The success of PLA as a food-package alternative is due to its low toxicity, high biodegradability and biocompatible thermoplastic, with high-strength, high-modulus and a good processability.[5] However, a drawback of PLA is its medium gas barrier properties. Combining PLA with other polymers,[6] or adding nanomaterials such as cellulose nanocrystals or nanowhiskers[3,7,8] may reduce such a problem, thus improving the characteristics of PLA-based packages.

Another possibility to improve the quality and safety of packed products is to include additives able to ameliorate polymer barrier properties by creating a bioactive material;[7] a polymer designed to deliberately incorporate components that would release or absorb substances into or from the packaged food or the environment surrounding the food.[9–11]

Due to the current global demand for minimally processed and preservative-free foodstuffs, new bioactive packaging techniques and ingredients from natural sources are gaining

---

* Correspondence to: José A González-Pérez, IRNAS-CSIC, Av. Reina Mercedes, 10, 41012 Seville, Spain. E-mail: jag@irnase.csic.es

a Area of Toxicology, Faculty of Pharmacy, Universidad de Sevilla, Profesor García González n° 2, 41012 Seville, Spain

b IRNAS-CSIC, Av. Reina Mercedes, 10, 41012 Seville, Spain

c Area of Packaging Materials and Systems, ITENE, C/ Albert Einstein 1, 46980 Paterna, Valencia, Spain

