

Pyrolysis-gas chromatography–isotope ratio mass spectrometry for monitoring natural additives in polylactic acid active food packages.

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## HIGHLIGHTS FOR REVIEW:

- Accurate and rapid determination of isotope composition ( $\delta^{13}\text{C}$ ) in PLA and additives
- The technique detect isotopically distinct additives within a bio-polymer matrix with a minimum of sample preparation
- First report of Py-CSIA application to the direct characterization of a bio-plastic

## Abstract

Compound-specific isotope analysis (CSIA) usually requires preparative steps (pretreatments, extraction, derivatization) to get amenable chromatographic analytes from bulk geological, biological or synthetic materials. Analytical pyrolysis (Py-GC/MS) can help to overcome such sample manipulation. This communication describe the results obtained by hyphenating analytical pyrolysis (Py-GC) with carbon isotope-ratio mass spectrometry (IRMS) for the analysis of a polylactic acid (PLA) a based bio-plastic extruded with variable quantities of a natural plant extract or oregano essential oil. The chemical structural information of pyrolysates was first determined by conventional analytical pyrolysis and the measure of  $\delta^{13}\text{C}$  in specific compounds was done by coupling a pyrolysis unit to a gas chromatograph connected to a continuous flow IRMS unit (Py-GC-C-IRMS). Using this Py-CSIA device it was possible to trace natural additives with depleted  $\delta^{13}\text{C}$  values produced by C3 photosystem vegetation (cymene:  $-26.7\text{‰} \pm 2.52$ ; terpinene:  $-27.1\text{‰} \pm 0.13$  and carvacrol:

-27.5‰ ± 1.80 from oregano and two unknown structures: -23.3‰ ± 3.32 and -24.4‰ ± 1.70 and butyl valerate: -24.1‰ ± 3.55 from *Allium* spp.), within the naturally isotopically enriched bio-plastic backbone derived from corn (C4 vegetation) starch (cyclopentanones: -14.2‰ ± 2.11; lactide enantiomers: -9.2‰ ± 1.56 and larger polymeric units: -17.2‰ ± 1.71).

This is the first application of Py-CSIA to characterize a bio-plastic and is shown as a promising tool to study such materials, providing not only a fingerprinting, but also valuable information about the origin of the materials, allowing the traceability of additives and minimizing sample preparation.

Keywords

Bioplastics; Analytical pyrolysis; Compound-specific isotope analysis; Carbon isotopes, Py-CSIA

## 1. Introduction

Due to increasing environmental concern, the study of new materials within a perspective of eco-design or sustainable development is a strategy that is currently applied in the food packaging industry [1]. Biodegradable polymers can be considered an environmentally safe alternative to petroleum-based conventional packaging which takes hundreds of years to decompose [2]. In this regard, polylactic acid (PLA) is emerging as an important green polymeric alternative due to its biodegradability, biocompatibility and process ability [3]. PLA is an aliphatic polyester made primarily from renewable agricultural resources (corn) following the fermentation of starch and further condensation of lactic acid [4]. Hence, considering that PLA is classified as GRAS (Generally recognized as safe) by the American Food and Drug Administration (FDA) and is authorized by the European Commission (Commission Regulation No 10/2011), this polymer is an excellent candidate for producing a commercial compostable food packaging material [5]. Moreover, PLA has desirable features for food packaging such as good mechanical and light barrier properties and is easily processed by injection, molding, blow molding, thermoforming or extrusion [6].

Active food contact materials were defined in Regulation No 1935/2004 of the Europe Parliament [7] and of the Council as “materials that are intended to extend

the shelf-life or to maintain or improve the condition of packaged food". They are designed to deliberately incorporate components that would release or absorb substances into or from the packaged food or the environment surrounding the food". In order to produce these materials, PLA incorporated with several substances such as the bacteriocin nisin, vitamin E ( $\alpha$ -tocopherol) or as copolymer with polyethylene glycol have been developed [8]. Similarly, natural extracts and essential oils (EOs) can be incorporated into PLA to develop active food packaging to extend shelf-life of perishable product due to the antimicrobial or antioxidant properties of these substances. In this sense, recently a commercial product based on *Allium* extract (Proallium-SO-DMC®) or oregano essential oil were incorporated by extrusion into PLA to develop active food materials for improving shelf life of ready-to-eat salads [5, 9]. However, due to its high volatility several authors reported that losses of essential oils were to be expected during the fabrication or storage of the active film or preformed packages [10, 11].

In order to assess that after manufacture the active packaging still contains effective concentration of the natural extracts, previous thermogravimetric analysis has been applied [9]. However, it is desirable to explore other more accurate and informative techniques to confirm the above fact.

Isotope ratio mass spectrometry (IRMS) is extensively used to trace the origin of biogenic materials and to enlighten relevant scientific and technical questions in food science and the industry, including aspects related to traceability and fraud detection [12–15]. The stable carbon isotopic composition ( $\delta^{13}\text{C}$ ) of plants depends on carbon fixation process such as the C3 or C4 cycle. Most plants, including *Origanum* sp. and *Allium* sp., utilize the C3 photosynthetic pathway to assimilate  $\text{CO}_2$ . The  $\delta^{13}\text{C}$  value of these C3 plants generally ranges from  $-24$  to  $-30$  ‰. However, corn is a tropical herb and a representative plant with C4 type photosystem known to be  $^{13}\text{C}$  enriched with  $\delta^{13}\text{C}$  values between  $-6$  and  $-19$ ‰ [16]. Thus, these differences in carbon isotopic composition between corn and essential oils can be used to detect and trace additives into bio-based polymeric matrices, such as PLA manufactured from C4 plants products.

While no or little sample preparation is required for bulk isotopic analyses, for the compound-specific isotope analysis (CSIA) variant, intermediate multi-step

preparative procedures are required in most cases prior to chromatographic analysis i.e., compounds must generally be first isolated from bulk sample materials, such as polymers, soils, sediments, or biological tissues. Non-volatile organic compounds usually require derivatization i.e. silylation, alkylation, acylation, esterification or other methods in order to enhance its volatility and improve chromatographic separation [17–19]. All these pretreatments may lead to artifacts formation, un-accuracies or misleading results. In particular, carbon isotopic composition can be changed due to additional atoms from the derivatization agents or by small fractionations that may occur during the derivatization process [20].

