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Decomposition of bio-degradable plastic polymer in a real on-farm composting process

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Abstract

Background: The current wide diffusion of bio-degradable plastic made up by starch-based polymeric composite has focused the attention on the allocation of bio-polymers for the direct recycling in composting processes. Actually, the acknowledged current methods to estimate the bio-degradability are mainly based on laboratory tests and measurements under controlled conditions, while scarce information are available on the effective transformation of bio-film derivatives in real composting facilities. The aim of this paper was to determine at molecular level the decomposition of specific starch-based thermoplastic mulching film for horticultural crops, in a real *on-farm* composting system for the attainment of mature compost for agricultural application.

Results: The initial and final molecular composition of both bulk biomasses and bio-plastic composite were evaluated through ^{13}C solid-state CPMAS-NMR spectroscopy and off-line thermochemolysis—gas chromatography–mass spectrometry. The effective decomposition of the bio-polymer was shown by mono-dimensional and pseudo-2D NMR experiments that revealed the alteration of the intermolecular linkages among the monomeric constituents, while the thermochemolysis confirmed the complete decomposition of starch components. Concomitantly, the molecular characterization of bulk compost indicated the typical selective preservation of hydrophobic components currently found in aerobic composting processes, with a significant increase (+50 %) for the yields of aromatic lignin derivatives and recalcitrant aliphatic compounds.

Conclusion: In addition to the classical testing methodologies, the detailed analytical investigation represents a powerful methodology to elucidate the molecular composition and modification of plastic bio-polymers thereby providing a valuable contribution to further promote the composting process as viable way to recycle the biodegradable polymeric materials.

Keywords: Bio-degradable polymer, On-farm composting, Molecular characterization, CPMAS–NMR spectroscopy, Off-line thermochemolysis GC–MS

Background

In the last decade, much of the plastic products for domestic, industrial, and agricultural applications have been progressively replaced by more environmentally compatible bio-decomposable polymers [1, 2]. However, these materials should be differentiated in either degradable or bio-degradable "plastic." In the former group, a fragmentation occurs by the action of heat, moisture, sunlight, and/or enzymes. The intermolecular bonds are weakened and the polymer chains shortened, although these residues may still reveal a biochemical recalcitrance and long-term persistence. Such an incomplete degradation process may last 12–24 months and does not yet make these materials fully compatible with a sustainability concept [3]. Conversely, when bio-plastic or their fragments present molecular properties which make them suitable for microbial degradation and become their carbon and energy sources, the polymer is fully considered bio-degradable and environmentally sustainable.

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A technological improvement was represented by the appearance of a bio-degradable plastic made up by starch-based polymeric composite, since starch is a totally decomposable, cheap, and abundant biopolymer available from different annual crops. Actually, the acknowledged current methods to estimate the biodegradability are mainly based on laboratory tests and measurements under controlled conditions, through respirometric tests (ASTM D5338-92, ISO/CD14855; ASTM D5209) structural disintegration during fermentation stage and evaluation of the toxicity effects of degradation products [4–6]. Currently, the wide diffusion of bio-plastics as bag container for the recyclable organic fraction of municipal solid waste, and the more recent application as bio-films for mulching treatment of horticultural crops, has focused the attention on the possible allocation of bio-degradable polymers for the direct recycling in composting processes [7].

In addition to the classical testing methodologies, a valuable contribution may hence be provided by the evaluation of the effective decomposition of bio-polymers on real scale composting plant under outdoor conditions, based on the analytical characterization of both recycled organic biomasses and bio-plastic molecular components.

The combined application of ^{13}C cross polarization magic angle spinning nuclear magnetic resonance spectroscopy (^{13}C-CPMAS-NMR), and off-line pyrolysis followed by gas chromatography–mass spectrometry (THM-GC–MS), represent updated and powerful tools for the molecular investigation of complex organic matrices. The nondestructive NMR techniques provide the distribution of organic carbons in a wide range of different materials and are properly applied to characterize composition and transformation of natural and synthetic macromolecules [8–10]. Besides the basic distribution of C functionalities, application of solid-state NMR allows the implementation of specific experiments dedicated to evaluate the steric arrangement and conformational behavior of crystalline and amorphous polymers [11, 12]. In particular, the analytical appraisal of specific parameters related to cross polarization dynamics, such as cross polarization time (*tCH*) and the spin–lattice proton relaxation in the rotating frame ($t1\rho H$), are suitable to evaluate structural properties and modification in complex substrates.

Pyrolysis in the presence of tetramethyl ammonium hydroxide (thermochemolysis) is commonly used to study the detailed molecular composition of either natural and synthetic biopolymers [13]. It involves the cleavage of covalent bonds combined with the solvolysis and methylation of ester and ether groups, in complex mixture of organic macromolecules and bio-polymers, thereby enhancing the thermal stability of acidic, alcoholic, and phenolic groups and allowing a suitable chromatographic detection of pyrolytic products [14].

The aim of this paper was to determine at molecular level the decomposition of specific starch-based thermoplastic mulching film for horticultural crops, combined with the simultaneous characterization of the organic biomasses for the attainment of mature compost for agricultural application, in a real *on-farm* composting system. The molecular characterization of original and final components of bulk compost and bio-polymer was analyzed by ^{13}C CPMAS NMR spectroscopy and off-line thermochemolysis gas-chromatography–mass-spectrometry.

Methods

Composting system and experimental set-up

The decomposing test was performed on starch-based thermoplastic bio-film for mulching application, developed in the Operative National Project PON Enerbiochem. The experiment was carried out in the *on-farm* composting facility built within the same project, at the Long Term Experimental field site of the University of Napoli, located at Castel-Volturno (CE). The composting method is based on a static pile receiving an upward air insufflation provided by a rotative pump and distributed through a frame of perforated rubber tubes of 10 m length. The tubes were placed on a adsorbing bed of dry corn residues of 4 × 6 m. The composting pile were made up by a mixture (base matrix) of buffalo manure and cow dung (70 % w/w) with maize straw and poplar trimming as structuring woody part (30 % w/w). The mixed material was uniformly spread by a power shovel and manure spreader to form a ground layer that covers the air distribution system. The thermoplastic blend included in the experimental bags had 20 μ of thickness and was cut into strips of 1.5 × 30 cm (Additional file 1: Figure S1b). Three experimental tests were performed by placing 9 litter bags, containing compost matrix and bio-film strips, inside the composting pile (Additional file 1: Figure S1a):

Test A) bags 1, 2, 3—bulk compost (base matrix) without bio-polymer.

