Molecular Characterization of Compost at Increasing Stages of Maturity. 2. Thermochemolysis—GC-MS and $^{13}$C-CPMAS-NMR Spectroscopy

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Off-line pyrolysis TMAH-GC-MS (thermochemolysis) and solid-state $^{13}$C NMR spectroscopy were applied for the direct molecular characterization of composted organic biomasses after 60, 90, and 150 days of maturity. Off-line thermochemolysis of both fresh and mature composts released various lignin-derived molecules, the quantitative measurement of which was used to calculate structural indices related to compost maturity. These indicated that most of the molecular transformation occurred within the first 60 days of the composting process, whereas slighter molecular variations were observed thereafter. Both $^{13}$C NMR spectra and offline pyrograms suggested that the process of compost maturity was characterized by a progressive decrease of alkyl components, whereas cellulose polysaccharides appeared to be more resistant and began to be transformed at a later composting period. The main components of the final mature compost were lignocellulosic material and hydrophobic alkyl moieties, inasmuch as that commonly found in well-humified organic matter of soils and sediments. The persistence of untransformed lignin-derived products and di- and triterpenoids throughout the maturity period suggested that these molecules are useful markers to both evaluate compost origin and trace its fate in the environment. Thermochemolysis provided the same characterization of molecules either unbound or bound to the compost matrix as that reached by a previously applied sequential chemical fractionation of the same compost materials, thereby indicating that thermochemolysis is a more rapid and equally efficient tool to assess compost molecular quality.

KEYWORDS: $^{13}$C-CPMAS-NMR; offline pyrolysis; TMAH; compost maturity; plant biomarkers

INTRODUCTION

Recycling of organic biomasses in compost is important for both environmental quality and sustainable agriculture. Although application of composted organic matter to soil may increase soil physical quality and plant nutrition, it may also reduce mineralization of biolabile compounds, thereby enhancing the role of soil organic matter (SOM) as sink of organic carbon (1, 2). Moreover, the content of humic matter in compost may be an important resource for soil remediation purposes (3, 4).

In recent years, the transformation of organic components during composting was investigated to understand the extent of the inherent biological stabilization. This research was mainly aimed to characterize separates from compost such as dissolved organic matter (DOM) (5), humic-like components (6, 7), extracted organic molecules (8), and lipid compounds (9). We concomitantly propose a chemical fractionation sequence to identify compost molecular components and differentiate the strength by which these are linked to the compost matrix (10). However, a direct molecular characterization of the bulk compost may also enlighten the process of organic matter stabilization by simultaneously determining all different organic molecules that are transformed during composting.

Both $^{13}$C cross-polarization magic angle spinning nuclear magnetic resonance spectroscopy ($^{13}$C-CPMAS-NMR) and offline pyrolysis with tetramethylammonium hydroxide (TMAH) followed by gas chromatography—mass spectrometry (Pyr-TMAH-GC-MS) are updated and powerful tools for the molecular investigation of complex natural organic matter (11, 12). The nondestructive solid-state $^{13}$C NMR technique provides the distribution of organic carbons in a wide range of solid matrices and is currently applied to characterize the composition, as well as the transformation, of plant tissues, litter, SOM, and humic substances.

Pyrolysis in the presence of TMAH is increasingly used to study natural biopolymers such as plant waxes, cutins, and woods, as well as humic materials and bulk soil organic matter (13–15). It involves the solvolysis and methylation of ester and ether bonds present in the complex mixture of organic macromolecules and biopolymers, thereby enhancing both the thermal stability of acidic, alcoholic, and phenolic groups and their chromatographic detection. This technique consists more of a simultaneous thermochemolysis reaction rather than a pyrolysis reaction.
followed by methylation of the polar compounds (16, 17). Lower temperatures are then required for the concomitant solvolysis and methylation reactions, thereby reducing the secondary pyrolytic rearrangements. Moreover, the Pyr-TMAH-GC-MS technique conducted in the offline mode allows a more effective determination and quantitative measurement of pyrolytic products (18).