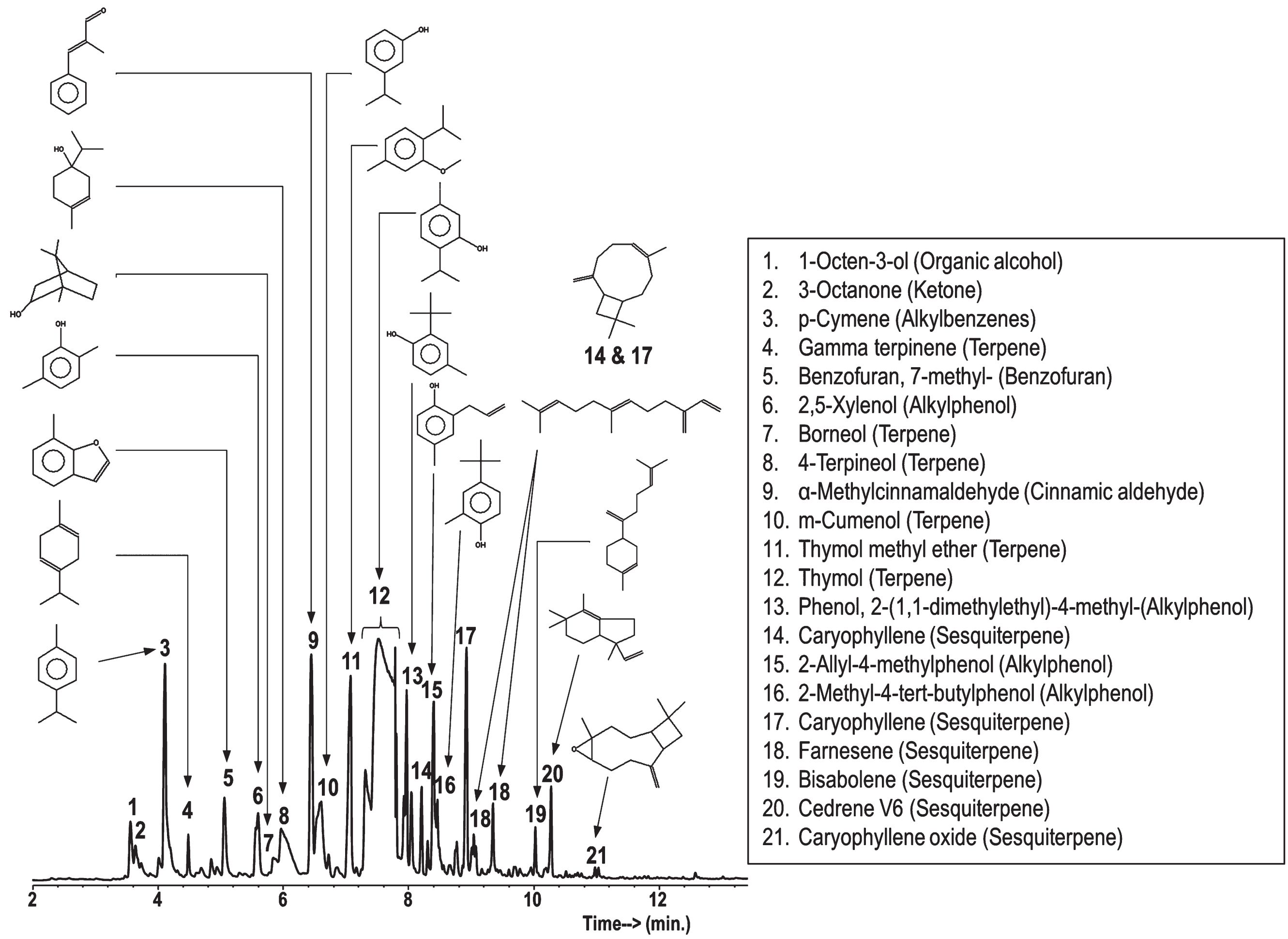

Figure 1. Total ion chromatogram (Py-GC-MS TIC) with an indication of the main pyrolysis products released at 500°C from oregano EO.

attention from the industry because these are perceived by consumers as low-health-risk materials. Therefore bioactive additives derived from essential oils (EOs), i.e. antimicrobial and antioxidants, are being increasingly used in food packaging.[12] Many of such EOs are plant secondary metabolites which have been extensively studied as natural food preservatives. Oregano essential oil is classified as 'generally recognised as safe' (GRAS) by the US Food and Drug Administration (FDA) and classified as a food additive by European Union (EU) legislation,[13,14] has been studied for its antimicrobial and antioxidant properties.[10,15] In fact, the monoterpenes carvacrol, thymol, cymene and terpinene, main constituents of oregano EO,[16,17] have been proven to have antimicrobial[18,19] and antioxidant properties.[20,21] In line with this, recent work revealed that carvacrol and thymol were able to protect Caco-2 cells against induced oxidative stress acting as an antioxidant agent *in vitro*.[22]

The use of EOs as food additives is sometimes limited due to unacceptable organoleptic properties.[14] However, the incorporation of EOs in food-packaging films allows the controlled release of active substances and reducing undesirable flavours caused by direct addition of EOs into food.[23,24] Due to the volatility of EOs and the conditions of film preparation, usually at elevated temperatures, it is necessary to ensure that the active compounds of oregano EO remain in the desired quantities in the final manufactured polymer. Analytical pyrolysis, defined as the thermochemical decomposition of organic materials at elevated temperatures in the absence of oxygen,[25] is a useful tool for the direct characterisation of polymers and additives within the polymer matrix. The products of pyrolysis (pyrolysate) are amenable to chromatographic separation and when combined with a mass spectrometry detector (Py-GC-MS), yields molecular information about the structure of complex mixtures of natural and synthetic macromolecular substances.[26] Other well-known advantages of the technique include the requirement of small sample sizes and little to no sample preparation needs. This makes analytical pyrolysis a convenient method for inexpensive and relatively rapid analyses of synthetic[27–30] and bio-based polymers including polylactic acid[31–36] and polybutylene succinate[37] plastics.

In this work direct analytical pyrolysis (Py-GC-MS) was used for a detailed characterisation ('fingerprinting') of both, an oregano EO and a bio-based 950 g kg^{-1} polylactic acid (PLA) plastic extruded with 50 g kg^{-1} polybutylene succinate (PBS) to ameliorate crystallinity. Also bio-plastic films extruded with add mixtures of essential oil (20, 50 and 100 g kg^{-1} in dry weight) were studied.

MATERIALS AND METHODS

Supplies and chemicals

The polymers used in this work were: polylactic acid (PLA) extrusion-grade (2003D) purchased in pellets from NatureWorks LLC (Minnetonka, MN, USA) and polybutylene succinate (PBS) GS Pla^{TM} FD92WD purchased from Mitshubishi Chemical Corporation

