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ARTICLE

Composition and Structural Characteristics of Humified Fractions during the Co-composting Process of Spent Mushroom Substrate and Wheat Straw

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A spent mushroom substrate (SMS) was mixed with wheat straw (WS) in three proportions, C1 (2:1), C2 (4:1), and C3 (6:1), and composted for 90 days in static piles with periodic turning to ensure adequate aeration. Samples from each pile were collected periodically (after 0, 30, 60, and 90 days), and the humic acid-like fractions (HAs) were isolated to determine their elemental composition (C, H, N, S, and O), acidic functional group (carboxylic and phenolic) content, and structural and functional characteristics using spectroscopic methods including ultraviolet−visible, Fourier transform infrared (FTIR), and fluorescence. The results of elemental and functional group analyses show that, with increasing time of composting, the N, O, and acidic functional group contents of HAs increase, whereas their C and H contents and C/N ratio decrease. The analysis of FTIR and fluorescence spectra shows that, with increasing composting time, the presence of aliphatic and polysaccharide-like structures in HAs decreases, whereas oxygenation, polycondensation, and polymerization increase. These results suggest that the chemical and structural characteristics of the HA fractions in the final composts resemble those typical of native soil HAs, which indicate that an adequate degree of maturity and stability is achieved after the end of composting. The results of the present study confirm that composting is an appropriate treatment to transform fresh organic matter (OM) in SMS into humified forms, thus enhancing their quality, agronomic efficiency, and environmental safety as a soil OM resource for application as soil amendment.

INTRODUCTION

Spent mushroom substrate (SMS) is a decomposed growing medium that results from mushroom growth in a blend of natural materials, generally represented by agricultural and animal wastes, such as wheat straw bedded horse manure, broiler chicken manure, cottonseed meal, gypsum, and other materials. In the past decade mushroom production increased up to around 60% in the world and 70% in Italy (1). Consequently, an adequate disposal method is needed for the high quantities of SMS generated in this agro-food industrial activity.

Recent proposals for waste policies in the European Union (EU) include the long-term goal of attaining a recycling society that uses wastes as a resource (2). In this sense, SMS represents a biowaste that may be recycled as an agronomically efficient organic amendment to restore, maintain, and/or improve the content of organic matter (OM) in soils. Furthermore, the European Thematic Strategy for Soil Protection calls for the

use of compost as one of the best sources of stable OM from which humus can be formed in soils (3).

However, fresh/immature organic wastes contain some components, such as inorganic solutes, which may inhibit plant growth (4), and unstable organic substances that would adversely affect the dynamics of soil OM (5). In addition, surface/groundwater resources may be threatened by runoff and/or leaching of soluble waste constituents of fresh SMS (6). Thus, the composting treatment of biowastes has become an attractive solution to enhance the agronomic efficacy and reduce the possible negative environmental impact by preventing the threat of their "eco-dumping" (2). Nevertheless, certain chemical and physical characteristics (low C/N ratio, poor physical structure) of fresh SMS could limit the efficiency of the composting process; thus, the use of a bulking agent that also provides organic C, such as wheat straw (WS), is expected to be a promising approach.

During composting, mineralization and humification processes transform the raw OM in the waste into a more stabilized material that contains the so-called "humic-like" substances (HS); the increasing presence of HS is suggested to be a reliable indicator of increasing stability and maturity achieved by OM

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during and at the end of the composting process (*7*, *8*). HS are the most important fractions of soil OM that promote soil fertility and soil protection from degradation (*9*). Thus, the quantity and adequate molecular and structural properties of HS-like components in the composted materials are believed to be of primary importance for the evaluation of the agronomic efficacy, environmental safety, and economic value of compost (*5*).

The EU recycling guidelines have the objective of introducing new kinds of wastes (i.e., SMS) to the compost industry; it is therefore relevant to evaluate the composting process of new specific substrates and/or mixtures and the quality and agricultural value of the final products (*2*). Furthermore, to strengthen user confidence and ensure the proper functioning of the recycling market, it is necessary to introduce common standards on biowaste treatment and compost quality (*10*).

The application of chemical analyses and advanced spectroscopic techniques able to evaluate satisfactorily the composting process and the compost quality can benefit compost research. Chemical methods such as elemental composition and functional group analyses and spectroscopy techniques such as UV−visible, Fourier transform infrared (FTIR), and fluorescence have been widely used to successfully characterize HS obtained from a variety of organic wastes (*11*) and composted materials (*12*−*17*) and to evaluate the effect of organic amendments on native OM in diverse types of soils (*18*−*21*). The suitability of the mentioned methods to assess the humification process during composting is also supported by previous works that combined them with other techniques such as thermal analysis (*22*−*24*), nuclear magnetic resonance, and molecular size chromatography (*25*−*27*).