The main objective of this work was to characterize the molecular components of bulk compost samples at different degrees of maturity and to evaluate their transformation during the stabilization process of composting. The direct characterization of bulk compost samples was attempted by applying both solid-state $^{13}$C NMR spectroscopy and offline Pyr-TMAH-GC-MS techniques, and the results were compared with those obtained by a previous stepwise chemical fractionation procedure (10).

MATERIALS AND METHODS

**Compost Samples.** The organic biomasses used in compost production (GeSeNu Srl., Perugia, Italy) had the following composition: 50% domestic organic wastes, 40% refuse from plant trimming, and 10% vegetal residues from tobacco and aromatic plants. These materials were mixed, ground, and sieved at 12 mm. Compost products were obtained aerobically after 30 days of a common active phase, followed by three curing phases of an additional 30, 60, or 120 days, which yielded, respectively, compost samples 60, 90, and 150, of different maturities. Before analysis, compost samples were oven-dried at 40 °C until constant weight and sieved at 500 μm.

**Solid-State $^{13}$C NMR Spectroscopy.** Solid-state NMR spectroscopy ($^{13}$C-CPMAS-NMR) was performed on a Bruker AV-300 equipped with a 4 mm wide-bore MAS probe. NMR spectra were obtained by applying the following parameters: 13000 Hz of rotor spin rate; 1 s of recycle time; 1 ms of contact time; 20 ms of acquisition time; 5000 scans. Samples were packed in 4 mm zirconia rotors with Kel-F caps. The pulse sequence was applied with a $^1$H ramp to account for nonhomonuclear coupling and for the Hartmann–Hahn condition at high spin rotor rates. The resulting spectra were compared with published spectra, and real standards.

**Offline Pyrolysis TMAH-GC-MS.** About 100 mg of dried compost was placed in a quartz boat and moistened with 1 mL of TMAH (25% in methanol) solution. After drying the mixture under a gentle stream of nitrogen for about 10 min, the sample was introduced into a Pyrex tubular reactor (50 cm × 3.5 cm i.d.) and heated at 400 °C for 30 min (Barnstead Thermolyne 21100 furnace). The released products of thermochemical decomposition were trapped in methanol and concentrated by rotoevaporation. The residue was redissolved in 1 mL of chloroform and transferred in a glass vial for GC-MS analysis. Three replicates of thermochemical were carried out for each compost sample.

The GC-MS analyses were conducted with a Perkin-Elmer Auto-system XL equipped with an RTX-5MS WCOT capillary column (Restek, 30 m × 0.25 mm; film thickness = 0.25 μm) and coupled, through a heated transfer line (300 °C), with a PE TurboMass-Gold quadrupole mass spectrometer. Chromatographic separation was achieved with the following temperature program: 60 °C (1 min isothermal), raised at 7 °C min$^{-1}$ to 100 °C and then at 4 °C min$^{-1}$ to 320 °C (10 min isothermal). Helium was the carrier gas at 1.90 mL/min, the injector temperature was at 250 °C, and the split injection mode had a 30 mL/min of split flow. Mass spectra were obtained in EI mode (70 eV), scanning in the range of m/z 45–650, with a cycle time of 1 s. Compound identification was based on comparison of mass spectra with the NIST library database, published spectra, and real standards.

For quantitative analysis, due to the large variety of detected compounds with different chromatographic responses, external calibration was applied. The relative area of each compound collected in each chromatographic peak was calculated with the PE-TurboMass software. The extraction curves were built by mixing methyl esters and/or methyl ethers of the following molecular standards: tridecanoic acid, octadecanoic acid, 16-hydroxyhexadecanoic acid, 16-docosandioic acid, β-sitosterol, and cinnamic acid. Increasing amounts of standard mixtures were placed in a quartz boat and moistened with 0.5 mL of TMAH (25% in methanol) solution. The same thermochemical conditions as for compost samples were applied for the standards. The percentage recovery of standards ranged from 82 to 91% of initial amount.