Conventional analytical pyrolysis (Py-GC/MS) is a well-established technique that can help overcome preparative manipulation of samples; requires small sample size with little or no preparation, thus being convenient for inexpensive and relatively rapid routine analyses. The technique has been proved to be particularly useful for the characterisation of different natural and synthetic polymers and additives [21–25] and also for bio-based polymers, including PLA [26–31] and polybutylene succinate (PBS) [31,32] plastics.

Recently we have effectively hyphenated pyrolysis (Py-GC) with light stable isotopes (C, H, N) IRMS (Py-GC–C/HT–IRMS). Early work demonstrated that pyrolysis process does not produce appreciable fractionation of stable isotopes and therefore the pyrolysis products can be considered isotopically representative of the starting material [33–35]. This technique allows on-line quantification of stable isotope proportions in chromatographically separated products released by pyrolysis and has been successfully applied to the study of widely different natural and industrial samples e.g., dyed polyethylene, sucrose from different origins [15, 25] or speleothems [36].

This work reports the use of conventional IRMS and Py-CSIA for a detailed study of the carbon stable isotope composition a polylactic acid:polybutylene succinate (PLA:PBS) based film extruded with variable quantities of natural plant extracts or essential oils for use in active food packaging.

## 2. Material and methods

### 2.1. Bio-polymer and additives

The plastic films were made of polylactic acid (PLA) with polybutylene succinate (PBS) (950 g kg<sup>-1</sup>:50 g kg<sup>-1</sup>) extruded with variable quantities of oregano essential oil (EO) or of the commercial additive (Proallium®) prepared from *Allium* spp. extracts.

The PLA extrusion-grade (2003D) was purchased in pellets from NatureWorks LLC (Minnetonka, MN, USA) and the PBS, GS Pla™ FD92WD from Mitsubishi Chemical Corporation (Tokyo, Japan).

Oregano essential oil (EO) was obtained from El Jarpil® (Almería, Spain). Commercial Proallium® (L14/7), extract obtained from *Allium* spp. was supplied by the manufacturer DOMCA S.A. (Alhendín, Granada, Spain). Chemicals for the different assays were purchased from Sigma-Aldrich (Spain) and VWR International Eurolab (Spain).

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<sup>-1</sup> which correspond to 2, 5 and 10% w/w, respectively) of oregano EO and (20, 50 and 65 g kg<sup>-1</sup> which correspond to 2, 5 and 6.5% w/w, respectively) of Proallium® 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<sup>-1</sup>. A control film was extruded in the same manner but with no oregano EO or Proallium® added. The average thickness of the final films was 80 µm (315 Gauge).

### 2.2. Bulk C stable isotopic analysis (IRMS)

Bulk isotopic composition of carbon ( $\delta^{13}\text{C}$ ) was analysed using a Flash 2000 HT (C, N, S) combustion (C) and (H, O) pyrolysis (TC) elemental micro-analyser coupled via a ConFlo IV interface unit to a continuous flow Delta V Advantage isotope ratio mass spectrometer (IRMS) (Thermo Scientific, Bremen, Germany) (C/TC-IRMS). Isotopic ratios are reported as parts per thousand (‰) deviations from appropriate standards recognized by the International Atomic Energy Agency (IAEA) [37]. The standard deviation of bulk  $\delta^{13}\text{C}$  was typically less than  $\pm 0.05\text{‰}$ .

The proportion of additive in the bioplastic was calculated using a mass balance equation as described in [38].

Proportion of additive in bio-plastic =  $100 \times (A-B)/(C-B)$

A:  $\delta^{13}\text{C}$  bioplastic with additive

B:  $\delta^{13}\text{C}$  bioplastic

C:  $\delta^{13}\text{C}$  additive

### 2.3. Conventional analytical pyrolysis (Py-GC/MS)

In order to obtain molecular information and unambiguously characterize the main pyrolysis products, direct pyrolysis-gas chromatography–mass spectrometry (Py-GC/MS) was performed using a double-shot pyrolyzer (Frontier Laboratories, model 2020i) attached to a GC/MS system Agilent 6890N. Samples (0.5 mg) were placed in small crucible capsules and introduced into a preheated micro-furnace at (500 °C) for 1 min. The volatile pyrolysates were then directly injected into the GC/MS for analysis. The gas chromatograph was equipped with a low polar-fused silica (5%-phenyl-methylpolysiloxane) capillary column (Agilent 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<sup>-1</sup>, from 100 °C to 300 °C at 10 °C min<sup>-1</sup>, and stabilized at 300 °C for 10 min with a total analysis time of 32 min. The carrier gas was helium at a controlled flow of 1 mL min<sup>-1</sup>. The detector consisted of an Agilent 5973 mass selective detector and mass spectra were acquired at 70 eV ionizing energy. Compound assignment was achieved by single-ion monitoring (SIM) for the major homologous series and by comparison with published data reported in the literature or stored in digital libraries (NIST and Wiley libraries).

### 2.4. Pyrolysis compound specific carbon isotope analysis (Py-CSIA)

Direct pyrolysis compounds specific isotope analysis (Py-CSIA) of carbon ( $\delta^{13}\text{C}$ ) was done by coupling a double-shot pyrolyzer (Frontier Laboratories, model 3030D) to a gas chromatograph (Thermo Fisher Scientific, model Trace GC Ultra) system. At the end of the chromatographic column the flux is directed to a GC-Isolink II System equipped with a micro-reactor for combustion set at 1000 °C (EA) and coupled via a ConFlo IV universal interface unit to a continuous flow Delta V Advantage isotope ratio mass spectrometer (IRMS) (Thermo Scientific, Bremen, Germany) (Py-GC-C-IRMS).

Samples of 1–2 mg in weight were placed in small stainless steel crucible capsules and introduced into a preheated micro-furnace at 500 °C for 1 min.

The gases were then directly injected into the GC/IRMS system for analysis. The gas chromatograph was equipped with the same column type and same chromatographic conditions to those described in section 2.3.