Test B) bags 4, 5, 6—bulk compost added with 1 % (w/w) of bio-polymer.

Test C) bags 7, 8, 9—bulk compost added with 2 % (w/w) of bio-polymer.

All the bags had a starting fresh weight of 5 kg (moisture 36 %). The experimental bags were made by a polyester net with a mesh size of 1 mm, currently used in forest litter studies [15]. For every test the bags were evenly distributed with respect to the insufflations tube, and finally covered with the composting mixture for a final pile height of approximately 1.5 m (Additional file 1: Figure S1c).

The composting process lasted 108 days, with a periodic automatic monitoring of external and internal temperature level (5 min interval) and oxygen percentage (60 min interval). During the first 50 days the minimum percentage of oxygen was set at 10 %, and at 5 % thereafter. At the end of the composting period, the experimental bags were weighed and a representative samples of bulk composts and residual bio-plastic were collected from each bag for the subsequent analyses.

^{13}C CPMAS NMR spectra

Fine-powdered composite samples of bulk composts and bio-plastic samples were analyzed by solid-state NMR spectroscopy (^{13}C CPMAS NMR) on a Bruker AV300 Spectrometer equipped with a 4 mm wide-bore MAS probe. The NMR spectra of initial and decomposed substrates were obtained by applying the following parameters: 13,000 Hz of rotor spin rate; 2 s of recycle time; 1H-power for CP 92.16 W: 1H 90° pulse 2.85 μs; ^{13}C power for CP 150,4 W; 1 ms of contact time; 30 ms of acquisition time; 4000 scans. Samples were packed in 4 mm zirconium rotors with Kel-F caps. The cross polarization pulse sequence was applied with a composite shaped "ramp" pulse on the 1H channel in order to account for the inhomogeneity of Hartmann-Hann condition at high rotor spin frequency. The Fourier transform was performed with 4 k data point and an exponential apodization of 50 Hz of line broadening.

The different carbon functionalities are conventionally grouped into the following chemical shift regions: alkyl-C: 0–45 ppm; methoxyl-C: 45–60 ppm; O-alkyl-C: 60–110 ppm; aryl-C: 110–145 ppm; phenol-C: 145–160 ppm, and carboxyl-C: 190–160 ppm. The relative contribution of each region was determined by integration (MestreNova 6.2.0 software, Mestre-lab Research, 2010), and expressed as percentage of the total area. In order to summarize the modification occurring with incubation time, two indices of the extent of decomposition, namely hydrophobic index (HB) and alkyl ratio (A/OA), were determined from the combination of relative area of specific NMR spectral regions (Table 2) as follows:

$$\mathrm{HB} = \sum[(0-45)+(45-60)+(110-160)]/\sum[(45-60)+(60-110)+(160-190)]$$

$$\mathrm{A/OA} = \sum(0-45)/\sum(60-110)$$

In order to determine the variation in structural properties of thermoplastic polymeric blend, variable contact time (VCT) experiments have been performed on starting sample and on the decomposed fragments retrieved at final composting time. The analytical conditions were the following: VCT: 13,000 Hz of rotor spin rate; 2 s of recycle time; 92.16 W 1H-CP pulse power; VCT: from 0.01 to 10 ms for a total of 20 steps; 30 ms of acquisition time; 2000 scans.

The equation used to fit the experimental data and to evaluate the molecular CP parameters was the following [16]:

$$I = [I0/\alpha] \times [\exp(-tCP/t1\rho H)] \times [1-(\exp(-\alpha tCP/tCH))]$$

where I is the experimental signal intensity; $I0$ is the theoretical max signal intensity; α is the $(1 - \mathrm{tCH/t1\rho H})]$; tCP is the instrumental variable contact time(s); tCH is the molecular cross polarization time; $t1\rho H$ is the molecular proton-lattice relaxation time (in the rotating frame).

The assignment of peak resonances in NMR spectra of thermoplastic components was based on published spectra and on reference spectra in NMR Library database http://www.sdbs.riodb.aist.go.jp (National Institute of Advanced Industrial Science and Technology date of access 23 June 2015; SDBS-^{13}C NMRS-DBS No. 18984CDS-02-509; SDBS-^{13}C NMRSDBS No. 1159CDS-12-449).

Thermochemolysis-GC–MS

For the off-line-THM-GC–MS, about 0.2 g of bulk composts and 0.2 g of bio-film strips were placed in a quartz boat with 0.5 mL of TMAH (25 % in methanol w/v) solution. After drying under a stream of nitrogen, the mixture was introduced into a Pyrex tubular reactor (50 × 3.5 cm i.d.) and heated at 400 °C for 30 min in a circular oven (Barnstead Thermolyne 21,100 Furnace, Barnstead International, Dubuque, IA, USA). The gaseous products from thermochemolysis were flowed into two chloroform (50 mL) traps in series, kept in ice/salt baths. The chloroform solutions were combined and rotoevaporated to dryness. The residue was dissolved in 1 mL of chloroform and transferred in a glass vial for GC–MS analysis. The GC–MS analyses were conducted with a Perkin Elmer Autosystem XL by using a RTX-5MS WCOT capillary column (Restek, 30 m × 0.25 mm; film thickness, 0.25 mm) that was coupled, through a heated transfer line (250 °C), to a PE Turbomass-Gold quadrupole mass spectrometer. The chromatographic separation was achieved with the following temperature program: 60 °C (1 min. isothermal), rate 7 °C min^{-1} to 320 °C (10 min. isothermal). Helium was used as carrier gas at 1.90 mL min^{-1}; the injector temperature was at 250 °C, and the split-injection mode had a 30 mL min^{-1} of split flow. Mass spectra were obtained in EI mode (70 eV), scanning in the range 45–650 m/z, with a cycle time of 0.2 s. Compound identification was based on comparison of mass spectra with the NIST-library database, published spectra, and real standards.

