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Molecular characterization of a bio-based active packaging containing *Origanum
vulgare* L. essential oil using pyrolysis gas chromatography/mass spectrometry (Py-
GC/MS).

Running title (33 characters): *Oregano oil in active biofilms*

María Llana-Ruiz-Cabello¹, Silvia Pichardo¹, Nicasio T. Jiménez-Morillo², José M.
Bermúdez³, Susana Aucejo³, Francisco J. González-Vila², Ana M. Cameán¹, José A.
González-Pérez^{2*}

¹Area of Toxicology, Faculty of Pharmacy, Universidad de Sevilla, Profesor García
González nº2, 41012-Seville, Spain.

² IRNAS-CSIC, Av. Reina Mercedes, 10, 41012-Seville, Spain.

³ Area of Packaging Materials and Systems, ITENE, C/ Albert Einstein 1, 46980-
Paterna (Valencia), Spain.

*Corresponding author:

Tel +34954624711; Fax +34 954644002; e-mail jag@irnase.csic.es

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 0.950R² 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 its 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 use of 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 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 PLA drawback is its medium gas barrier properties and the combination with other polymers,⁶ the addition of nanomaterials like cellulose nanocrystals or nanowhiskers^{3,7,8} may reduce such problem 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 food stuffs, new bioactive packaging techniques and ingredients from natural sources are gaining 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 recognized as GRAS by 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 additive is sometimes limited due to unacceptable organoleptic properties.¹⁴ However, the incorporation of EOs in food-packaging films allows the a controlled release of active substances whereas reducing undesirable flavors caused by direct EOs addition into food.^{23,24} Due to the volatility of EOs and the conditions of films 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 oxygen²⁵, is a useful tool for the direct characterization 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 synthetic²⁷⁻³⁰ and bio-based polymers including polylactic acid³¹⁻³⁶ and polybutylene succinate³⁷ plastics.

In this work direct analytical pyrolysis (Py-GC/MS) was used for a detailed characterization (“fingerprinting”) of both, an oregano EO and a bio-based 950 g kg⁻¹ polylactic acid plastic (PLA) 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, USA) and polybutylene succinate (PBS) GS PlaTM FD92WD purchased from Mitshubishi Chemical Corporation (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, 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 through 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 pyrolyzer F-Labs model 2020i (Frontier Laboratories, Fukushima, Japan) attached to a GC/MS system Agilent 6890N (Agilent Technologies Inc., Santa. Clara, California, 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%-phenyl-methylpolysiloxane) capillary column J&W HP-5ms Ultra Inert, of 30 m $\times$ 250 μ m $\times$ 0.25 μ m 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 stabilized at 300 °C for 10 min. The carrier gas used was helium at a controlled flow of 1 cm³ min⁻¹. The detector was an Agilent 5973 (Agilent Technologies Inc., Santa. Clara, California, USA) mass selective detector, and mass spectra were acquired at 70 eV ionizing 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.

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 (TIC) 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 (min. 7 to 8) [12] that corresponds to a mixture of thymol [Phenol, 5-methyl-2-(1-methylethyl)-] with the isomer carvacrol [Phenol, 2-methyl-5-(1-methylethyl)-] which, under the chromatographic conditions used, could not be resolved. Other major pyrolysis products included the alkylbenzene *p*-cymene [3], other terpenes like γ -terpinene [4], terpineol [8], α -methylcinnamaldehyde [9], thymol/carvacrol methyl ester [11] and a number of known sesquiterpenes i.e. caryophyllene [14, 17], farnesene [18], bisabolene [19] and cedrene [20].

A detailed pyrogram of the PLA:PBS (950 g kg⁻¹:50 g kg⁻¹) biodegradable film is shown in Figure 2. The main pyrolysis products were a broad peak at min. 6 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 $PM=56+(n \times 72)$. 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 [1], furan, tetrahydro-2,5-dimethyl [2], 2-propenoic acid, (1-propionato)ethyl ester [3], succinic acid anhydride [4] and 4-pentenoic acid, 2-acetyl-, ethyl ester [5], observed in the TIC

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 Figure 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 (min. c. 7.55), the alkyl benzene cymene (min 4.30) and, less apparent mainly at lower EO doses, a third peak corresponding to the terpene terpinene (min. 4.65). These three peaks can be considered as diagnostic/marker peaks to trace the added oregano EO within the bioplastic matrix. In fact, Pearson linear correlation coefficients of better than 0.950 R² value ($p < 0.001$) were found between the chromatographic area of these three main marker peaks and the amount of oregano EO (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 demonstrate the potent antimicrobial and antioxidant activities of oregano EO^{16,17,20,38} and its use is increasing as a natural component of many food stuffs and also of non-edible materials of use in the food industry i.e. plastic films used in bio-active packaging. Previous results³¹ 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.

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FIGURE CAPTIONS

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

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.

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

Figure 4. Relation between the main oregano EO diagnostic peaks and the percentage of added EO in the PLA. A) Cymene peak at min. 4.30; B) Terpinene peak at min. 4.65: C) Carvacrol/Thymol peak at min 7.55.

Fig.1

Fig.2

Fig.3

Fig.4