**Labile Carbohydrates.** To evaluate the biolability of carbohydrate compounds, alkyl compounds were first removed from compost samples by the sequential procedure outlined earlier (19), which consisted of (1) extraction in an organic solvent, (2) BF$_3$–MeOH transesterification, and (3) KOH–MeOH solvolysis. Easily hydrolyzable carbohydrates were removed from this simplified compost matrix by an overnight extraction with dilute sulfuric acid (0.5 M, 1/10 w/v) (19). The resulting solid residue was extensively washed with deionized water until neutral pH and freeze-dried before being subjected to $^{13}$C-CPMAS-NMR spectroscopy.

RESULTS AND DISCUSSION

**NMR Spectra.** The $^{13}$C-CPMAS-NMR spectra of bulk compost samples at increasing stage of maturities are shown in Figure 1, whereas the relative distribution of signal areas is reported in Table 1. The spectrum of the less mature compost 60 was dominated by the signal in the alkyl-C (0–60 ppm) and O-alkyl-C (60–110 ppm) regions. The former region is characteristic of plant woody tissues, whereas the alkyl group area is related to the presence of aliphatic groups (16). The resulting solid residue was extensively washed with deionized water until neutral pH and freeze-dried before being subjected to $^{13}$C-CPMAS-NMR spectroscopy.

Table 1. Relative Distribution (Percent) of Signal Area over Chemical Shift Regions (Parts per Million) in $^{13}$C-CPMAS-NMR Spectra of Compost Samples

<table>
<thead>
<tr>
<th>sample</th>
<th>0–60</th>
<th>60–110</th>
<th>110–160</th>
<th>160–200</th>
<th>HB/HI$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>compost 60</td>
<td>37.6</td>
<td>50.8</td>
<td>7.2</td>
<td>4.3</td>
<td>0.81</td>
</tr>
<tr>
<td>compost 90</td>
<td>30.6</td>
<td>56.1</td>
<td>7.3</td>
<td>6.1</td>
<td>0.61</td>
</tr>
<tr>
<td>compost 150</td>
<td>45.3</td>
<td>37.6</td>
<td>9.6</td>
<td>7.4</td>
<td>1.22</td>
</tr>
</tbody>
</table>

$^a$ Hydrophobic carbons/hydrophilic carbons = [(0−60) + (110−160)]/(60−110) + (160−200).

**Figure 1.** $^{13}$CPMAS-NMR spectra of bulk compost samples at increasing maturity stages: (A) compost 60; (B) compost 90; (C) compost 150.
ppm shoulder of methoxy groups on aromatic rings of guaiacyl and syringyl units of lignin structures (20). The resonances in the O-alkyl-C region are assigned to monomeric units in oligo- and polysaccharidic chains of plant woody tissues (21). The intense signal around 72 ppm corresponds to the overlapping resonances of C2, C3, and C5 carbons in the pyranoside structure of cellulose and hemicellulose, whereas the signals at 106 ppm (sharp), 65 ppm, and 82–85 ppm (shoulders) are assigned to the anomeric C1 carbon and the C6 and C4 carbons, respectively, the latter being split in the presence of both amorphous and crystalline forms of cellulose (22). The aromatic region (110–160 ppm) did not reveal the distinct resonances of ring carbons in lignin structures (23). Only a weak broad band around 130 ppm may be related to p-hydroxyphenyl rings of cinnamic units in both lignin and suberin biopolymers. A prominent signal for quaternary carbons at 172 ppm is currently assigned to carboxyl groups.