Results and discussion

Bulk compost

Mass balance and elemental analyses

At final experimental time, all composting tests showed an intense decomposition of the original raw material, with a 43–50 % losses of dry weight (Table 1). The elemental analysis revealed a slight increase for both total organic carbon (TOC) content and C/N ratio, while a significative decrease was observed in the H/C ratio (Table 1). This finding suggested the preferential removal of decomposable aliphatic compounds, mainly attributable to bioavailable free lipids and carbohydrates [17], combined with the preservation of less protonated molecules, likely represented by the lignin aromatic derivatives included in manure biomass and woody structuring components.

CPMAS-NMR

The NMR spectra of the original biomass were characterized by an initial composition dominated by polysaccharides and carbohydrates (Fig. 1), whose abundance accounted for 58 % of total spectral area (Table 2). The different resonances in the O-alkyl-C region (60–110 ppm) are currently assigned to monomeric units in oligo and polysaccharides of plant tissue [15]. The intense signal around 72 ppm corresponds to the overlapping resonances of carbon 2, 3, and 5 in the pyranoside structures in cellulose and hemicelluloses, whereas the signal at 105 ppm is the specific mark of anomeric carbon 1 of glucose chains in cellulose. The shoulders at 62/64 and 82/88 ppm derive from carbon 6 and 4 of carbohydrate rings, respectively, for which the low field resonances (larger chemical shift) of each couple indicate the crystalline forms of cellulose, whereas the high field ones (lower chemical shift) are typical of amorphous forms of cellulose and/or hemicellulose structures.

In the alkyl-C interval (0–45 ppm), the peaks at 19 and 31 ppm suggested, respectively, the terminal methyl groups and bulk methylenes (CH_2) segments of aliphatic chains present in various lipid molecules, such as wax, sterol, and cutin (Fig. 1). The shoulder around 26 ppm was associated to the CH_2 group in β and γ position of the alcohol segment in aliphatic esters [15]. The broader signals at 39–40 ppm were attributed to the inclusion of tertiary (CH) and quaternary (C-R) carbons in assembled rings of sterol derivatives. The resonance centered at 56 ppm identifies the methoxyl substituents on the aromatic rings of guaiacyl and syringyl units in lignin, with the possible partial contribution of C-N groups in peptides [18]. The wide bands included in the 110–130 ppm shift interval are derived from protonated and C-substituted phenyl carbons in either lignin monomers, lignans or flavonoids. The less pronounced shoulders shown in the phenolic region (140–160 ppm) are indicative of an initial smaller relative contribution of O-substituted aromatic carbons, pertaining to lignin and lignan components (Fig. 1). Finally, the signals in the carbonyl region (190–160 ppm) result from the overlapping of different carboxyl groups related to aliphatic acids, amide groups in amino acid moieties and acetyl substituents in hemicellulose.

The NMR spectra obtained from different litter bags at the final composting time revealed an effective stabilization of organic biomasses, as suggested by the decomposition of bio-labile fractions and relative increase of recalcitrant molecules (Fig. 1). The evaluation of NMR characteristics has revealed to be reliable probing tool to estimate, either, reactivity or recalcitrance of decomposing biomasses such as composts, litters, and soil organic matter inputs [15, 19] The marked decrease of O-alkyl-carbon atoms (60–110 ppm), combined with the raising contribution of NMR signals of alkyl (0–45), aromatic (110–160), and methoxyl (45–60) regions, found in each composting test (Table 2), is in line with the selective preservation of stable hydrophobic organic compounds

Table 1 Amount (dry weight) and elemental composition of bulk compost and bio-polymer at initial and final experimental time

	Dry w. (kg)	C (%)	N (%)	H (%)	C/N	H/C
Compost						
t0	3.22 (0.01)	23.3 (0.8)	1.97 (0.06)	4.22 (0.04)	13.8 (0.09)	2.2 (0.06)
Test A	1.61 (0.06)	26.7 (0.9)	2.13 (0.10)	3.95 (0.33)	14.6 (0.64)	1.8 (0.10)
Test B	1.78 (0.25)	27.2 (0.9)	2.20 (0.06)	4.04 (0.08)	14.4 (0.37)	1.8 (0.07)
Test C	1.78 (0.09)	26.7 (0.9)	2.00 (0.10)	4.05 (0.09)	15.5 (0.50)	1.8 (0.06)
Bio-polymer						
t0	0.05/0.1	57.8 (2.25)	0.28 (0.14)	6.80 (0.13)	ND	1.41 (0.03)
Test B	ND	55.5 (2.04)	0.41 (0.12)	6.24 (0.08)	ND	1.35 (0.05)
Test C	ND	54.8 (1.69)	0.30 (0.09)	6.12 (0.15)	ND	1.35 (0.03)

ND not detectable

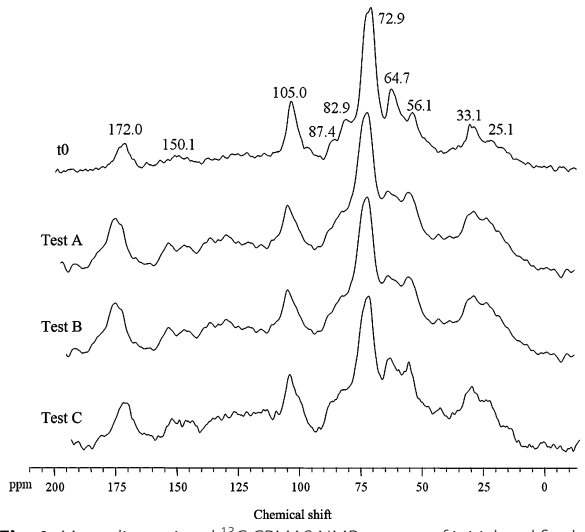

Fig. 1 Monodimensional ^{13}C CPMAS NMR spectra of initial and final bulk compost matrices

in final mature composts, that is currently observed in aerobic composting processes [17, 20]. The degradation of polysaccharide constituents was further highlighted by the progressive lower intensity of the shoulders related to C4 nuclei of glycosidic bond (82–84 ppm), and associated with an increasing broadening of C1 resonances (104–105 ppm) (Fig. 1). This findings suggest the occurrence of progressive cleavage of 1-4 C bonds and the consequent shift of the residual unbound carbons to less deshielded position [15]. The molecular modification of bulk compost samples was summarized by the increase of the structural HB and A/AO indexes, thereby confirming the significant improvement of the overall hydrophobic character associated with the OM stabilization of composting matrices in each experimental test (Table 2).