Stable isotope composition is given as parts per thousand (‰) deviations from appropriate standards recognized by the IAEA [37]. The standard deviation of compound specific  $\delta^{13}\text{C}$  was typically less than  $\pm 0.1\text{‰}$ . To ensure accurate isotope composition readings,  $\delta^{13}\text{C}$  values are only given for peaks for which an appropriate chromatographic resolution ( $R_s > 1.3$ ) was achieved.

Structural features of specific peaks were inferred by comparing and matching the mass spectra obtained by conventional Py-GC/MS with the Py-GC-C-IRMS chromatograms obtained using the same column type and identical chromatographic conditions.

To ensure that no isotope fractionation occurred under our experimental pyrolysis and chromatographic conditions, Py-CSIA analyses were also performed in the same way but including 0.3 mg of sugarbeet sucrose as internal standard (IS). The pyrolytic behaviour of the chosen IS is well characterized producing major peaks of furfural (F) and hydroxymethyl furfural (HMF) of known  $\delta^{13}\text{C}$  ( $F = -24.7\text{‰} \pm 0.8$  and  $\delta^{13}\text{C HMF} = -22.1\text{‰} \pm 0.4$ ) [15]. These peaks elute separated from the main PLA and additive analytes, minimizing possible interferences. Note that the less abundant lactide enantiomer (L3) was not detected in the Py-CSIA+IS chromatogram, this may be due to small conformational changes that may occur during the co-pyrolysis with the sucrose.

### **3. Results and discussion**

#### **3.1. PLA analytical pyrolysis (Py-GC/MS)**



Detailed structural study of the polymer (PLA:PBS) alone or with variable quantities of the additives within the film matrix using conventional analytical pyrolysis (Py-GC/MS) has been recently described in detail [31, 39]. In summary, the 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 carvacrol (mixture of isomers carvacrol/thymol) peaks as major diagnostic peaks. Proallium® commercial additive main pyrolysis products, were oligomers of polyethyleneglycol, alkyl ethers and oleic acid, 3-hydroxypropyl ester compatible with a polysorbate surfactant and the sulphur compound, propyl sulphide that was a diagnostic peak for tracing the additive in the polymer.

Analytical pyrolysis was sensitive in detecting the increasing amounts of additive in the plastic. When comparing the chromatographic area of the main diagnostic peaks -both for OE and Proallium®- with the amount of additive added to the bioplastic, good correlations with coefficient values better than 0.950  $R^2$  ( $P < 0.001$ ) were always found.

Examples of the chromatograms obtained by direct pyrolysis of the biopolymer (PLA:PBS) and of films containing additives are shown in Fig.1. Labels on peaks corresponds to compounds listed in Table 1.

### 3.2 PLA bulk isotopic composition

The carbon isotope composition of the biopolymer was found clearly  $^{13}\text{C}$ -enriched ( $\delta^{13}\text{C} = -10.7 \pm 0.63\text{‰}$ ) indicative of a main C4 vegetation origin, probably from corn starch. On the other hand, both additives showed depleted  $\delta^{13}\text{C}$  values (Proallium®  $-28.9 \pm 0.07\text{‰}$ ; oregano OE  $-28.2 \pm 0.05\text{‰}$ ) reflecting biogenic origin from C3 photosystem vegetation.

Consistent  $\delta^{13}\text{C}$  shifts were observed in the PLA:PBS film extruded with variable quantities of additives, becoming more negative ( $^{13}\text{C}$ -depleted) with increasing content. This carbon isotopic composition parallels the contribution from the additive light carbon within the heavier carbon PLA:PBS matrix and fits well, with correlations coefficient values better than 0.980  $R^2$ , to a linear model in the case of oregano EO and to a quadratic model for Proallium® (Fig. 2A).

From the carbon isotope composition of the mixtures it was possible to calculate the real amount of the additive included in the bioplastic. Using a simple mass balance equation, we were able to assess the amount of additive that was ultimately and effectively incorporated in the final casting of the active-biopolymer. The estimation of the content of the two additives in the bioplastic was highly correlated with the alleged concentration (Fig. 2B). However, the IRMS measurements overestimate the declared quantity of additive in the biopolymer in c. 20%.

### 3.3. PLA compound specific carbon isotope analysis (Py-CSIA)

The major chromatographic diagnostic peaks elute at the beginning of the chromatograms (from min 2 to 8) and appropriate chromatographic resolution could be achieved under our experimental conditions. An example of the different chromatograms used for this study; Py-GC/MS for structural elucidation and its correspondence with the the Py-CSIA chromatogram to associate C isotope composition to the diagnostic peaks is shown in Fig. 3. Also included is a Py-CSIA control run with added sugarbeet sucrose and  $\delta^{13}\text{C}$  readings on IS and diagnostic peaks. The inclusion of the Is warrant the consistency of given Py-CSIA  $\delta^{13}\text{C}$  values and demonstrate that no appreciable isotope fractionation occurs under the conditions of our experiment.

Due to the differential C isotope composition between the biopolymer ( $^{13}\text{C}$ -enriched) and the additives ( $^{13}\text{C}$ -depleted), it was possible to detect specific compounds of the additive within the polymer matrix using direct Py-CSIA, as well as to estimate their  $\delta^{13}\text{C}$  values.

In Fig. 4, scatter plots of chromatographic retention time vs C isotope composition of selected peaks are shown for pure PLA:PBS film and additives (Proallium<sup>®</sup> and oregano EO) as well as for active food packaging film including variable quantities of the additives. The stable C isotope composition of specific bio-plastic compounds released by direct pyrolysis was consistent with the film bulk values and indicative of a C4 vegetation (corn) origin. Tentative  $\delta^{13}\text{C}$  values ranged from  $-7.7$  and  $-19.9\text{‰}$ , with an average stable C isotope composition for lactide enantiomers significantly heavier (L1-L3:  $\delta^{13}\text{C} = -9.2\text{‰} \pm$

1.56) than for cyclopentanones (C1-C2:  $\delta^{13}\text{C} = -14.2\text{‰} \pm 2.11$ ) and these heavier than for larger polymeric units ( $\delta^{13}\text{C} = -17.2\text{‰} \pm 1.71$ ).