A decrease of alkyl carbons was shown for the sample after 90 days of composting (Figure 1B and Table 1), suggesting an overall mineralization of the most bioavailable lipid compounds. No equivalent changes were revealed for carbohydrate carbons, except for the significant reduction of the 83 ppm shoulder assigned to noncrystalline cellulose. Conversely, a relatively larger decrease of the carbohydrate region was shown for compost at the final stage of maturity (Figure 1C and Table 1). In this sample, the significant residual carbohydrate and alkyl carbons, as well as the increased content of methoxyl and aromatic carbons, suggested a predominance of lignocellulosic and recalcitrant hydrophobic materials. The increased hydrophobicity of compost 150 with respect to less mature samples is shown by the hydrophobic C/hydrophilic C ratio (HB/HI) in Table 1.

To evaluate whether the carbohydrate carbons belonged to labile mono- or recalcitrant polysaccharides, the compost materials were subjected to a mild stepwise purification procedure (10) and removal of labile carbohydrates, which should have left unaltered the glycosidic bonds of polysaccharides. The 60–110 ppm region of NMR spectra of all compost residues after such purification (Figure 2A–C) closely resembled that of cellulose from plant woody tissues (21, 24), suggesting that a large part of carbohydrates consisted of polysaccharide components of plant cellulose fibers. This indicated a biological stability of cellulose content in the less stable compost and only an incipient decomposition during the last period of the compost curing phase. Conversely, the lack of changes for both aromatic and methoxyl lignin components was confirmed also in these purified compost samples.

**Offline Pyr-TMAH-GC-MS.** The total ion chromatograms (TIC) derived from the thermochemolysis of less mature compost 60 and mature compost 150 are shown in Figure 3. The compounds identified in the compost samples are listed in Table 2, whereas their quantitative evaluation is shown in Table 3. Thermochemolysis of the bulk composts released more than 100 different molecules, which were identified as methyl ethers and esters of natural compounds (Table 2). The majority of these compounds originated from higher plants and were represented by lignin, waxes, and aliphatic biopolymers. These findings on compost samples are similar to other results on bio- and geochemical materials (25, 26) and validate the effectiveness of the offline Py-TMAH-GC-MS technique in the investigation of complex organic materials.

Contrary to the indications of $^{13}$C NMR spectra, low amounts of carbohydrates were found among the pyrolysis products. Only a few peaks reducible to polysaccharides, such as trimethoxy benzene isomers and methyl ether derivatives of monosaccharides, were found in the various chromatograms (Table 2). A pyrolysis product, which was possibly assigned to an oligosaccharide, was found only in the pyrogram of compost 150 (Figure 3B). The lack of polysaccharidic compounds was already noted in applications of thermochemolysis on plant woody tissues and soil organic matter (13, 27). It was pointed out that although conventional flash pyrolysis may produce carbohydrate derivatives, the offline TMAH pyrolysis appears to be less prone to the detection of polysaccharides in complex geochemical matrices. Works on model compounds showed that parameters such as TMAH content and temperature and time of pyrolysis should be carefully set up for different substrates if carbohydrate products are to be made visible in pyrograms (28). In the present work, the evaluation of OC content in compost samples before and after pyrolysis revealed that about 50% of the initial OC remained in the solid residue. It is thus conceivable that, also for compost samples, the current setup of thermochemolysis parameters is highly selective for lignin and alkyl components and reduces the simultaneous identification of carbohydrate units from cellulose.

**Lignin Compounds.** The number of lignin components released by thermochemolysis of compost 60 (Figure 3A) closely resembles that obtained for lignocellulose fractions of plant tissues and plant debris (21, 29). The different lignin derivatives (Tables 2 and 3) are associated with current symbolism in thermochemolysis analysis for lignin basic structures: P, p-hydroxyphenyl; G, guaiacyl (3-methoxy, 4-hydroxyphenyl); and S, syringyl (3,5-dimethoxy, 4-hydroxyphenyl) (23, 30).