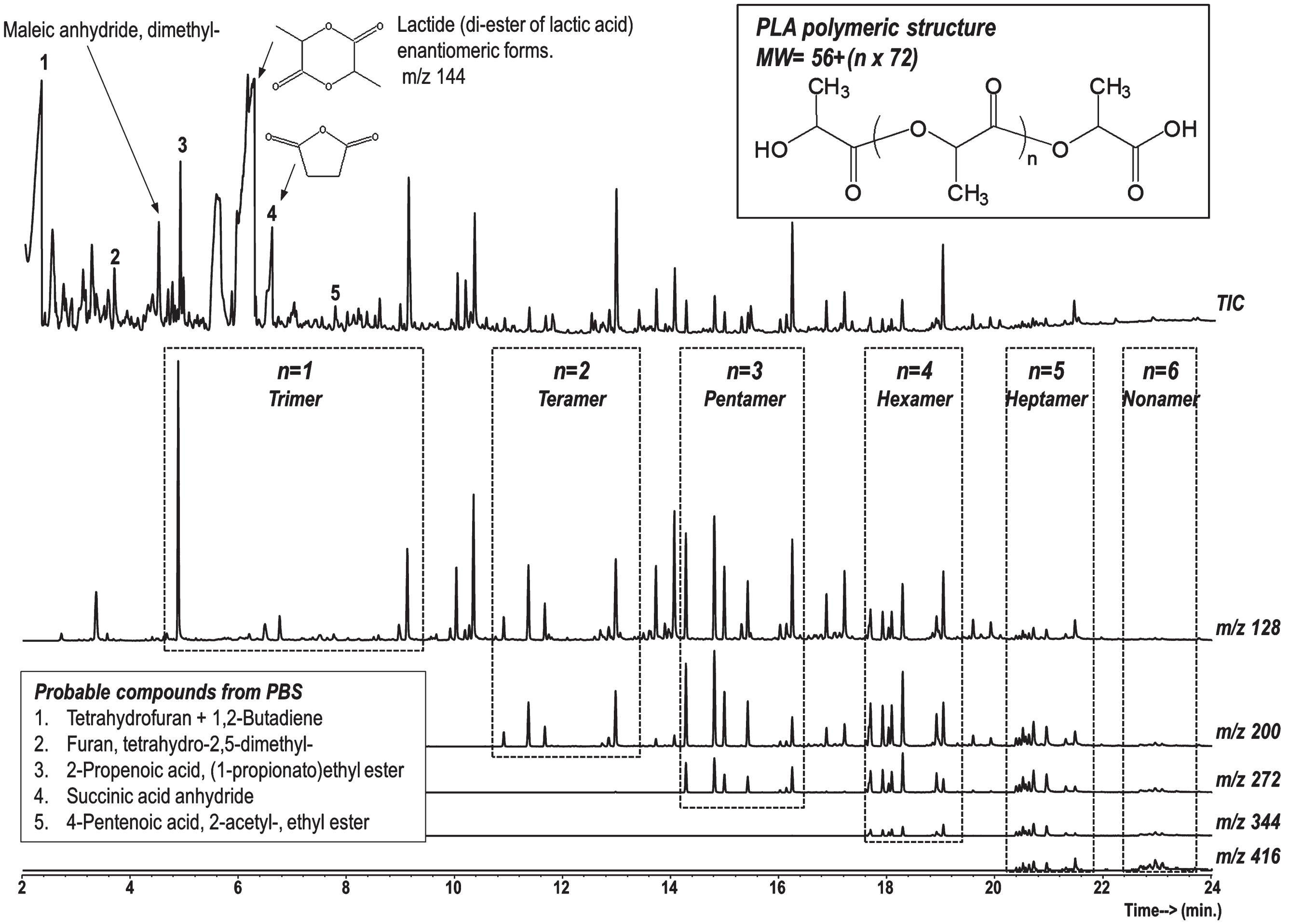

Figure 2. Total ion and selected ion monitoring chromatograms (Py-GC-MS TIC and SIM) of a biodegradable polymer blend PLA:PBS (950 g kg^{-1}:50 g kg^{-1}) with an indication of the main PLA polymeric units and probable PBS derived compounds. *m/z*, selected ion mass to charge ratio.

(Tokyo, Japan). Oregano essential oil (EO) was obtained from El Jarpil® (Almería, Spain).

Film preparation

The different active PLA films were obtained by melt blending in a twin-screw extruder (DSE 20-40D; Brabender, Duisburg, Germany). Different concentrations (20, 50 and 100 g kg^{-1} which correspond to 2, 5 and 10% w/w, respectively) of oregano EO were fed into the barrel trough the lateral liquid port at L/D 10 in order to reduce possible volatility and degradation losses. Barrel temperatures were set at 200–205°C working at a screw speed of 70 min^{-1}. A control film was extruded in the same manner but with no oregano EO added. The average thickness of the final films was 80 μm (c. 315 gauges).