This simple data representation of chromatographic retention time vs  $\delta^{13}\text{C}$  allows us —at a first sight— to detect the compounds from the additive present in the film as  $^{13}\text{C}$ -depleted outliers within the heavier compounds from the bio-plastic (Figs. 3 and 4). Specifically, for oregano EO, even at the lower concentration used (2%) it was possible to identify up to three specific marker compounds (A-C): the alkylbenzene cymene (A:  $\delta^{13}\text{C} = -26.7\text{‰} \pm 2.52$ ) and the monoterpenes terpinene (B:  $\delta^{13}\text{C} = -27.1\text{‰} \pm 0.13$ ) and carvacrol (C:  $\delta^{13}\text{C} = -27.5\text{‰} \pm 1.80$ ). For Proallium<sup>®</sup> specific marker compounds were detected only when the additive concentration was 5% or higher: two unknown structures (P1:  $\delta^{13}\text{C} = -23.3\text{‰} \pm 3.32$ ; P3:  $\delta^{13}\text{C} = -24.4\text{‰} \pm 1.70$ ) and butyl valerate (P2:  $\delta^{13}\text{C} = -24.1\text{‰} \pm 3.55$ ) (Table 1 and Fig. 1).

## 4. Conclusions

Using pyrolysis-gas chromatography—<sup>13</sup>C isotope ratio mass spectrometry (Py-CSIA) we were able to obtain, in addition to a comprehensive molecular fingerprinting, accurate isotopic composition ( $\delta^{13}\text{C}$ ) for specific polylactic acid based bio-plastic pyrolytic fragments (cyclopentanones:  $-14.2\text{‰} \pm 2.11$ ; lactide enantiomers:  $-9.2\text{‰} \pm 1.56$  and polymeric units:  $-17.2\text{‰} \pm 1.71$ ), as well as for specific markers of the natural products added oregano EO (cymene:  $-26.7\text{‰} \pm 2.52$ ; terpinene:  $-27.1\text{‰} \pm 0.13$  and carvacrol:  $-27.5\text{‰} \pm 1.80$ ) and Proallium<sup>®</sup> (two unknown structures:  $-23.3\text{‰} \pm 3.32$  and  $-24.4\text{‰} \pm 1.70$  and butyl valerate:  $-24.1\text{‰} \pm 3.55$ ). These values were consistent with measured bulk  $\delta^{13}\text{C}$  values. A simple data representation of chromatographic retention time vs  $\delta^{13}\text{C}$  of peaks allows detecting compounds from isotopically distinct additives contained within the bio-polymer. Py-CSIA is found a valuable technique to detect and trace natural additives in plastic films produced from corn starch with a minimum need for sample preparation. Nonetheless, we believe that there is plenty of room to improve the accuracy of this hyphenated Py-CSIA technique, mainly through the optimization of the chromatographic separation minimizing compound coelution that may affect the isotopic composition i.e. appropriate stationary phases, longer and thinner columns, or even using less conventional multidimensional GC systems.

## Acknowledgements

M. Llana-Ruiz-Cabello wish to acknowledge 'Junta de Andalucía' grant associated to AGR-7252 Junta de Andalucía group and N.T Jiménez-Morillo is funded by a FPI research grant (BES-2013-062573). Projects CSIC10-1E-448 and INTERCARBON (CGL2016-78937-R) co-financed by FEDER Funds. Alba Carmona Navarro and Desire Monis Carrere are recognized for their technical assistance. Four anonymous referees are acknowledged for their helpful comments on the manuscript.

