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Evolution of Composting Process in Maize Biomass Revealed by Analytical Pyrolysis (Py-GC/MS) and Pyrolysis Compound Specific Isotope Analysis (Py-CSIA)

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Abstract: An innovative approach based on the combination of analytical pyrolysis coupled with gas chromatography-mass spectrometry (Py-GC/MS) with compound-specific isotope analysis (Py-CSIA) is used to study the composting process of maize biomass. This multidisciplinary approach aims to elucidate the decomposition rate of the main biogenic materials (lignin, cellulose, proteins, lipids, and waxes) responses to the composting process. According to Py-GC/MS data/structural composition, a noticeable and significant decrease during the first stage of the composting process of carbohydrates and aromatic compounds is found, followed by a gradual increase of all compounds till the end of the experiment. This trend, along with an increase of fatty acids methyl-ester at the first composting stage, sustains the microbial activity and its stabilization over time. Py-CSIA data showed a significant enrichment in ^{13}C in all identified compounds over time, supporting the semi-quantitative results and the decomposition of initial biomass throughout the composting process. This trend is also perceptible in lignin moieties, long-chain aliphatic structures, and isoprenoids, as highly recalcitrant compounds, presumably due to depolymerization and carbon translocation of side-chain molecules during the composting process. Compound-specific isotope values showed a good correlation with the bulk isotope data, and this served as validation of the technique. However, bulk values showed higher heterogeneity because those represent an average of all organic compounds in the sample. By combining isotopic and structural information using Py-GC/MS and Py-CSIA, we are able to provide further information and a more detailed approach to the study of the decomposition process of biomass by considering the diverse dynamics of the main biogenic compounds.

Keywords: compost; lignocellulose characterization; stable carbon isotopes; compound-specific $\delta^{13}C$

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Table S1. Pyrolysis products and relative abundance1 of biomass and compost samples taken at different times, yielded by Py-GC/MS.

RT (min)	T0	T1	T2	T3	T4	Compound	MW	ORIGIN[2]
2.44	0.87	1.84	2.99	0.91	1.50	1-Penten-3-one	84	PS
2.68	0.98	0.99	0.86	1.32	1.37	Pentanal	86	PS
2.77	1.32	0.39	0.62	1.32	0.61	1-methoxy-1,2-butadiene	84	PS
2.92*	2.86	2.41	2.89	2.38	3.98	Furfural	96	PS
3.04	2.07	0.67	1.17	1.68	0.96	2-Furanmethanol	98	PS
3.56	0.00	0.00	1.70	0.00	1.88	2(5H)-Furanone	84	PS
3.64*	5.79	1.23	2.49	2.54	3.52	1,3-Cyclopentanedione	98	PS
3.96*	2.18	0.97	1.04	1.24	1.50	2-Furancarboxaldehyde, 5-methyl-	110	PS
4.10	1.62	2.00	1.85	1.30	2.33	Phenol	94	ARO
4.31*	3.34	1.87	1.99	2.87	4.70	3-Methyl hydantoin	114	N
4.48	1.16	0.65	0.84	0.72	0.97	2-Cyclopenten-1-one, 2-hydroxy-3-methyl-	112	PS
4.61*	2.06	1.40	1.57	1.58	1.32	1,2-Cyclopentanedione, 3-methyl- (Isomer?)	112	PS
5.08	1.11	1.92	1.97	0.97	1.82	Phenol, x-methyl	108	LH
5.28*	2.07	2.64	2.92	2.38	3.35	Phenol, 2-methoxy- (Guaiacol)	124	LG
5.68*	1.43	0.54	0.82	1.57	1.30	4H-Pyran-4-one, 3-hydroxy-2-methyl- (Maltol)	126	PS
5.85	1.47	0.58	0.86	1.46	1.22	2H-Pyran-3(4H)-one, dihydro-6-methyl-	114	PS
6.13	2.44	1.23	2.32	1.19	1.69	Phenol, x-ethyl-	122	LH
6.31	0.70	0.00	0.77	1.63	0.51	2,3-Dihydroxybenzaldehyde	138	ARO
6.49*	0.77	1.38	1.48	1.11	2.86	Methylguaiacol	138	LG
6.68	2.32	0.69	0.00	0.95	1.48	4H-Pyran-4-one, 3,5-dihydroxy-2-methyl-	142	PS
6.78*	6.38	6.09	7.66	9.74	13.96	4-Vinylphenol	120	LH
6.96*	2.62	1.16	1.46	1.63	0.83	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	126	PS
7.42	1.23	1.80	2.45	1.43	2.55	1,2-Benzenediol, 3-methoxy-	140	ARO
7.55*	0.85	1.41	1.43	1.35	1.68	Ethylguaiacol	152	LG