The amount of various methylated p-hydroxyphenyl, guaiacyl, and syringyl derivatives released from compost confirms the origin of lignin from different higher plants. The softwood of gymnosperms is made up almost exclusively of guaiacyl subunits, whereas both syringyl and guaiacyl units constitute the hardwood of perennial angiosperm, and all three components are building blocks of grass lignin, the p-hydroxyphenyl unit being the major constituent (31).
The main lignin components released from compost 60 were the respective oxidized products of both di- and trimethoxy phenylpropane molecules, such as benzaldehyde (G4, S4), acetonophenone (G5, S5), and benzoic acid (G6, S6) units (Table 2). Other important and classical products of lignin thermochemolysis were the cis and trans isomers of 1-(3,4-dimethoxy-phenyl)-2-methoxyethylene (G7, G8) and 1-(3,4,5-trimethoxy-phenyl)-2-methoxyethylene (S7, S8), as well as the enantiomers of 1-(3,4-dimethoxyphenyl)-1,2,3-trimethoxypropane (G14 and G15), and 1-(3,4,5-trimethoxyphenyl)-1,2,3-trimethoxypropane (S14 and S15). 3-(4-Methoxyphenyl)-2-propenoic acid (P18) was the most abundant P product, which may have resulted from both the oxidation of \( p \)-hydroxyphenyl units in lignin and the aromatic domains of suberin biopolymers in plant woody tissues. Compost maturity did not imply a significant variation in the total amount of guaiacyl and syringyl lignin components. Conversely, a decrease was noted for the \( p \)-hydroxyphenyl units (Table 3), which were represented only by the P6 and P18 components in the final compost 150. This behavior may be explained by the less complex structure of grass lignin, which leaves the phenylpropanoid units more exposed to bio-oxidation.

All of these released products may be used as plant biomarkers to trace the biodegradation of plant straw and woody tissues and evaluate the fate of organic matter in soils (30, 32). The extent of lignin degradation may thus be estimated by structural indices that are based on the relative amount of specific guaiacyl and syringyl thermochemolysis products (23). These include the G4,S4 and G6,S6 derivatives, as well as the threo/erythro isomers of 1-(3,4-dimethoxyphenyl)-1,2,3-tri-methoxypropane (G14 and G15) and 1-(3,4,5-trimethoxyphenyl)-1,2,3-trimethoxypropane (S14 and S15) (Table 2). Whereas the aldehydic and acidic forms of guaiacyl and syringyl structures result from progressive lignin oxidation, the corresponding homologues with a methoxylated side chain are indicative of unaltered lignin components, which retain the propyl ether intermolecular linkages. Therefore, Ad/Al and \( \Gamma \) indices are, respectively, the ratio of peak areas of acidic structures over that of the corresponding aldehydes (G6/G4, S6/S4) and over the sum of peak areas for the threo/erythro isomers (\( \Gamma_G = G6/[G14 + G15] \); \( \Gamma_S = S6/[S14 + S15] \)). These indices are considered to be good indicators of the bio-oxidative transformation of lignin polymers (21).

In this work, the chromatographic coelution of S15 with the front of the large hexadecanoic acid methyl ester peak (Figure 3A) undermined the correct evaluation of the S15 peak area, thereby hindering the full estimation of the \( \Gamma_S \) parameter. Moreover, the use of mass base peaks for methyl palmitate (m/z 74) and S15 (m/z 211) to evaluate their different contributions, as suggested elsewhere (30), was not helpful due to the predominance of the fatty acid methyl ester and consequent masking of the S15 peak. Nevertheless, both Ad/Al and \( \Gamma \) ratios for the less mature compost 60 (Table 3), as compared to analogous data reported in the literature for both fresh and decomposed woody tissues (30, 33), suggest an advanced lignin decomposition during the first composting period. Conversely, no significant changes were revealed by these structural indices.