Analytical pyrolysis (Py-GC-MS)

Direct pyrolysis gas chromatography–mass spectrometry (Py-GC-MS) analysis was performed using a double-shot pyrolyser F-Labs model 2020i (Frontier Laboratories, Fukushima, Japan) attached to a GC-MS system Agilent 6890N (Agilent Technologies Inc., Santa Clara, CA, USA). Samples (0.5 mg) were placed in small crucible capsules and introduced into a preheated micro-furnace at 500°C for 1 min. The evolved gases were transferred into the GC-MS for analysis. The gas chromatograph was equipped with a low polar-fused silica (5% phenylmethylpolysiloxane) capillary column J&W HP-5ms Ultra Inert, of 30 m × 250 μm × 0.25 μm film thickness. The oven temperature was held at 50°C for 1 min and then increased to 100°C at 30°C min^{-1}, from 100°C to 300°C at 10°C min^{-1}, and stabilised at 300°C for 10 min. The carrier gas used was helium at a controlled flow of 1 cm^3 min^{-1}. The detector was an Agilent 5973 (Agilent Technologies Inc.) mass selective detector, and mass spectra were acquired at 70 eV ionising energy. Compound assignment was achieved by single-ion monitoring for various homologous series, low-resolution mass spectrometry, and via comparison with published and stored (NIST05 and WILEY7N libraries) data.

The Pearson correlation coefficient was used to assess the significance of the EO added to the plastic and the chromatographic peak areas of EO derived peaks. The analysis was conducted using the PEARSON function in MS Excel 2010 software.

RESULTS AND DISCUSSION

Figure 1 shows the total ion chromatogram of the pyrolysis products (pyrogram) release at 500°C from oregano EO, with an indication to the chemical identities of the main pyrolysis products. These are a typical mixture of aromatic and hydroaromatic structures dominated by monoterpenes and sesquiterpenes with a conspicuous broad peak (at 7–8 min) (peak 12) that corresponds to a mixture of thymol [phenol, 5-methyl-2-(1-methylethyl)-] with the isomer carvacrol [phenol, 2-methyl-5-(1-methylethyl)-]