7.78	0.00	1.57	1.18	0.00	0.00	Indole	117	N
7.87	1.73	0.00	0.00	1.23	1.06	1,2-Benzenediol, 4-methyl-	124	ARO
8.01*	5.31	3.66	4.33	5.56	6.39	4-Vinylguaiacol	150	LG
8.44*	1.37	2.52	2.88	2.11	3.61	Syringol	154	LS
8.53	0.52	0.63	0.46	0.83	0.62	Eugenol	164	LG
8.63	0.92	0.76	0.93	0.77	0.65	Isomer	154	LS
8.95	1.25	0.79	0.91	0.91	0.73	1H-Indole, 2-methyl-	131	N
9.10*	1.43	1.15	1.89	2.10	1.46	Vanillin	152	LG
9.16	0.00	0.64	0.00	0.00	0.00	Isomer eugenol (trans-Isoeugenol)	164	LG
9.61*	0.74	0.97	1.09	0.84	0.77	Methylsyringol	168	LS
9.68*	1.15	1.57	1.09	1.64	1.03	Isoeugenol	164	LG
10.16*	1.17	1.62	2.52	1.03	1.36	Acetoguaiacone	166	LG
10.50*	0.22	1.30	0.00	0.41	0.54	1,6-Anhydro-.beta.-D-glucopyranose (levoglucosan)	162	PS
10.52	0.50	0.00	0.54	0.37	0.70	Vanillic acid, methyl ester	182	LG
10.58	0.49	1.47	0.88	0.50	0.80	Ethylsyringol	182	LS
10.65	0.56	0.00	0.00	0.52	0.00	Lignin unknown compound #89*	164	LG
10.71	0.83	1.52	1.36	0.75	1.10	Guaiacylacetone	180	LG
10.91	1.67	0.99	1.30	1.09	0.99	Dodecanoic acid (FA12)	200	FA
11.04*	1.80	2.13	2.56	2.34	2.34	Vinylsyringol	180	LS
11.46*	0.68	0.84	0.96	0.70	0.51	Propenylsyringol (PS)	194	LS
11.89	0.85	0.69	0.00	0.52	0.42	Hexadecane, 2,6,10,14-tetramethyl-	282	ISO
12.03	0.68	0.79	1.09	0.91	0.43	Isomer of PS	194	LS
12.17	0.97	0.93	1.32	0.96	0.81	Syringaldehyde	182	LS
12.39	0.92	0.84	1.09	0.79	0.64	Propynylsyringol (PS2)	192	LS
12.47	0.00	0.67	0.80	0.50	0.37	Isomer of (PS2)	192	LS
12.58*	1.02	1.76	2.26	1.86	2.03	Isomer of PS	194	LS
12.80*	1.64	1.36	1.25	0.82	0.79	cis-2-Methyl-7-octadecene	266	ISO
12.98*	1.03	1.35	1.65	0.82	1.25	Acetosyringone	196	LS