Thermochemolysis-GC–MS

The thermochemolysis applied to initial and final bulk compost samples released more than hundred recognizable different molecules, which were identified as methyl ethers and esters of natural compounds (Fig. 2; Additional file 1: Table S1). They were mostly represented by lignin derivatives, fatty acids, aliphatic biopolymers, hydrocarbons, and alcohols. The amount and distribution of the most representative monomers found in compost samples (Table 3) were comparable with previous results obtained from the thermochemolysis of different organic biomasses [19, 21]. In respect to NMR data, a significant lower yield of carbohydrates was found among the pyrolysis products of composts. This finding has been related to the poor efficiency of pyrolysis techniques to reliably evaluate carbohydrate units in complex matrices [22]. The thermal behavior and pyrolitic rearrangement of poly-hydroxy components, combined with the alkaline reaction condition of the TMAH reagent solution, produce a negative interference on the volatilization and subsequent chromatographic detection of carbohydrates and polysaccharides.

The identified lignin constituents (Table S1) are associated with current symbolism applied in thermochemolysis analyses to identify the main basic structures [19, 20]: P, p-hydroxyphenyl; G, guaiacyl (3-methoxy, 4-hydroxyphenyl); and S, syringyl (3,5-dimethoxy, 4-hydroxyphenyl). As expected from the initial composition of the original biomasses (cattle and buffalo manure as well as maize straw), the even contribution of the three different lignin structural units to pyrolitic products suggests herbaceous and grass plants as main sources of organic woody inputs. This finding was confirmed by the prevalence in the pyrogram of the propenoic acid derivative [2-propenoic acid, 3-(4-methoxyphenyl)-methyl ester] (P18), that is a basic component of lignified tissues of annual crops and grasses.

An average increase of total yield of lignin units of about 58 % in respect to initial biomasses was found for each mature compost sample (Table 3), thereby showing a selective preservation of aromatic and phenolic components during the OM stabilization process. The extent of lignin preservation or decomposition may be estimated by the

Table 2 Relative distribution (%) of signal area over chemical shift regions (ppm) in ^{13}C-CPMAS-NMR spectra of bulk compost samples at initial and final experimental time

Sample	Carbonyl 190–160	O-aromatic 160–145	Aromatic 145–110	O-alkyl 110–60	CH_3O/CN 60–45	Alkyl 45–0	HB[a]	A/AO[b]
t0	4.1	3.0	8.6	58.4	10.5	15.4	0.50	0.26
Test A	6.7	3.4	12.0	48.0	11.4	18.5	0.69	0.39
Test B	7.1	4.0	12.3	47.9	11.1	17.6	0.68	0.37
Test C	5.7	3.6	11.6	49.2	11.0	18.9	0.68	0.38

[a] HB = Σ[(0–45) + (45–60) + (110–160)]/Σ[(45–60) + (60–110) + (160–190)]

[b] A/OA = Σ(0–45)/Σ(60–110)

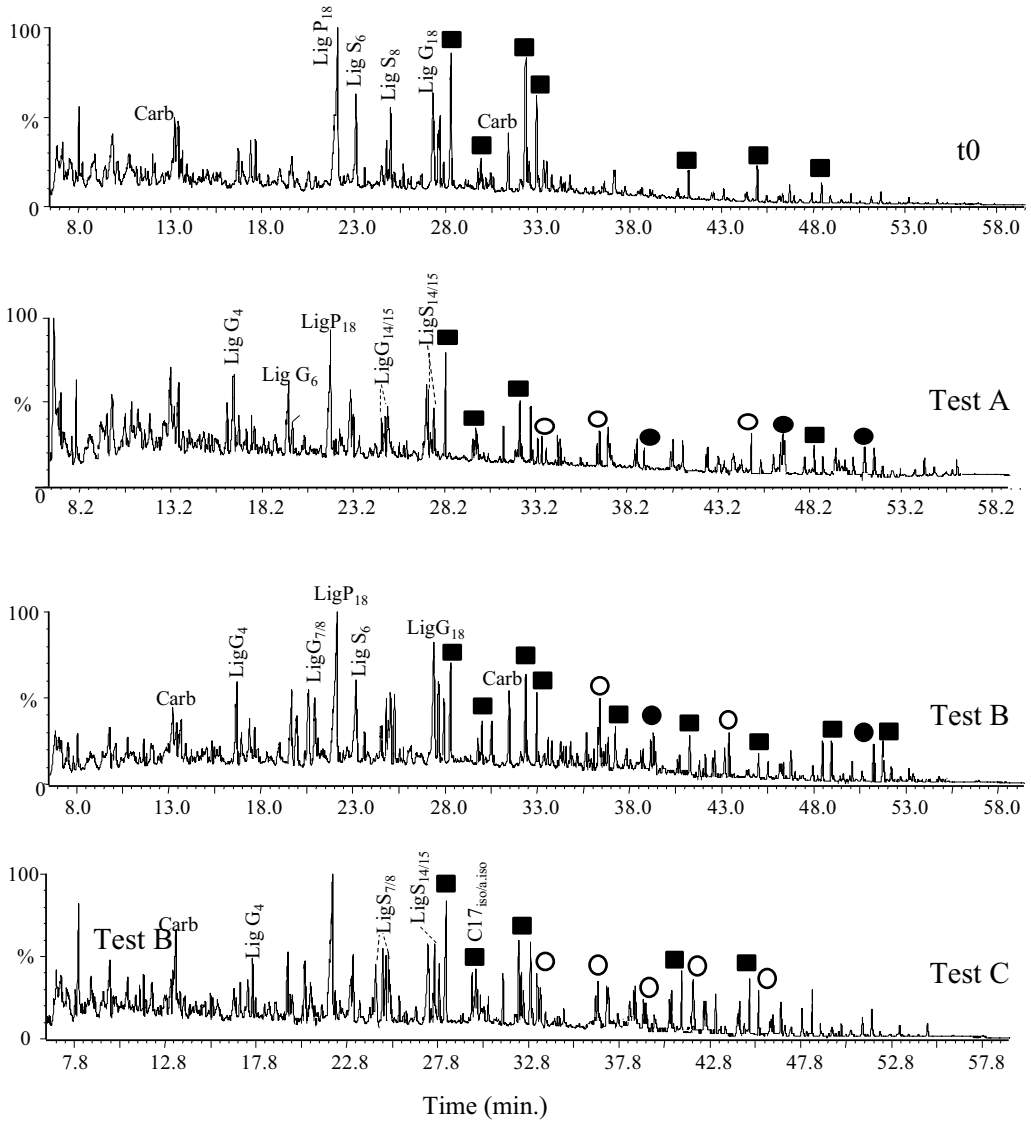

Fig. 2 Total ion chromatograms of thermochemolysis products released from initial and final compost samples Lig lignin, *filled square* fatty acids, *filled circle* alkyl-dioic acids, *circle* hydroxy acids