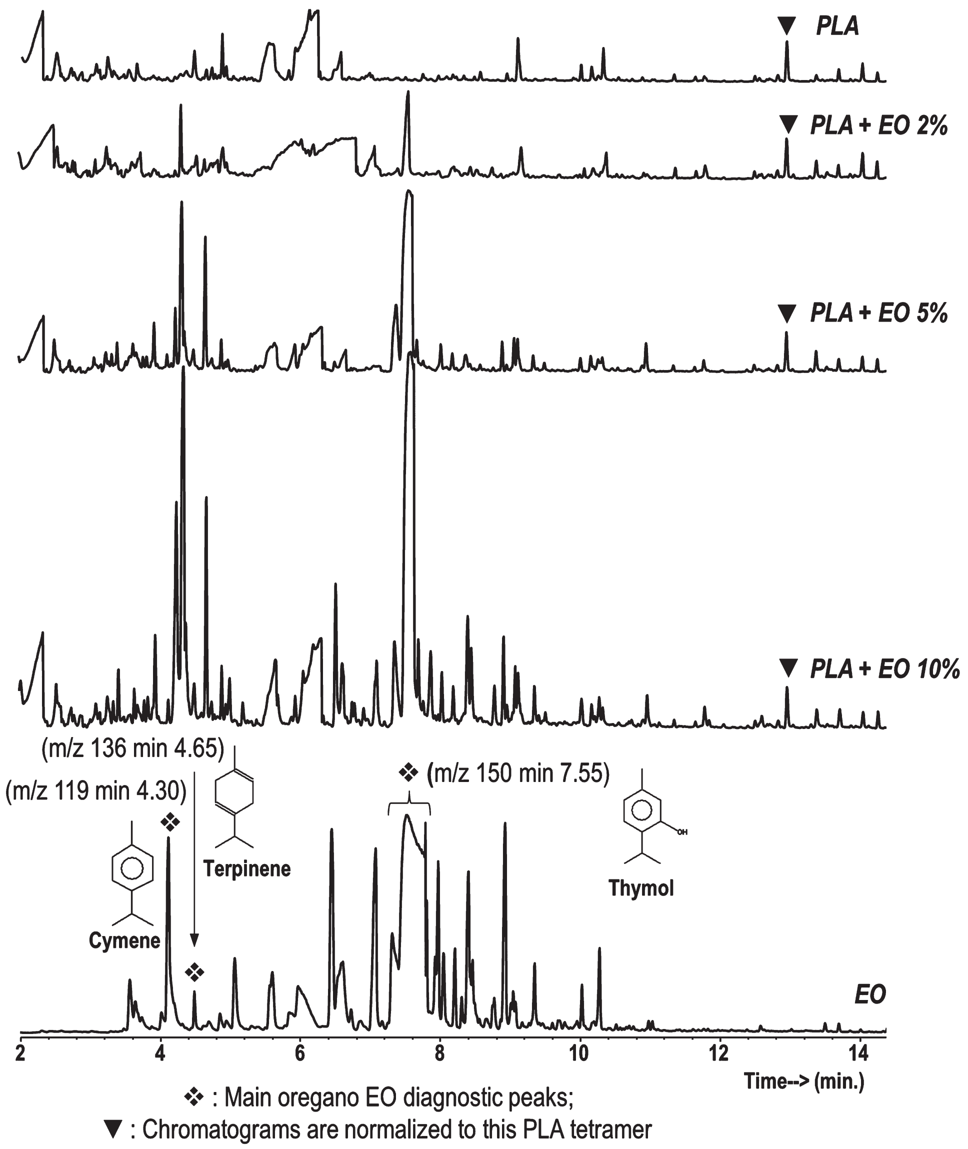

Figure 3. PLA and oregano EO total ion chromatograms (Py-GC-MS TIC) and of added mixtures of 20, 50 and 100 g kg^{-1} which correspond to 2, 5 and 10% w/w, respectively oregano EO in PLA.

which, under the chromatographic conditions used, could not be resolved. Other major pyrolysis products included the alkylbenzene *p*-cymene (peak 3), other terpenes such as γ-terpinene, (peak 4), terpineol (peak 8), α-methylcinnamaldehyde, (peak 9), thymol/carvacrol methyl ester (peak 11) and a number of known sesquiterpenes, i.e. caryophyllene (peaks 14 and 17), farnesene (peak 18), bisabolene (peak 19) and cedrene (peak 20).

A detailed pyrogram of the PLA:PBS (950 g kg^{-1}:50 g kg^{-1}) biodegradable film is shown in Fig. 2. The main pyrolysis products were a broad peak at 6 min that corresponds to lactide (di-ester of lactic acid or 1,4-dioxane-2,5-dione, 3,6-dimethyl-) and their enantiomeric forms. Besides, cyclic oligomers were clearly detected in the PLA pyrolysates when searching for specific ions following the polymer general formula: $MW = 56 + (n \times 72)$, where MW is molecular weight. Under the chromatographic conditions used up to nine monomer units with a maximum molecular weight of 488 Da were detected. These findings are in line with previous pyrolysis and PLA thermal degradation studies.[36] In addition, a number of other peaks, tetrahydrofuran and 1,2-butadiene (peak 1), furan, tetrahydro-2,5-dimethyl (peak 2), 2-propenoic acid, (1-propionato)ethyl ester (peak 3), succinic acid anhydride (peak 4) and 4-pentenoic acid, 2-acetyl-, ethyl ester (peak 5), observed in the total ion chromatogram trace were identical to those previously identified in PBS pyrolysates,[37] i.e. they most probably derive from the minor PBS fraction present in the biodegradable plastic blend used for enhancing PLA crystallinity.

In Fig. 3, the PLA and oregano EO total ion chromatograms are depicted together with the bio-based active film manufactured with oregano EO add mixtures (20, 50 and 100 g kg^{-1}) in the biodegradable PLA. Conspicuous peaks, obviously derived from the added oregano EO are clearly visible in the active film even in that with the lowest EO doses (20 g kg^{-1}). These peaks corresponded to the major oregano EO terpene thymol/carvacrol mixture (at approx. 7.55 min), the alkyl benzene cymene (at 4.30 min) and, less apparent mainly at lower EO doses, a third peak corresponding to the terpene terpinene (at 4.65 min). These three peaks can be considered as diagnostic/marker peaks to trace the added oregano EO within the bioplastic matrix. In fact, Pearson linear