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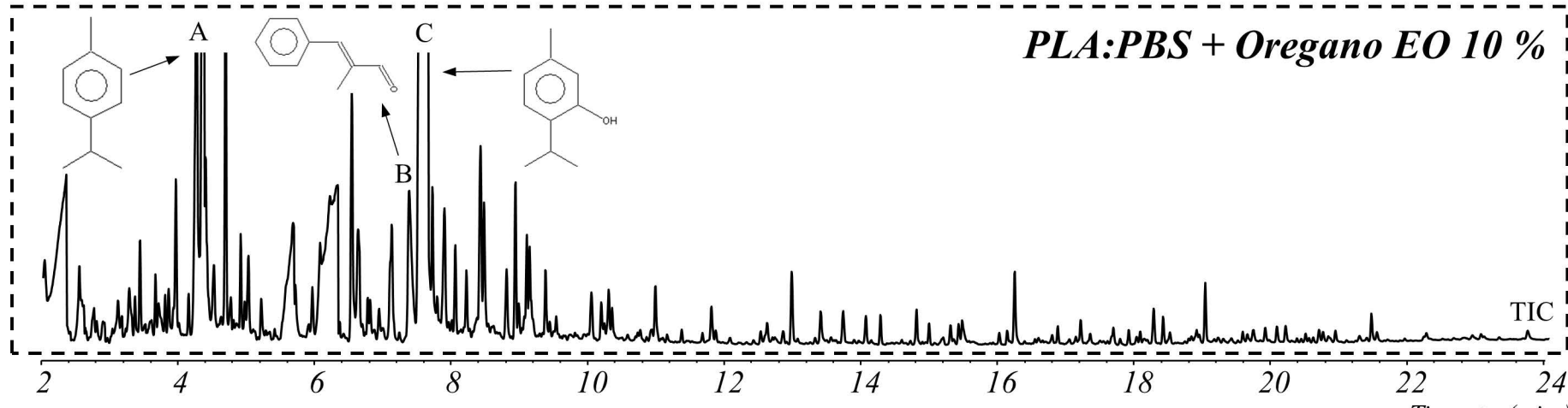
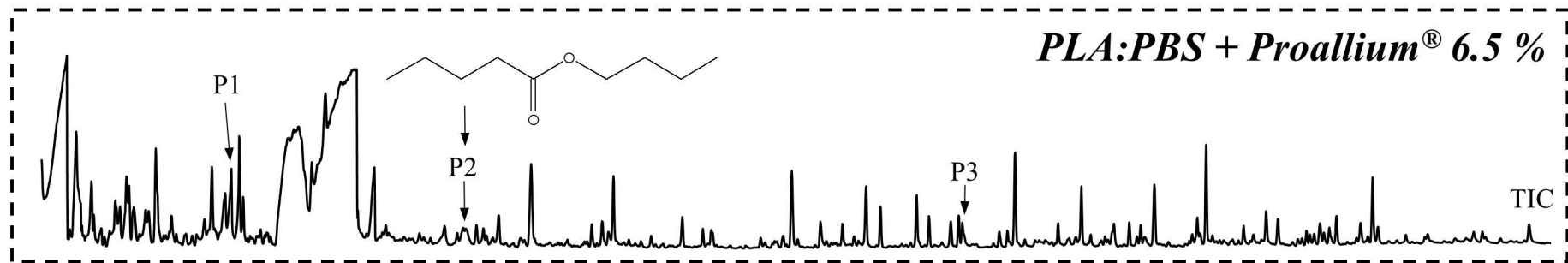
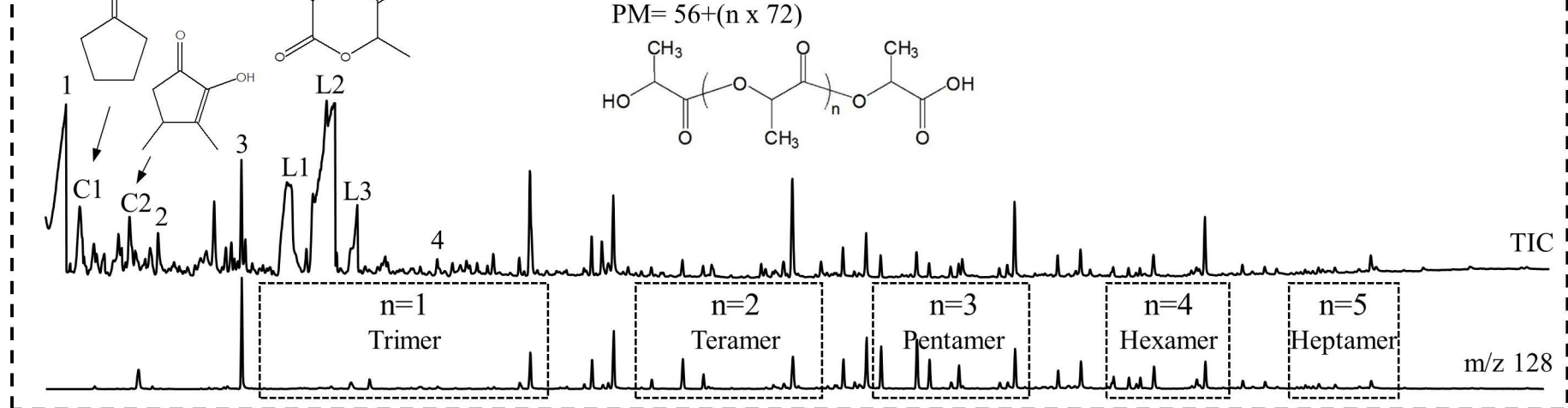
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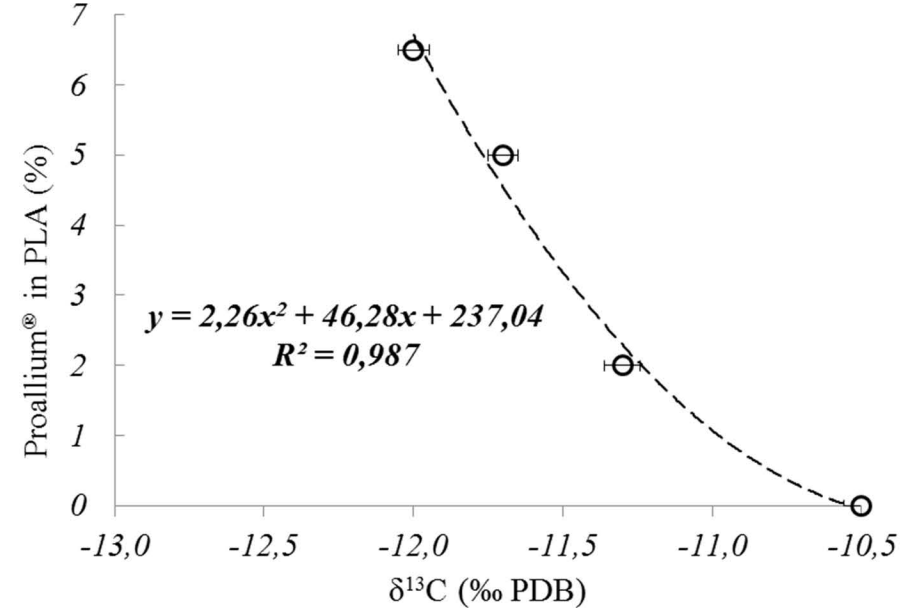
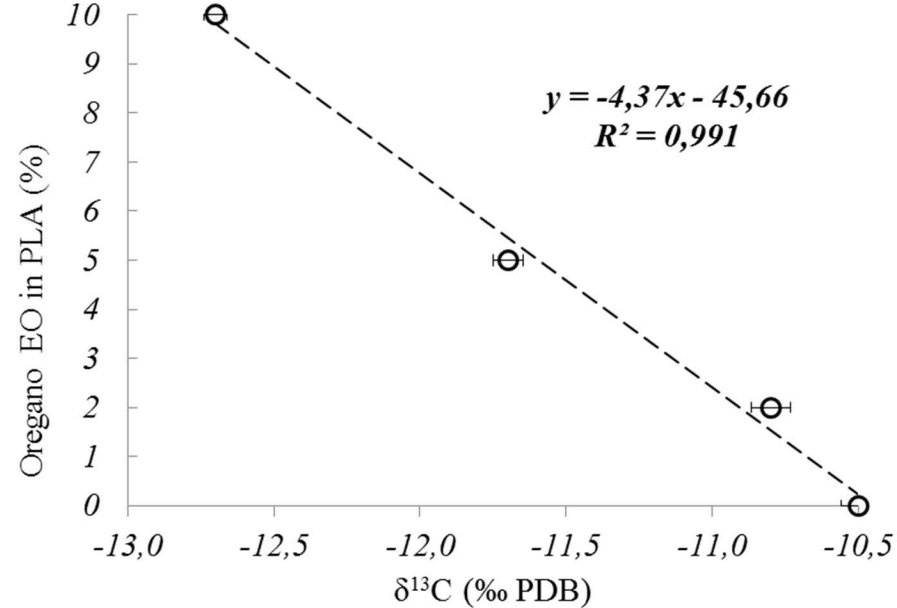