Figure 3. Total ion chromatograms of thermochemolysis products: (A) compost 60; (B) compost 150; (■) FAME; (□) \( \alpha \)-hydroxy-FAME; (●) alkanedioic acid DIME; (▲) n-alkanes.
in compost 90 and in the final compost 150, thereby indicating that lignin was hardly oxidized after the first 60 days of compost maturity. These findings, together with the steady total content of guaiacyl and syringyl compounds and the presence of less altered molecules (G14/15, S14/15) in the final compost 150 (Table 2 and Figure 3B), confirm the overall stability of lignin components during composting, as already suggested by NMR spectra.

Alkyl Compounds. Alkyl compounds were the most abundant thermochromolysis products released from compost samples (Table 2). The total release yield of compounds, their dimensional ranges, and the dominant homologues are shown in Table 3.

As indicated by NMR spectra, the Pyr-TMAH-GC-MS results suggest that hydrophobic alkyl moieties were the main constituents of the less mature compost (Figure 3A and Table 3).
Compost 60 mostly released methyl esters of linear fatty acid (FAME), with chain length ranging from C_{10} to C_{30}. About 80% of total FAME was made by both saturated and unsaturated hexadecanoic and octadecanoic acids (Figure 3A), which are ubiquitous components in living and decayed organisms. The marked predominance of even over odd carbon atoms in FAME indicates the contribution of higher plants to this compost (34, 35). This is supported also by the presence of longer chain FAME, which derive from the breakdown of aliphatic esters and constitute, together with n-alkanes and sterols, the external protective wax layer in aerobial plant tissues (35). Conversely, a direct input from microbial activity was revealed by the detection of branched chain FAME. Among these, the most abundant compounds were the 12- and 13-methyl tetradecanoic and hexadecanoic acids (iso and anteiso C_{15} and C_{17} FAME in Table 2), which are common microbial constituents of natural organic matter (NOM) in terrestrial and marine environments.

Other alkyl molecules in compost 60 were, in order of abundance, the methylated form of midchain hydroxyalkanoic acids, ω-hydroxyalkanoic acids, and alkanedioic acids (Table 3). The monoo-, dio-, and trihydroxy acids were represented only by the C_{16} and C_{18} homologues, with 9,16- and 10,16-dihydroxyhexadecanoic isomers being the most abundant products, followed by 9,10,18-trihydroxyoctadecanoic acid and other C_{16} and C_{18} components in minor amounts (Figure 3A). The hexadecanoic and monounsaturated octadecanoic acids were the main ω-hydroxyalkanoic acids, the minor components (C_{14}–C_{20}) of which showed a wide range of even carbon numbered units. The alkanedioic acids were characterized by two well-distinct short-chain (C_{6}–C_{10}) and long-chain (C_{18}–C_{24a}) groups, the latter also including the monounsaturated C_{18:1} acid as the principal product (Table 2). Among the heaviest components, another C_{18} homologue was tentatively identified, on the basis of mass spectra, as the 9,10-dihydroxyoctadecanedioc acid.

A significant amount of straight-chain alkanes was released from compost 60. Their exclusive presence as long-chain hydrocarbons (Table 2), the distinct predominance of odd versus even carbon numbers (Figure 3A), and the large amount of nonacosane, hentriacontane, and tritriacontane all suggested a prevalent origin of these alkanes from wax layers of higher plants (36).

Tricyclic diterpenes and tetra- and pentacyclic triterpenes were distinctly identified, although in low amount, among the thermochromolysis products from compost 60 (Tables 2 and 3). The abietic, pimaric, and isopimaric acids were the main original precursors found among the diterpenoid molecules, followed by their diagenetic products, such as dehydroabietane and dehydroabietic acid and labdane acid derivatives. The diterpenic acids, especially those with abietane and pimarane skeletons, are the most representative component of natural diterpenoids, currently found in resins of various higher plants such as Coniferae and Leguminosae families (37). The tetracyclic triterpenes found in compost 60 were represented by methyl ethers and esters of methyl/ethyl cholesten-3-ol derivatives, whereas pentacyclic triterpenes were the ursane, lupeane, and oleanane structures. Both sterol and triterpenol compounds are among the most abundant lipid components of plant tissues (38, 39). It has been already pointed out that sterol and triterpenol of plant residues undergo a rapid decline once exposed to microbial activity (35, 38). Therefore, their relatively low amount in compost 60 may be explained by the biotic and abiotic degradation occurring at the initial active phase of composting. Both diterpenoid and triterpenoid compounds are useful indicators of coniferous and angiosperm plants in terrestrial and marine environments (40), because the structural characteristics of their original precursors are maintained despite the various degradation processes undergone by the plant species (41). Conversely, biomarkers of microbial activity in altered natural organic matter are the 2-hydroxyalkanoic acids (26), which were also found in low amount in compost samples (Table 2).