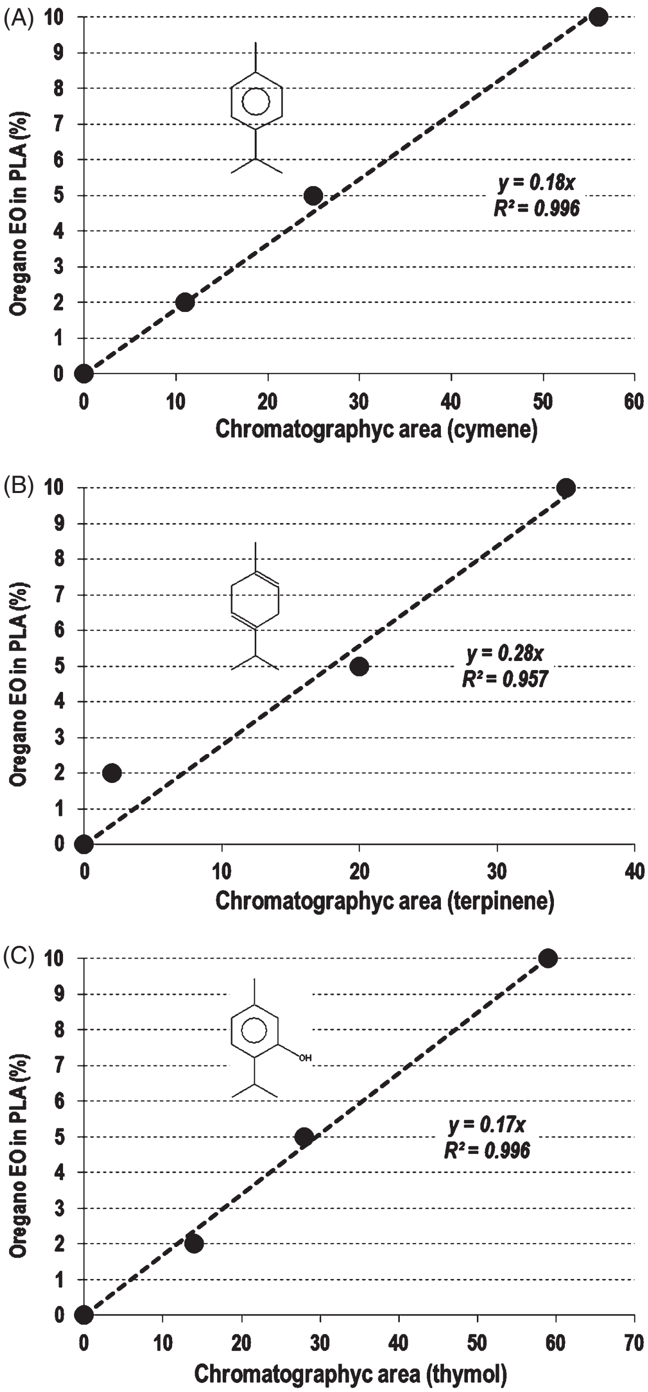

Figure 4. Relation between the main oregano EO diagnostic peaks and the percentage of added EO in the PLA. (A) Cymene peak at 4.30 min; (B) terpinene peak at 4.65 min; and (C) carvacrol/thymol peak at 7.55 min.

correlation coefficients of better than 0.950R^2 value ($P < 0.001$) were found between the chromatographic area of these three main marker peaks and the amount of oregano EO (in g kg^{-1}) added to the biodegradable plastic to extrude the active film (Fig. 4).

Although the primary use of EOs in the food industry is as flavourings, these oils also represent a source of natural food preservatives. Many studies have demonstrated the potent antimicrobial and antioxidant activities of oregano EO[16,17,20,38] and its use is increasing as a natural component of many foodstuffs and also of non-edible materials of use in the food industry, i.e. plastic films used in bio-active packaging. Previous results[31] and those described here indicate that analytical pyrolysis (Py-GC-MS) can provide rapid and accurate information about the composition, quality and even a precise fingerprinting of EOs contained in active packages made with biogenic polymers like PLA:PBS. It is also foreseen that the technique will be of use to study other EOs and additives included in a wide variety of other natural or synthetic polymeric matrices.

ACKNOWLEDGEMENTS

This work was partly funded by the Spanish 'Ministerio de Economía y Competitividad' through projects CGL2012-38655-C04-01 and AGL2012-38357-C02-01 co-financed by FEDER Funds, and Junta de Andalucía (AGR-7252). N.T. Jiménez-Morillo is funded by a FPI research grant (BES-2013-062573).