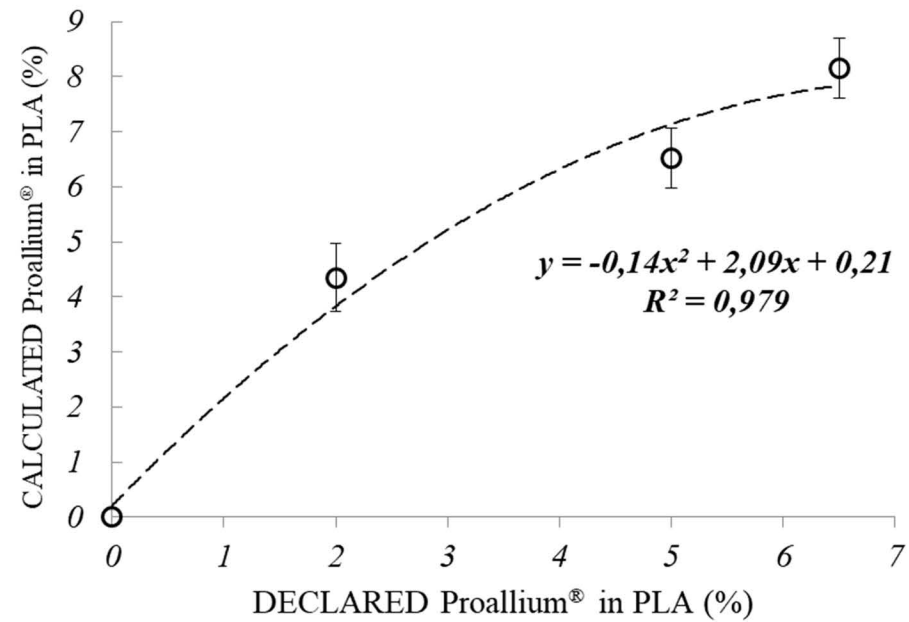
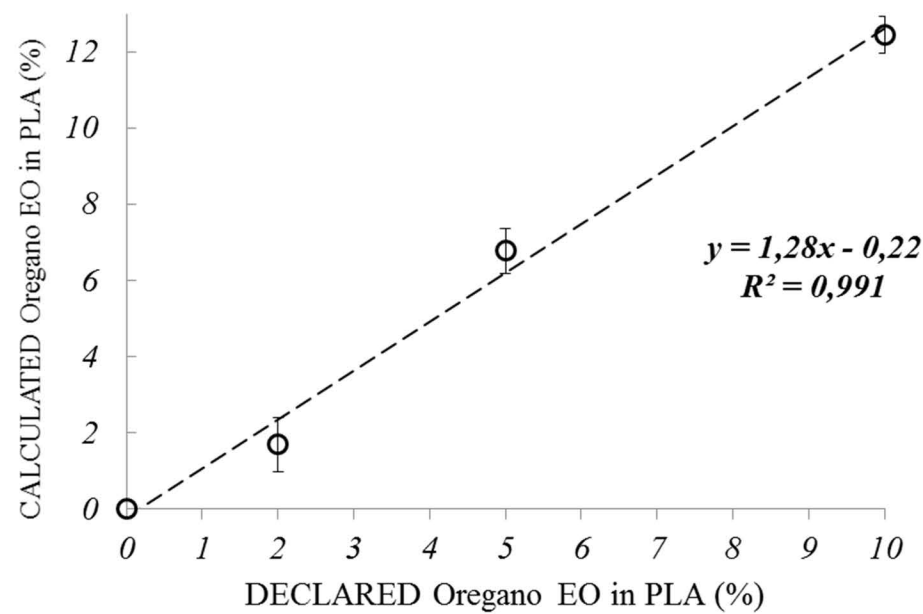
Fig. 1. Examples of the chromatograms obtained by direct pyrolysis (Py-GC/MS) of the biopolymer (PLA:PBS) and of films containing additives. Labels on peaks corresponds to compounds listed in Table 1.