structural indexes that are based on the relative amount of specific thermochemolysis products, which are associated with either microbially derived organic compounds or fresh plant debris. In particular, the aldehyde (G4 and S4) and acid (G6 and S6) forms of guaiacyl and siryngyl molecules derive from the degradation of lignin polymer, which involve the progressive oxidation of propyl chain. Conversely, the corresponding homologues with integral hydroxylated side chains (G14/15, S14/15) are indicative of unaltered lignin components, which retain the typical β-O-4 ether intermolecular linkages (Additional file 1:Table S1). Therefore, the index obtained (Table 3) by dividing the amount of acidic structures (G6 and S6) by, respectively, that of G4 and S4 aldehydes (Ad/AlG = G6/G4, Ad/AlS = S6/S4), and the index for the global yield of threo/erythro isomers (ΓG = G6/[G14 + G15]; ΓS = S6/[S14 + S15]) are regarded as suitable indicators of the bio-oxidative transformation of lignin polymers [14, 20]. The larger the dimensionless indexes, the wider the decomposition process of lignin substrates. The low intensity and substantial similar level shown by these decomposing indexes over composting time (Table 3) further supported the overall progressive preservation of hydrophobic aromatic and phenolic constituents.

Aliphatic and alicyclic lipids were the principal alkyl molecules found in the pyrograms of compost samples

Table 3 Composition and yields (μg g^{-1}) of main thermochemolysis products released from bulk compost at initial and final experimental time

Compound	t0	Test A	Test B	Test C
Lignin[a]	2186	3474	3433	3258
Ad/Al_G	1.2	0.9	1.3	1.1
Γ_G	1.4	1.5	1.9	1.6
Ad/Al_S	2.4	2.7	2.7	2.4
Γ_S	1.4	1.3	1.2	1.3
Linear fatty acids C_{12}–C_{30} ($C_{18:1}$)	3822	2737	2820	2685
Microbial fatty acids C15–C24 (C_{17})	785	889	963	792
Hydroxy/dioic acids C_{16}–C_{26} (C_{16})	2159	5209	4968	5120
Alkanes C_{17}–C_{31}	301	418	527	562
Alcohols C_{22}–C_{26}	135	92	120	105
Sterols	98	195	210	234

Total range varying from Ci to Cj; compounds in parentheses are the most dominant homologues; numbers after colon refer to double bond

[a] Structural indices: Ad/Al = G6/G4, S6/S4; ΓG = G6/(G14 + G15); ΓS = S6/(S14 + S15)

(Additional file 1:Table S1). The most abundant products were the methyl ester of linear fatty acids, dominated by the hexadecanoic and octadecanoic saturated and unsaturated homologues. The off-line pyrolysis of initial biomass produced also a notable yield of the methylated form of ω-hydroxy alkanoic acids and alkan-dioic acids (Table 3). These molecules are the main building blocks of the external hydrophobic protective barriers of fresh and lignified plant tissues, namely cutin and suberin [23]. No clear predominance of particular monomer was found for both these compound classes, which instead showed an almost uniform distribution of even carbon-numbered long chain components (Table S1). Conversely, the di- and tri-hydroxy substituents of the C16 and C18 homologues were the unique representatives of mid-chain-hydroxy alkanoic acids (Tables 3, 4). The relatively least abundant lipid fraction was the high molecular weight tetra- and pentacyclic triterpenes (Table 3), which have been tentatively identified as methyl ethers and esters of methyl/ethyl cholest(di)en-3-ol structures, and of ursane, lupeane, and oleanane derivatives.

The contribution of microbial input was shown by the pyrolytic release of phospho-lipid fatty acids (PLFA) and 2-hydroxy aliphatic acids, which are basic structural components of microbial cells (Additional file 1:Table S1). The most representative PLFA monomers were, in order of elution, the 12- and 13-methyl tetradecanoic (iso/anteiso pentadecanoic), the 14- and 15-methyl hexadecanoic (iso/anteiso heptadecanoic) acids and the cyclopropane-(2-hexyl)-octanoic acid (C17 cy FAME), which are characteristic microbial markers of natural organic matter [24].

Notwithstanding the contrasting behavior in the yield of specific lipid components released by the thermochemolysis of final compost samples, an overall increase of about 50 % on total dry weight basis was found for the relative amount of total alkyl molecules (Table 3). The marked decrease for the yield of linear fatty acids may be attributed to the decomposition of bio-available free components, which undergo to a most favorable decomposition during the active phase of composting processes [17]. Conversely, a substantial preservation was found for the hydrophobic and structural recalcitrant biopolyester constituents, made up by hydroxyl and alkyl dioic acids (Table 3), which form the stable alkyl fraction of inert soil organic matter pools [25, 26].

Bio-polymer

Mass balance and elemental analyses

The elemental composition of the initial bio-polymer samples indicated the almost exclusive presence of organic components, with a prevalence of aliphatic chains, as suggested by the atomic H/C ratio (Table 1). The intense decomposition sustained by the bio-plastic strips during the composting tests hampered a suitable collection of residual fragments for the gravimetric analysis at the final experimental time, thereby suggesting severe physical fragmentation and chemical modification in both decomposition tests with 1 and 2 % of bio-film concentration (Additional file 1: Figure S1d). A slight variation was observed in the elemental composition of thermoplastic blend at final composting time in each decomposition test, as characterized by a slight decrease of TOC content and H/C ratio (Table 1).