The alkyl composition found here by thermochromolysis agrees with the results shown by Spaccini and Piccolo (10), who characterized the molecular composition of the same compost materials by applying a sequential chemical fractionation (10). The linear fatty acids, alkanes, sterol, and diterpenes released by thermochromolysis closely resembled the components extracted from compost by an organic solvent (10), thereby indicating that the thermal treatment effectively favored the transfer in the gas phase of the alkyl molecules, which were only physically entrapped in the compost matrix. Both hydroxyalkanoic and alkanedioic acids were released from compost by progressively stronger hydrolytic treatments and, thus, assumed to be chemically bound to the compost matrix (10). Also in the case of the thermochromolysis applied here, whereas the short-chain alkanedioic acids may result from biotic and abiotic

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**Table 3. Yields (Micrograms per Gram of Dry Weight)\(^a\) and Composition\(^b\) of Main Thermochromolysis Products Released from Compost Material at Different Compost Maturities**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Compost 60</th>
<th>Compost 90</th>
<th>Compost 150</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lignin Products</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(p)-hydroxyphenyl</td>
<td>1480</td>
<td>870</td>
<td>650</td>
</tr>
<tr>
<td>guaiacyl</td>
<td>3240</td>
<td>2920</td>
<td>2830</td>
</tr>
<tr>
<td>(\text{Ad/Al})(^c)</td>
<td>3.8 (0.3)</td>
<td>4.1 (0.2)</td>
<td>4.3 (0.3)</td>
</tr>
<tr>
<td>(\Gamma)(^c)</td>
<td>2.9 (0.2)</td>
<td>2.8 (0.2)</td>
<td>3.0 (0.1)</td>
</tr>
<tr>
<td>syringyl</td>
<td>2950</td>
<td>2710</td>
<td>2730</td>
</tr>
<tr>
<td>(\text{Ad/Al})(^c)</td>
<td>5.0 (0.2)</td>
<td>4.9 (0.3)</td>
<td>5.1 (0.2)</td>
</tr>
<tr>
<td>Fatty acids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(&gt;\text{C}_{20}) (%)</td>
<td>6.1</td>
<td>14.3</td>
<td>31.2</td>
</tr>
<tr>
<td>(\omega)-hydroxy acids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mid-chain hydroxy acids</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Alkanedioic acids</td>
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<td>n-Alkanes</td>
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<tr>
<td>Diterpenoids</td>
<td></td>
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</tbody>
</table>

\(^a\) \(n = 3\). Overall coefficient of variations: lignin lower than 10%; alkyl lower than 15%. \(^b\) Total range varying from C\(_{i}\) to C\(_{j}\); compounds in parentheses are the most dominant homologues; number after colon refers to double bond. \(^c\) Structural indices: Ad/Al = G6/G4, S6/S4; \(\Gamma\) = G6/(G14 + G15); standard deviation in parentheses.
oxidation of longer chain monounsaturated acids (25), the released longer chain molecules represent the building block of plant bio-polyesters (42, 43) and, thus, compost components strongly bound in an extensive network of intra- and intermolecular ester bonds. Therefore, the large range and high yield of methyl ether/ester derivatives of alkyl monomers released by thermochemolysis of compost (Tables 2 and 3) indicate the Pyr-TMAH-GC-MS technique is a rapid and effective alternative to the chemical stepwise fractionation for the molecular characterization of complex compost substrates.