13.12*	2.10	0.92	1.19	1.37	0.71	Tetradecanoic acid (FA14)	228	FA
13.35*	0.91	1.03	1.31	0.94	1.04	Syringylacetone	210	LS
13.95*	0.62	0.65	0.59	0.45	0.55	Neophytadiene	278	ISO
14.02*	0.77	0.57	0.53	0.37	0.26	2-Pentadecanone, 6,10,14-trimethyl-	268	ISO
14.08	0.57	0.59	0.65	0.43	0.18	2-Propenoic acid, 3-(4-hydroxy-3-methoxyphenyl)-, methyl ester	208	LG
14.39	0.68	0.54	0.46	0.37	0.12	Bicyclo[10.8.0]eicosane, (E)-	278	LIP
14.81	0.51	0.77	0.79	0.25	0.12	Hexadecanoic acid, methyl ester	270	FAME
15.18*	3.66	2.37	2.14	1.84	1.84	n-Hexadecanoic acid (FA16)	256	FA
16.50	0.44	0.65	0.26	0.20	0.14	Alk/Alk C21	296	Alk
16.72	0.47	0.77	0.00	0.31	0.06	Phytol/methyl stearate (sólo nov18)	296	ISO
16.86*	3.23	2.33	2.27	1.64	1.40	Octadecadienoic + Octadecenoic acid (FA18:2 FA18:1)	280	FA
17.04*	1.60	1.06	1.30	0.81	0.55	Octadecanoic acid (FA18)	284	FA
17.36	0.43	0.64	0.65	0.34	0.12	Alk/Alk C22	310	Alk
18.23*	0.64	0.47	0.38	0.32	0.16	Alk/Alk C23	324	Alk
18.62	0.51	0.81	0.63	0.59	0.42	Unknown (m/z 83, 280)	280	UNK
18.77*	0.56	0.40	0.37	0.75	0.13	4,8,12,16-Tetramethylheptadecan-4-olide	324	TOC
19.05	0.34	0.37	0.34	0.29	0.10	Alk/Alk C24	338	Alk
19.84*	0.31	0.40	0.32	0.34	0.16	Alk/Alk C25	352	Alk
20.09	0.25	0.32	0.32	0.44	0.07	Docosanoic acid, methyl ester	354	FAME
20.28	0.31	0.50	0.24	0.18	0.10	Unknown (m/z 93, 346)	346	UNK
20.38	0.36	0.82	0.40	0.55	0.11	Docosanoic acid (FA22)	340	FA
20.61	0.43	0.54	0.26	0.27	0.13	Alk/Alk C26	366	Alk
21.15	0.16	0.93	0.36	0.51	0.03	Tricosanoic acid (FA23)	354	FA
21.35*	0.45	0.78	0.41	0.62	0.26	Alk/Alk C27	380	Alk
21.58	0.17	0.89	0.44	0.41	0.09	Tetracosanoic acid, methyl ester	382	FAME
21.88	0.21	0.75	0.39	0.52	0.10	Tetracosanoic acid (FA24)	368	FA
22.05	0.33	1.08	0.72	0.63	0.18	Alk/Alk C28	394	Alk
22.75*	0.27	0.69	0.31	0.45	0.23	Alk/Alk C29	408	Alk

23.50	0.14	0.61	0.27	0.36	0.20	Alk/Alk C30	422	Alk
24.04	0.10	0.00	0.00	0.00	0.00	Stigmasta-4,7,22-trien-3.beta.-ol	410	EST
24.15*	0.14	0.75	0.30	0.79	0.09	.gamma.-Tocopherol	416	TOC
24.35*	0.21	0.75	0.30	0.54	0.24	Alk/Alk C31	436	Alk
24.43	0.17	0.52	0.21	0.33	0.16	Ergosta-4,6,22-trien-3.beta.-ol	396	EST
24.57*	0.31	0.77	0.24	0.70	0.00	Unknown (m/z 379, 394)	394	UNK
24.77*	0.32	1.08	0.23	0.97	0.12	Stigmasta-3,5-diene	396	EST
24.92*	0.16	1.32	0.41	1.04	0.09	dl-.alpha.-Tocopherol	430	TOC
26.17	0.01	0.84	0.26	0.82	0.11	5-Cholestene-3-ol, 24-methyl-	400	EST
26.46	0.02	0.41	0.10	0.35	0.07	Alk/Alk C33	436	Alk
26.55	0.01	0.60	0.18	0.52	0.10	Stigmasterol	412	EST
26.80	0.01	0.45	0.00	0.45	0.00	Ursa-9(11),12-dien-3-ol	424	EST
27.30	0.04	2.02	0.62	1.48	0.10	.gamma.-Sitosterol	414	EST
27.46	0.00	0.00	0.00	0.25	0.00	Stigmastanol	416	EST
27.93	0.01	0.34	0.05	0.31	0.00	.beta.-Amyrin	426	EST
28.71	0.01	0.32	0.15	0.39	0.00	Stigmasta-3,5-dien-7-one (Tremulone)	410	EST
29.36	0.00	0.21	0.11	0.37	0.07	Stigmast-4-en-3-one (.gamma.-Sitostenone)	412	EST