CPMAS-NMR

The ^{13}C-NMR spectra of starting bio-film strips (Fig. 3) revealed a basic structural feature commonly associated to decomposable thermoplastic blend, formed by starch, polyester derivatives, and plasticizers additives. The prevalent role of starch components was stressed by the peaks in the O-alkyl-C region (60–110 ppm) which represented the 53 % of the total relative area (Table 4). The different resonances showed the typical correspondence with the C atoms of glucose rings in polysaccharide chain. The signals at 60 and 80 ppm are associated with the C6 and C4 carbons, respectively, while the large band centered at 72 ppm arise from the coalescence of signals for the C2, C3, and C5 positions, and the di-O-alkyl anomeric C1 is deshielded at 104 ppm. Although the starch molecules were the main O-alkyl-C elements in NMR spectra of initial bio-polymeric sample, the exclusive inclusion of glucose rings, in the 60-110 ppm chemical shift range, should have produced a rough relative ratio of 1:1:3:1, for the spectral areas associated with the peaks

Table 4 Relative signal area (%) and VCT parameters (ms) over chemical shift regions (ppm) in CPMAS-NMR spectra of bio-polymer samples at initial and final experimental time

	Carbonyl-C n		Aromatic-C h		O-Alkyl-C u				Alkyl-C f	
	180–170	170–160	140–130	130–120	110–100	90–80	80–70	70–60	40–30	30–20
t0	2.6	3.4	5.4	10.4	6.0	6.1	20.5	20.3	7.4	17.7
Test B	4.1	5.3	8.0	18.6	–	–	–	24.2	11.1	28.7
Test C	4.0	5.3	8.6	18.9	–	–	–	23.5	9.7	30.0
VCT parameters										
	Aromatic-C (160–110)				O-alkyl-C (110–60)				Alkyl-C (45–0)	
	tCH		t1rH		tCH		t1rH		tCH	t1rH
t0	0.07		100		0.06		78		0.08	100
Test B										
Sample 4	0.05		62		0.03		12		0.06	25
Sample 5	0.05		60		0.03		15		0.05	28
Sample 6	0.04		64		0.03		15		0.06	32
Test C										
Sample 7	0.04		78		0.03		14		0.05	33
Sample 8	0.04		65		0.03		15		0.05	27
Sample 9	0.05		70		0.03		13		0.05	32

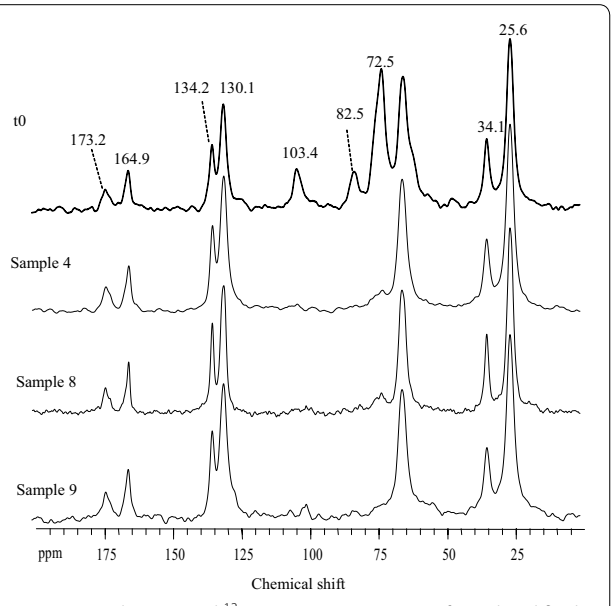

Fig. 3 Monodimensional ^{13}C CPMAS NMR spectra of initial and final bio-polymer components

at 105, 82/88, 73, 62/64 ppm, in the order, according with the corresponding number of carbon nuclei held in the pyranoside structures. With respect to the theoretical expected amounts, a significant overrate was revealed for the integrated area in the 60–70 ppm interval (Table 4), thus suggesting an additional contribution of O-alkyl compounds deriving form other molecules present in thermoplastic composite.

The alkyl C region (0-45 ppm) of initial NMR spectra was characterized by two signals at 26 and 35 ppm, assigned to CH_2 groups in methylenic chain and in α/β position, respectively, to the carbonyl of ester bonds in short chain polyester derivatives, which may be combined with starch in the thermoplastic bio-film products (Fig. 3). The peak at 173 ppm is related to the carbonyl groups of ester bonds, while the corresponding esterified terminal alcoholic carbon of each monomeric unit of polyester components may be associated with a chemical shift around 63–65 ppm [27], thus contributing to the observed intense overlapping resonance in the 60–70 ppm range.

The chemical shift of the remaining peaks, placed in the olefinic and carbonyl regions, at 129, 135 and 164 ppm, respectively, indicated the inclusion of aromatic ring carbons and carboxylic functions of esterified 1,4 benzen di-carboxylic acid (teraphthalic acids) moieties, used as common plasticizer additives.

A large variation in chemical composition was found in the NMR spectra of bio-polymer fragments retrieved at end of the composting process (Fig. 3). The bio-film remains, collected from each testing bag, were characterized by a sharp degradation of starch components, as highlighted by the almost complete disappearance of O-alkyl-C signals of glucose units at 67, 72, 84, and 103 ppm (Fig. 3). However, the permanence of evident

signal in the 60–70 ppm range suggests that a persistence of carbohydrate carbons cannot be excluded (Table 4). The resonances associated to residual compounds were mainly those of carboxylic, aromatic, and alkyl carbons in either plasticizer additives or aliphatic polyesters, thereby confirming the contribution of esterified the variation, with composting process, of structural properties of polymeric chain, was further assessed by the evaluation of cross polarization dynamics, performed with pseudo-bidimensional solid-state VCT NMR experiments. The analytical approaches, in solid-state NMR technique, are based on $^{1}H–^{1}H$ and $^{1}H–^{13}C$ dipolar nuclear interactions and structural mobility, which largely determine the signal behavior in cross polarization experiments [15]. In complex organic macromolecules, the structural properties and the magnetization features of secondary, tertiary, and quaternary C functionalities may produce striking differences on resonance intensity, signal resolution, and relaxation trend of various components. On the other hand, the occurrence of local high proton density and the steric proximity of protonated functional groups allow a powerful sharing of magnetization properties (*spin diffusion*), with an effective averaging and matching of cross polarization parameters [16, 28]. Therefore, since the specific sample properties correspond to characteristic response of VCT NMR parameters, the delays required for both the complete $^{1}H–^{13}C$ polarization transfer to maximize the signal intensity (*cross polarization time-tCH*), and the decay of proton magnetization (*spin–lattice proton relaxation time-t1ρH*) is currently used to evaluate the spatial homogeneity and conformational constraints of molecular domains [12, 29].