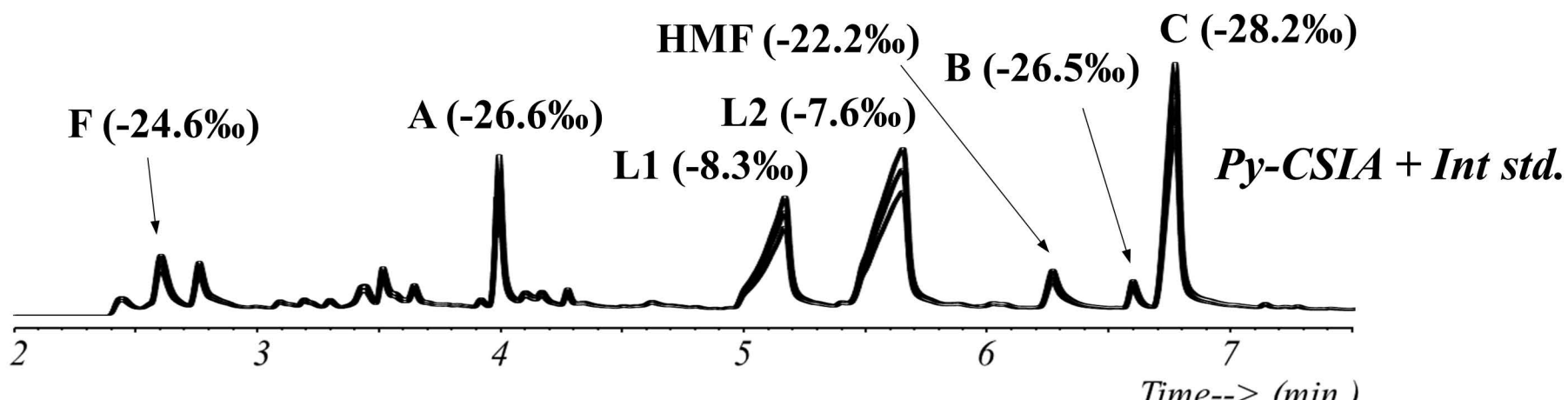
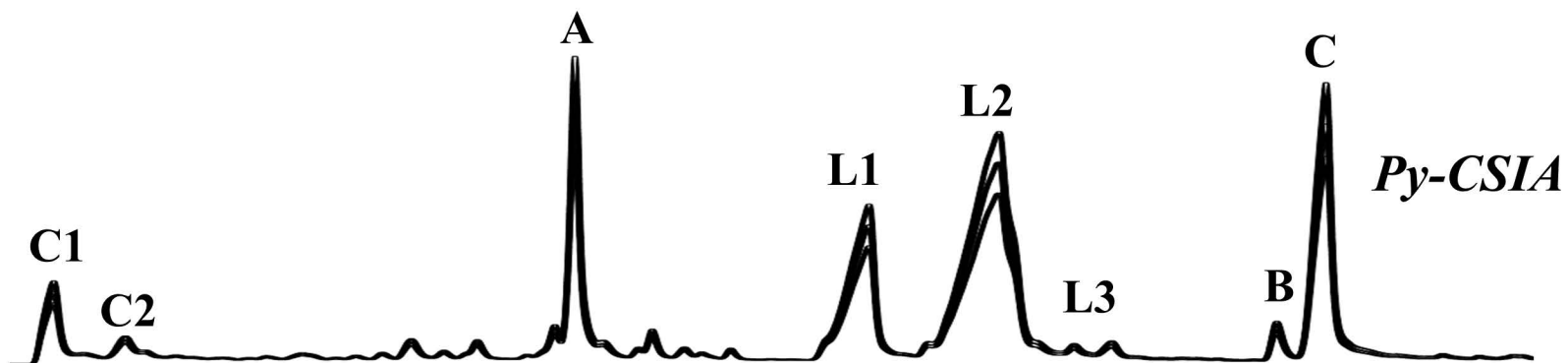
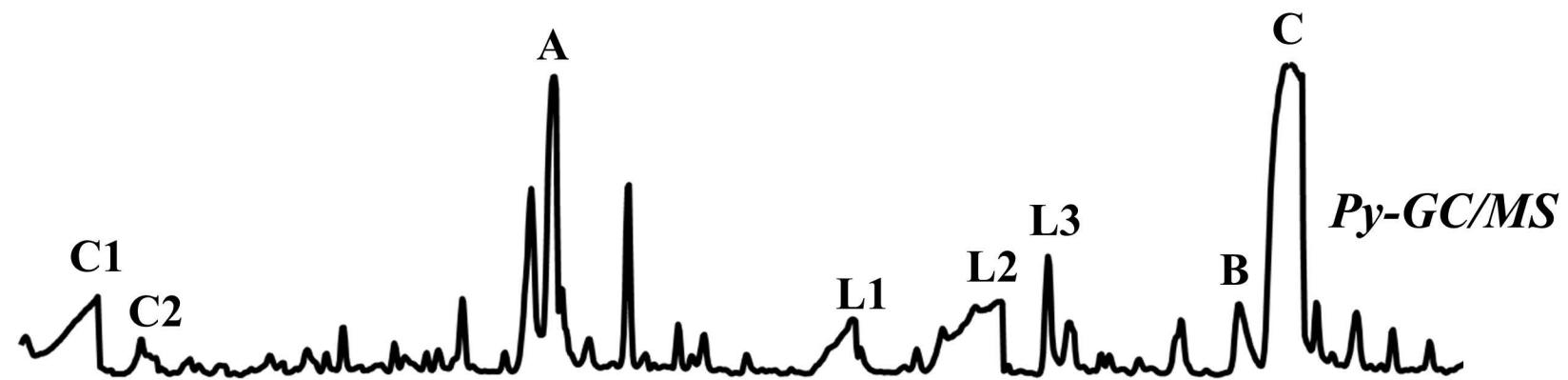
Fig. 2. Estimation of the additive in the PLA based in shifts in carbon isotopic composition ( $\delta^{13}\text{C}$ ). (A) Relation between  $\delta^{13}\text{C}$  and additive added; (B) Relation between declared additive in bioplastic and the calculated values based in a mass balance relation. Error bars indicate the mean STD ( $n = 3$ ).