[1]Calculated as percentage of total ion chromatographic area. Average values of pyrochromatograms according to their compound families indicated in Table 1.

[2]PS: polysaccharides, LH: hydroxyphenyl-units from lignin, LG: guaiacyl-units from lignin, LS: syringyl-units from lignin, N: nitrogen-derived compounds, ARO: aromatics, UNK: unknown compounds, LIP: mid-chain lipids, FA: fatty acids, FAME: fatty acid-methyl ester, ISO: isoprenoids, ALK: *n*-alkanes, EST: sterols.

Table S2. Compound specific δ13C (‰) average values of compost pyrolysis products at different sampling time. Values are reported in ‰ [V-PDB]. Mean standard deviation is shown in brackets (n=3).

Origin[1]	T1	T2	T3	T4
PS	-16.1 (1.0) ^{a} *	-11.5 (1.2) ^{a}	-11.3 (1.6) ^{a}	-9.8 (1.3) ^{a}
N	-14.6 (0.73) ^{a}	-10.6 (0.55) ^{a}	-8.9 (1.3) ^{ab}	-8.0 (0.21) ^{ab}
LH	-18.9 (0.05) ^{ab}	-13.9 (0.87) ^{ab}	-13.9 (0.80) ^{ab}	-12.7 (0.57) ^{ab}
LG	-18.4 (1.1) ^{a}	-13.3 (0.80) ^{ab}	-12.4 (1.2) ^{a}	-12.5 (1.1) ^{ab}
LS	-18.6 (1.0) ^{ab}	-14.2 (0.45) ^{b}	-13.5 (0.50) ^{a}	-13.5 (0.55) ^{ab}
FA	-19.2 (0.93) ^{ab}	-16.0 (3.8) ^{b}	-15.0 (1.7) ^{b}	-14.0 (0.52) ^{bc}
ISO	-17.5 (2.0) ^{ab}	-14.7 (0.20) ^{b}	-14.0 (0.2) ^{ab}	--14.0 (0.29) ^{bc}
ALK	-25.7 (3.7) ^{b}	-25.6 (3.1) ^{b}	-22.8 (2.4) ^{b}	-21.4 (4.2) ^{c}
TOC	-19.1 (4.1) ^{ab}	-19.22 (11.1) ^{b}	-19.3 (7.8) ^{bc}	-15.2 (2.6) ^{bc}
UNK	-23.5 (4.4) ^{ab}	-15.01 (1.5) ^{b}	-13.8 (0.85) ^{ab}	-18.3 (2.2) ^{bc}
EST	-24.2 (1.0) ^{ab}	-23.79 (1.3) ^{b}	-16.3 (1.2) ^{ab}	-15.5 (1.2) ^{ab}

[1]PS: polysaccharides, N: nitrogen-derived compounds, LH: lignin hydroxyphenyl-units, LG: lignin guaiacyl-units, LS: lignin syringyl-units, FA: fatty acids, ISO: isoprenoids, ALK: *n*-alkanes, TOC: tocopherols, UNK: unknown compounds, EST: sterols. Treatments with the same letters indicate no significant differences (Kruskal-Wallis test, $p<0.05$, Dunn post-hoc) among compound families within the same sampling time.