A significant difference was shown by VCT experiments performed on intact bio-polymer composite and residual fragments of each composting test (Table 4; Figs. 4, 5). An almost uniform cross polarization dynamics was found for the three main organic components identified in initial bio-plastic materials (Fig. 4), which revealed similar VCT curves with averaged cross polarization (*tCH*) and relaxation (*t1ρH)* times larger than 0.06 and 70 ms, respectively (Table 4). The effective mixing of spectroscopic properties suggested the presence of homogeneous contiguous molecular domains, in which the functional groups of thermoplastic blend are placed in close proximity to each other [11]. The spin communication between protonated groups of starch and aliphatic esters, allowed an effective sharing of NMR properties. However, the density of carbonyl quaternary carbons that formed the esterified connecting network, alternated with the inclusion of less protonated aromatic moieties, may have lowered the efficacy of spin diffusion and slackened the *t1ρH* relaxation rate, even approaching the dynamics typical of "remote" protonation systems [16]. Moreover, in opposition to rigid polymeric chains and crystalline macromolecules, the amorphous characteristic and large molecular flexibility of decomposable bio-film materials may have further contributed to hinder the intermolecular nuclear interactions, thus slowing down the overall cross polarization process [8, 12].

At the end of the composting process, a noticeable variation was found among cross polarization parameters, as it is highlighted by the VCT curves of residual resonances in NMR spectra of the remaining bio-film residues (Table 4; Fig. 5; Additional file 1: Figures. S2, S3). The low protonated aromatic components (110–160 ppm) were still characterized by large cross polarization time *(tCH*) and relaxation rate *(t1ρH*), while the signals in the O-alkyl (60–70 ppm) and alkyl (0–45 ppm) regions revealed an effective recovery of spin diffusion with a faster decay of proton magnetization (Fig. 6; Additional file 1: Figure S2) and a striking decrease corresponding relaxation times (Table 4).

The differences in cross polarization dynamics for the specific functional groups at final composting time, in respect to the initial behavior, revealed the progressive loosening of intermolecular interactions and decrease of structural homogeneity. This finding confirms that the composting process promoted an intense alteration of the intermolecular linkages among the organic components of the thermoplastic blend, thus allowing the progressive decomposition of simple constituents.

Thermochemolysis-GC–MS

The results of the Gas Chromatography–Mass Spectrometry analysis of products released by the off-line pyrolysis of initial bio-film strips (Fig. 6) were fully consistent with the structural information derived from solid-state NMR spectra. The mass spectra associated with the two intense peaks, eluting at 10.1 and 17.2 min, were assigned to the methylated forms of hexanedioic acid and 1,4 benzendicarboxylic acid, respectively, while the intermediate lower peaks at about 13.6 min of retention time were related to the methyl ether derivatives of oligosaccharide molecules (Fig. 6). With respect to the basic components identified in NMR spectra, the strong basic condition conveyed by the TMAH reagent may have promoted the solvolysis and the oxidation of the terminal hydroxyl groups of short chain aliphatic ester linkages, with the formation of the C6 alkyl dioic homologues.

The different pyrolytic behaviors of thermoplastic components did not allow a suitable quantitative estimation of the detected organic compounds. As previously noted, the thermally unstable poly-hydroxy molecules undergo unfavorable pyrolytic rearrangements, which strongly reduce the pyrolytic yield of carbohydrates and polysaccharides. Conversely, the volatility of benzene-carboxylic

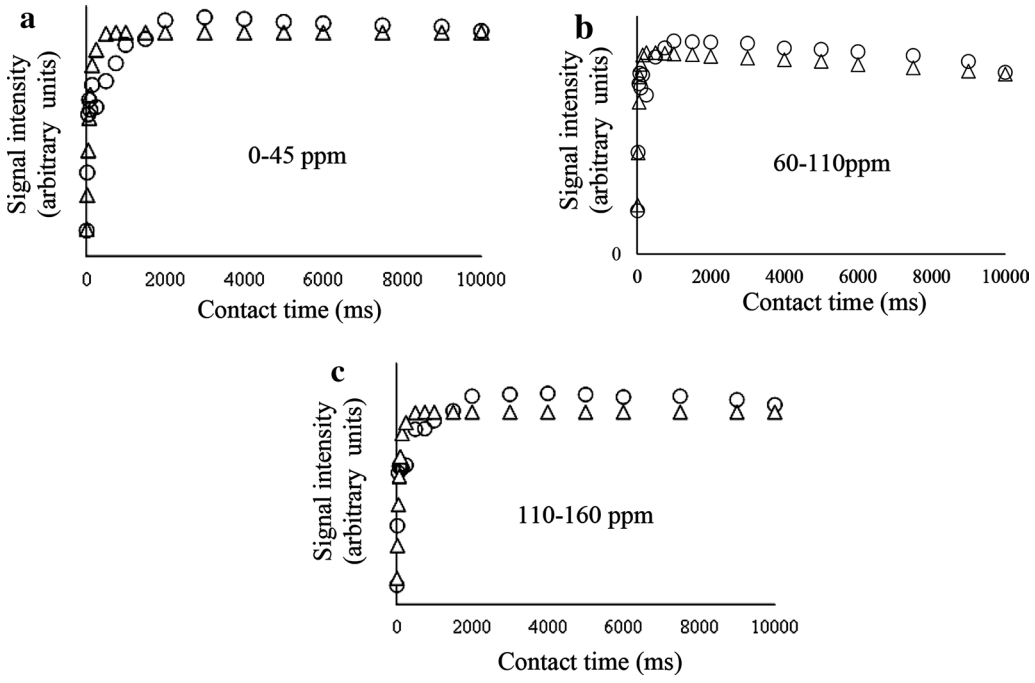

Fig. 4 ^{13}C CPMAS NMR VCT experiments of initial bio-polymer components: **a** VCT curves of alkyl (0–45 ppm) molecules; **b** VCT curves of O-alkyl (60–110) molecules; **c** VCT curves of aromatic (110–160) molecules; (*circle* experimental data; *triangle* fitting curve)