The amount of components released by Pyr-TMAH-GC-MS decreased progressively with increasing maturity of compost samples. A much larger loss of alkyl compounds was noted by passing from compost 60 to 90 than from compost 90 to the final mature compost 150 (Table 3). However, the thermochemolysis products released from compost 150 were still dominated by alkyl compounds but with a different relative distribution (Figure 3).

Linear fatty acids and hydrocarbons decreased by 65 and 72% after 150 days of compost stabilization, respectively. The hexadecanoic and octadecanoic acids were most significantly affected by the composting process, whereas greater stability and, thus, detectability were shown by longer chain fatty acids, which were even increased by 25% relative to the total amount of FAME (Table 3). A small variation with composting was revealed by the overall n-alkane distribution, with a slightly higher preservation of the heaviest components.

A larger persistence with compost maturity was noted for the various products deriving from aliphatic bio-polyesters, as is revealed by the relative increase of signal intensity in the compost 150 pyrogram (Figure 3B). Total yields showed their partial decrease passing from compost 60 to 90, although about 70% of components found in compost 60 were still released from compost 150 (Table 3). For ω-hydroxy- and alkanedioic acids, the degradation was more selective for the monounsaturated C18 compounds, owing to the biochemical lability of the internal double bond, whereas lower losses during composting were shown by the long-chain saturated homologues. Despite the decrease of some minor components, an unexpected high amount of midchain hydroxyalkanoic acids was found in compost 150. This is in contrast with the reported intrinsic biodegradability of the midchain oxygen functionality of these compounds (44, 45).

The largest alkyl loss, which occurred between 60 and 90 days of composting (Table 3), is in line with the large decrease of alkyl carbons that was observed in NMR spectra of compost 90 (Table 1 and Figure 1B). This may be due to the decomposition of structurally unbound, and thus easily bioavailable, fatty acids and n-alkanes. The greater access of linear alkyl acids and hydrocarbons to microbial decomposition agrees with the fact that these components are preferentially extracted in organic solvents as unbound components (10, 35, 38).

The relative persistence of different alkyl bio-polyesters, at increasing compost maturity, is in agreement with previous findings on NOM. Both hydroxyalkanoic acids and alkanedioic acids are currently found among the main thermochemolysis products of persistent soil organic fractions such as humic acids and humin (25, 26). Moreover, the selective preservation of these hydrophobic molecules was recognized among the main factors leading to the accumulation of recalcitrant organic compounds in soil (18, 46). Our findings confirm the key role of hydrophobic compounds in the stabilization of complex natural organic matter, such as compost, during biological transformation.

No significant changes with compost maturity were found in amount and composition of tricyclic diterpenes and tetra- and pentacyclic triterpenes. Sterols and triterpenols were in fact among the main Pyr-TMAH-GC-MS products of compost 150 (Table 3). This confirms that triterpenoid and diterpenoid compounds may survive relatively unaltered the biological decay of dead tissues and represent useful biomarkers to trace molecular inputs of higher plants into NOM (40).

Our results indicate that the offline Pyr-TMAH-GC-MS technique is a rapid and effective method to obtain direct qualitative and quantitative evaluation of complex organic materials. This technique can be efficiently applied for the direct molecular characterization of bulk compost substrates, thereby replacing, with equal molecular resolution, a more lengthy, although detailed, stepwise chemical fractionation procedure. Plant biopolymers such as lignin, waxes, and aliphatic polyesters were recognized as the main sources of composted organic material. Moreover, the offline Pyr-TMAH-GC-MS technique provided a detailed evaluation of the compost molecular modifications during the maturity process and of the various lignin and alkyl biomarkers, which are useful to trace both the origin of compost substrates and the fate of compost in the environment.

**LITERATURE CITED**


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