REFERENCES

1 Bohlman GM, General characteristics, processability, industrial applications and market evolution of biodegradable polymers, in *Handbook of Biodegradable Polymers*, 1st edition, ed. by Bastioli C. Rapra Technology Ltd, Shropshire, UK, pp. 183–218 (2005).
2 Debiagi F, Kobayashi RKT, Nakazato G, Panagio LA and Mali S, Biodegradable active packaging based on cassava bagasse, polyvinyl alcohol and essential oils. *Ind Crop Prod* **52**:664–670 (2014).
3 Fortunati E, Peltzer M, Armentano I, Torre L, Jimenez A and Kenny JM, Effects of modified cellulose nanocrystals on the barrier and migration properties of PLA nano-biocomposites. *Carbohydr Polym* **90**:948–956 (2012).
4 Lim LT, Auras R and Rubino M, Processing technologies for poly(lactic acid). *Prog Polym Sci* **33**:820–852 (2008).
5 Rhim J-W, Mohanty AK, Singh SP and Ng PKW, Effect of the processing methods on the performance of polylactide films: Thermocompression versus solvent casting. *J Appl Polym Sci* **101**:3736–3742 (2006).
6 Jamshidian M, Tehrany EA, Imran M, Jacquot M and Desobry S, Poly-lactic acid: production, applications, nanocomposites, and release studies. *Compr Rev Food Sci F* **9**:523–571 (2010).
7 Salmieri S, Islam F, Khan RA, Hossain FM, Ibrahim HMM, Miao C *et al.*, Antimicrobial nanocomposite films made of poly(lactic acid)–cellulose nanocrystals (PLA–CNC) in food applications: part A – effect of nisin release on the inactivation of *Listeria monocytogenes* in ham. *Cellulose* **21**:1837–1850 (2014).
8 Cabedo L, Feijoo JL, Villanueva MP, Lagaron JM and Gimenez E, Optimization of biodegradable nanocomposites based on PLA/PCL blends for food packaging applications. *Macromol Symp* **233**:191–197 (2006).
9 European Commission, Regulation (EC) No. 1935/2004 of the European Parliament and the Council of 27 October 2004, on materials and articles intended to come into contact with food and repealing Directives 80/509/EEC and 89/109/EEC. *Off J Eur Comm* **338**:4–17 (2004).
10 López P, Sánchez C, Batlle R and Nerín C, Development of flexible antimicrobial films using essential oils as active agents. *J Agric Food Chem* **55**:8814–8824 (2007).
11 Salmieri S, Islam F, Khan RA, Hossain FM, Ibrahim HMM, Miao C *et al.*, Antimicrobial nanocomposite films made of poly(lactic acid)–cellulose nanocrystals (PLA–CNC) in food applications: part B – effect of oregano essential oil release on the inactivation of *Listeria monocytogenes* in mixed vegetables. *Cellulose* **21**:4271–4285 (2014).
12 Llana-Ruiz-Cabello M, Maisanaba S, Puerto M, Prieto AI, Pichardo S, Jos A *et al.*, Evaluation of the mutagenicity and genotoxic potential of carvacrol and thymol using the Ames *Salmonella* test and alkaline, Endo III- and FPG-modified comet assays with the human cell line Caco-2. *Food Chem Toxicol* **72**:122–128 (2014).
13 Manso S, Pezo D, Gómez-Lus R and Nerín C, Diminution of aflatoxin B1 production caused by an active packaging containing cinnamon essential oil. *Food Control* **45**:101–108 (2014).

14 Muriel-Galet V, Cran MJ, Bigger SW, Hernández-Muñóz P and Gavara R, Antioxidant and antimicrobial properties of ethylene vinyl alcohol copolymer films based on the release of oregano essential oil and green tea extract components. *J Food Eng* **149**:9–16 (2015).

15 Sánchez-González L, Vargas M, González-Martínez C, Chiralt A and Cháfer M, Use of essential oils in bioactive edible coatings. *Food Eng Rev* **3**:1–16 (2011).

16 Lambert RJW, Skandamis PN, Coote PJ and Nychas G-JE, A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. *J Appl Microbiol* **91**:453–462 (2001).

17 Burt S, Essential oils: Their antibacterial properties and potential applications in foods. *Int J Food Microbiol* **94**:223–253 (2004).

18 Zivanovic S, Chi S and Draughon AF, Antimicrobial activity of chitosan films enriched with essential oils. *J Food Sci* **70**:M45–M51 (2005).

19 Ozkalp B, Sevgi F, Ozcan M and Ozcan MM, The antibacterial activity of essential oil of oregano (*Origanum vulgare* L.). *J Food Agric Environ* **8**:272–274 (2010).