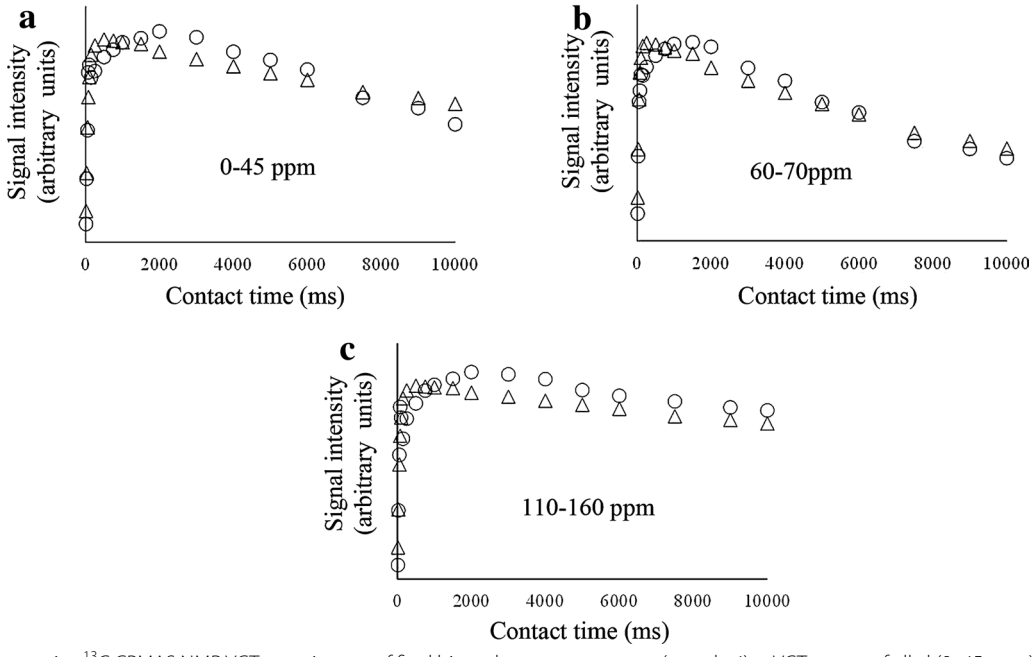

Fig. 5 Representative ^{13}C CPMAS NMR VCT experiments of final bio-polymer components (sample 4): **a** VCT curves of alkyl (0–45 ppm) molecules; **b** VCT curves of O-alkyl (60–110) molecules; **c** VCT curves of aromatic (110–160) molecules; (*circle* experimental data; *triangle* fitting curve)

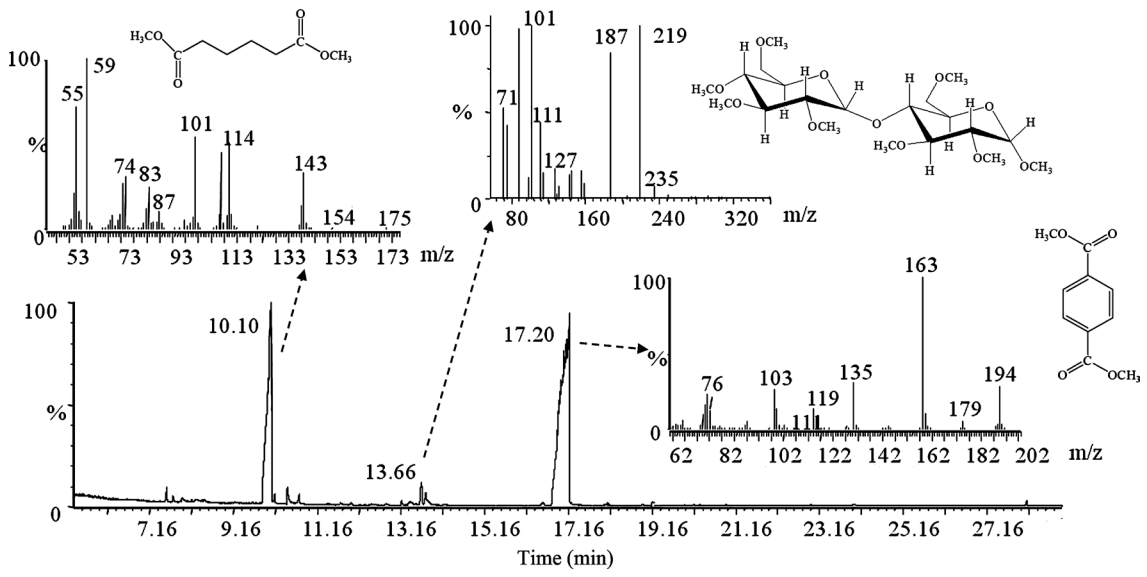

Fig. 6 Total ion chromatograms and mass spectra of thermochemolysis products released from initial bio-polymer samples

and short chain aliphatic acids determine the almost complete release of low molecular weight thermoplastic additives in the gas phase, and their consequent relative overestimation.

The pyrograms of final bio-film residues confirmed the effective chemical dissolution of the thermoplastic blend initially introduced into the composting pile. In fact, the thermochemolytic products obtained from each testing bag showed the partial persistence of low molecular weight alkyl and aromatic components, but the complete disappearance of the chromatographic peaks associated to carbohydrates (Additional file 1: Figure S4).

Conclusion

The effective degradation of the starch-based biopolymer used to make mulching films was successfully proved within an on-farm composting system. Solid-state NMR spectroscopy and thermochemolysis revealed a noticeable variation of both polymeric components and molecular properties. The composting process showed the typical modifications associated with OM stabilization, that is characterized by the decrease of bio-labile compounds and the selective preservation of recalcitrant alkyl and aromatic molecules. In addition to classical protocols to test biodegradation of materials, the experiments by real composting process of this work followed by the analytical characterization of residues appear as an useful technique to elucidate the molecular properties of starch-based polymers, and to highlight that the composting process is a viable way to recycle the residues of biodegradable materials.

Additional file

Additional file 1. Supporting information.

Authors' contributions

RS carried out the NMR and Thermochemolysis analytical characterizations and drafted the manuscript. DT set out the experimental plan and performed the composting process. MD participated to the interpretation of NMR and GC-MS data. AN participated to the interpretation of NMR data. AP set out the experimental plan and the analytical protocols, participated to the manuscript drafting. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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