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

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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 $\delta^{13}C$ 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 $\delta^{13}C$ values produced by C3 photosystem vegetation (cymene: −26.7‰ ± 2.52; terpinene: −27.1‰ ± 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.

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 toxtend 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

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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 (α-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 ($\delta^{13}C$) 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 CO_2. The $\delta^{13}C$ 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}C enriched with $\delta^{13}C$ 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 usefull 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^{-1}:50 g kg^{-1}) 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 Mitshubishi Chemical Corporation (Tokyo, Japan).

Oregano essential oil (EO) was obtained from El Jarpil® (Almería, Spain). Com-mercial 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^{-1} which correspond to 2, 5 and 10% w/w, respectively) of oregano EO and (20, 50 and 65 g kg^{-1} which correspond to 2, 5 and 6.5% w/w, respectively) of Proallium® 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 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 ($\delta^{13}C$) 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 $\delta^{13}C$ was typically less than ± 0.05‰.

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 × (A–B)/(C–B)

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

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

C: $\delta^{13}C$ 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

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