20 Kulisic T, Radonic A, Katalinic V and Milos M, Use of different methods for testing antioxidative activity of oregano essential oil. *Food Chem* **85**:633–640 (2004).

21 Amorati R, Foti MC and Valgimigli L, Antioxidant activity of essential oils. *J Agric Food Chem* **61**:10835–10847 (2013).

22 Llana-Ruiz-Cabello M, Gutiérrez-Praena D, Puerto M, Pichardo S, Jos A and Cameán AM, *In vitro* pro-oxidant/antioxidant role of carvacrol, thymol and their mixture in the intestinal Caco-2 cell line. *Toxicol In Vitro* **29**:647–656 (2015).

23 Llana-Ruiz-Cabello M, Gutiérrez-Praena D, Pichardo S, Moreno FJ, Bermúdez JM, Aucejo S *et al.*, Cytotoxicity and morphological effects induced by carvacrol and thymol on the human cell line Caco-2. *Food Chem Toxicol* **64**:281–290 (2014).

24 Ramos M, Jiménez A, Peltzer M and Garrigós MC, Characterization and antimicrobial activity studies of polypropylene films with carvacrol and thymol for active packaging. *J Food Eng* **109**:513–519 (2012).

25 Irwin WJ, Analytical pyrolysis – A comprehensive guide, in Chromatographic Science, *Series 22*, ed. by Cazes J. Marcel Dekker, New York, Chapter 6 (1982).

26 González-Pérez JA, González-Vila FJ, Almendros G, Knicker H, De La Rosa JM and Hernández Z, Revisiting structural insights provided by analytical pyrolysis about humic substances and related bio- and geopolymers, in *Functions of Natural Organic Matter in a Changing Environment*, ed. by Xu J, Wu J and He Y. Zhejiang University Press and Springer Science + Business Media, Dordrecht, pp. 3–6 (2013).

27 Blazsó M, Recent trends in analytical and applied pyrolysis of polymers. *J Anal Appl Pyrolysis* **39**:1–25(1997).

28 Tsuge S and Ohtani H, Structural characterization of polymeric materials by pyrolysis-GC/MS. *Polym Degrad Stab* **58**:109–130 (1997).

29 Haken JK, Pyrolysis gas chromatography of synthetic polymers – A bibliography. *J Chromatogr A* **825**:171–187 (1998).

30 González-Pérez JA, Jiménez-Morillo NT, de La Rosa JM, Almendros G and González-Vila FJ, Pyrolysis-gas chromatography–isotope ratio mass spectrometry of polyethylene. *J Chromatogr A* **1388**:236–243 (2015).

31 Arrieta MP, López J, Ferrándiz Sand Peltzer MA, Characterization of PLA–limonene blends for food packaging applications. *Polym Test* **32**:760–768 (2013).

32 Arrieta MP, Parres F, López J and Jiménez A, Development of a novel pyrolysis-gas chromatography/mass spectrometry method for the analysis of poly(lactic acid) thermal degradation products. *J Anal Appl Pyrolysis* **101**:150–155 (2013).

33 Vuorinen E and Hakkarainen M, Method development for the analysis of biodegradable polymers. *Int J Metrol Qual Eng* **1**:29–32 (2010).

34 Aoyagi Y, Yamashita K and Doi Y, Thermal degradation of poly[(*R*)-3-hydroxybutyrate], poly[ε-caprolactone], and poly[(*S*)-lactide]. *Polym Degrad Stab* **76**:53–59 (2002).

35 Westphal C, Perrot C and Karlsson S, Py-GC/MS as a means to predict degree of degradation by giving microstructural changes modelled on LDPE and PLA. *Polym Degrad Stab* **73**:281–287 (2001).

36 Kopinke F-D and Mackenzie K, Mechanistic aspects of the thermal degradation of poly(lactic acid) and poly(β-hydroxybutyric acid). *J Anal Appl Pyrolysis* **40–41**:43–53 (1997).

37 Papageorgiou DG, Roumeli E, Chrissafis K, Lioutas C, Triantafyllidis K, Bikiaris D *et al.*, Thermal degradation kinetics and decomposition mechanism of PBSu nanocomposites with silica-nanotubes and strontium hydroxyapatite nanorods. *Phys Chem Chem Phys* **16**:4830–4842 (2014).

38 Bakkali F, Averbeck S, Averbeck D and Idaomar M, Biological effects of essential oils – A review. *Food Chem Toxicol* **46**:446–475 (2008).