Fig. 3. Partial chromatograms (min 2-8) of sample PLA:PBS + Oregano EO 10%. Py-GC/MS, Py-CSIA chromatograms and a Py-CSIA control chromatogram with internal standard (sugarbeet sucrose). Labels on peaks corresponds to diagnostic compounds listed in Table 1. Internal standard pyrolysis peaks F: furfural; HMF: hydroxymethyl furfural. Numbers in brackets are  $\delta^{13}\text{C}$  (‰ VPDB) readings.

Fig. 4. Py-CSIA analysis; scatter plots of chromatographic retention time vs carbon isotopic composition ( $\delta^{13}\text{C}$ ) of selected peaks for pure PLA:PBS biopolymer film and pure additives (Proallium® and oregano EO) as well as for biopolymer film including variable quantities of the additives. Labels on dots corresponds to compounds listed in Table 1.



**A)****B)**



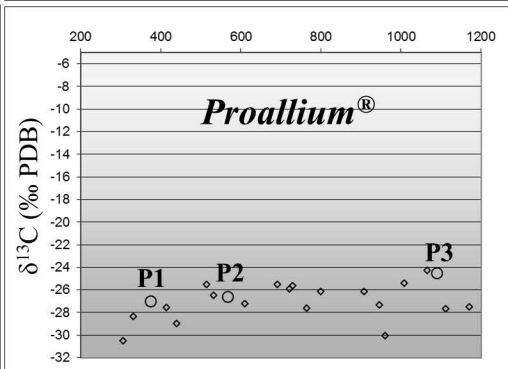
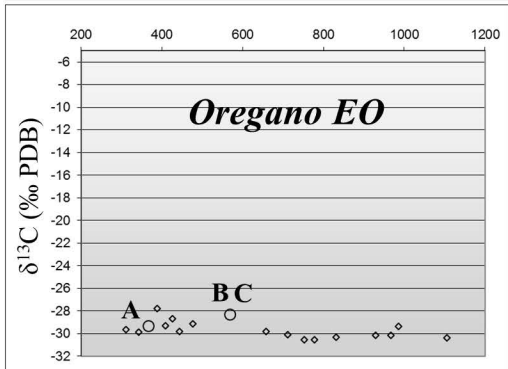
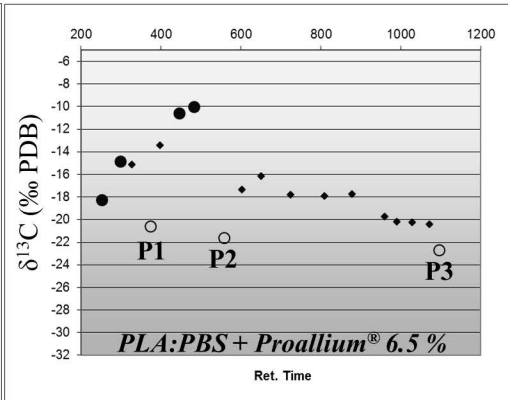
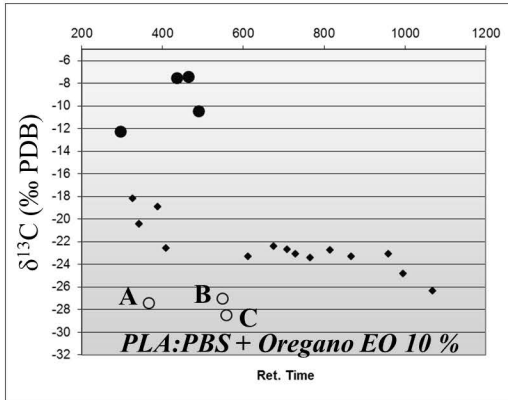
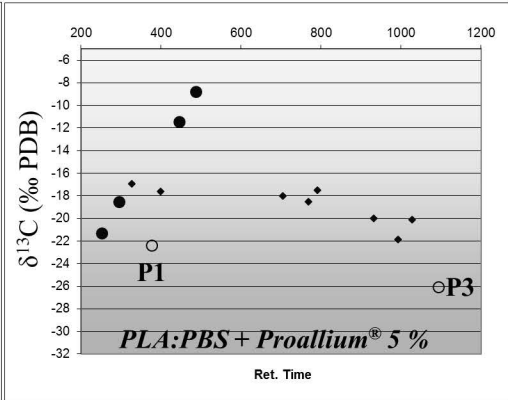
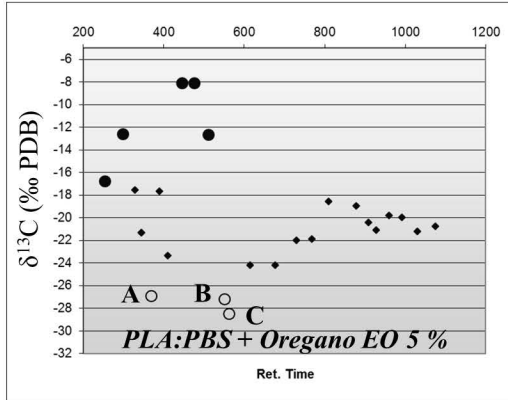
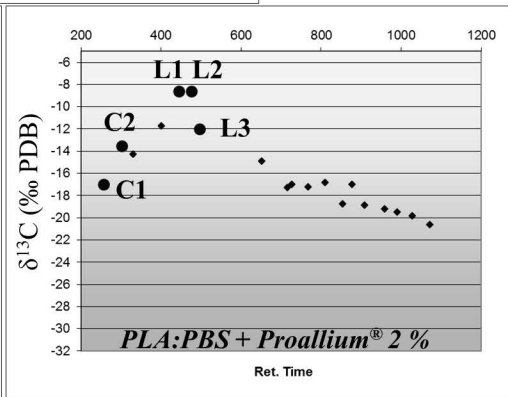
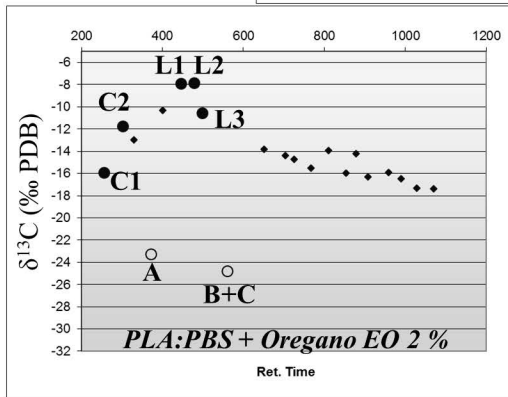
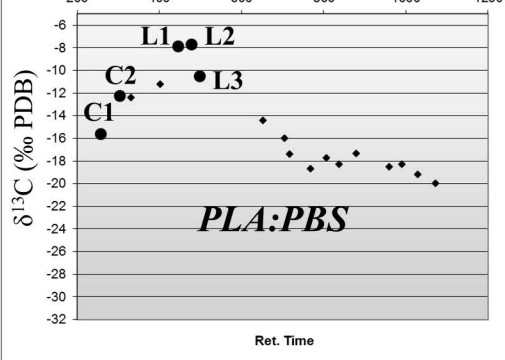


Table 1. Main compounds identified by Py-GC/MS in the biopolymer (PLA:PBS), in the additives and in the films containing additives (main marker compounds). PLA: Polylactic acid; PBS: Polybutylen succinate

PLA main marker compounds	
C1	Cyclopentanone
C2	2-Cyclopenten-1-one, 2-hydroxy-3,4-dimethyl-
L1-L3	Lactide enantiomeric forms
Compounds that probably derive from PBS	
1	Tetrahydrofuran + 1,2-Butadiene
2	Furan, tetrahydro-2,5-dimethyl-
3	2-Propenoic acid, (1-propionato)ethyl ester
4	4-Pentenoic acid, 2-acetyl-, ethyl ester
Proallium <sup>®</sup> main marker compounds	
P1	Unknown (min 4.94 m/z 57, 101, 130)
P2	Pentanoic acid, butyl ester (min 8.20 m/z 57, 85,103, 158) [Butyl valerate]
P3	Unknown (min 15.42 m/z 129, 157, 187)
Oregano EO main marker compounds	
A	Benzene, 1-methyl-4-(1-methylethyl)- [Cymene]
B	1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)- [Terpinene]
C	Phenol, 5-methyl-2-(1-methylethyl)- [Carvacrol]