Because organic wastes (and bulking agents) differ greatly depending on their source, physical characteristics, and chemical composition, specific studies are needed for each type of waste and possible mixtures. To our knowledge, no results are available in the current literature on SMS composting; thus, the objectives of the present study were (a) to investigate OM transformations during the composting process of three different mixtures of SMS and WS, (b) to evaluate OM stability and maturity degrees of the composted materials, and (c) to use various chemical and spectroscopic techniques to assess the quality of humic acid (HA)-like fractions formed during composting and present in the final compost for possible use as soil organic amendments.

MATERIALS AND METHODS

Substrates and Procedure of Composting. The experiment was developed in Rutigliano, Bari province, Italy, in the facilities of Fungo Puglia S.r.l., a company that mainly produces mushrooms (*Agaricus bisporus*) grown on various kinds of substrates. The SMS residue obtained at the end of the production had a relatively low C/N ratio; thus, to have an adequate composting process, an appropriate amount of an organic C source (WS) was added to increase the C/N ratio to 25−35. The main physical and chemical properties of the SMS and WS samples used in this study are shown in **Table 1**.

A static pile composting procedure was applied using three different ratios of SMS/WS (w/w) on a dry basis: C1 (2:1), C2 (4:1), and C3 (6:1). Furthermore, an adequate amount of urea was added in the C1 and C2 experiments to obtain the same initial content of N in the three piles. However, other types of available N sources can be used. For example, in a recent study (*28*) dried legume trimming residues have been used as N source.

After the components had been mixed in the mentioned proportions, trapezoidal piles (4.5 m long and 2.5 m high) were constructed, and water was added to provide the appropriate moisture content (about 65% of total weight). Temperature and moisture were checked daily in 10 different points of each composting pile to avoid losses of organic C and maintain the active microbial populations. The piles were turned periodically to provide adequate aeration and prevent anaerobic processes

Table 1. Chemical Properties (on a Dry Matter Basis) of Spent Mushroom Substrate (SMS) and Wheat Straw (WS)

	SMS	WS
pH	7.2 ± 0.1	6.8 ± 0.1
EC (dS/m)	4.8 ± 0.2	1.4 ± 0.1
dry matter (g/kg)	479 ± 1	944 ± 2
ashes (g/kg)	510 ± 2	95 ± 1
volatile solids (g/kg)	490 ± 2	904 ± 3
nitrogen (g/kg)	13.0 ± 0.3	4.4 ± 0.2
organic carbon (g/kg)	274 ± 5	526 ± 6
C/N ratio	21.1	119.5

in the composting mass. The process was considered to be completed after 3 months (90 days) according to the criteria established by Italian law (*29*).

At the beginning of the composting process (0 days) and after 15, 30, 60, and 90 days (end of the process), five subsamples were collected randomly from five sites of each pile along the whole substrate from the top to the bottom, avoiding the surface 5−8 cm. Composite samples were then prepared by mixing equal amounts of the five corresponding subsamples. The analytical results obtained allowed the evolution of the composting process to be evaluated and any possible deviation observed during the process to be corrected.

Extraction and Purification of HA-like Fraction. The HA-like fractions were isolated from the three substrates sampled during the composting process using a conventional procedure (*30*). In brief, samples were added with an alkaline solution (0.1 M $Na_4P_2O_7$ and 0.5 M NaOH) using a sample/extractant ratio of 1:20 (w/v), and the mixtures were shaken mechanically in capped plastic bottles under N_2 for 24 h at room temperature (293 ± 2 K). The alkaline supernatants were separated by centrifugation (9600*g* for 30 min) and collected after the extraction procedure had been repeated on each sample three times. The combined supernatants were then acidified (to pH ~1) with 6 M HCl, left for 24 h in a refrigerator to achieve a satisfactory coagulation of the HAs, and then centrifuged at 30400*g* for 20 min. The precipitated HAs were then purified by dissolution in 0.3 M KCl and 0.2 M KOH under N_2, centrifugation at 30400*g* for 20 min, and subsequent acidification (pH 1) of the supernatants with 6 M HCl. The obtained suspensions were left standing at room temperature for 24 h and then centrifuged at 30400*g* for 20 min. The purification procedure was performed three times on each sample. Finally, the precipitated HAs were recovered and suspended in distilled water, dialyzed until free of Cl^- ions, freeze-dried, and stored in plastic vials at room temperature inside a desiccator.

HA Analyses. The HA samples were heated overnight at 105 °C to determine the moisture content and then at 550 °C for 2 h to determine the ash content. The C, H, N, and S contents were measured in triplicate using a Fisons Instruments (model EA 1108) elemental analyzer. Oxygen content was calculated by difference: O% = 100 − (C + H + N + S) %. The Ba(OH)$_2$ method was used to determine the total acidity and the Ca(CH$_3$COO)$_2$ method to analyze the carboxyl group content, and the content of phenolic hydroxyl groups was then calculated by difference between total acidity and carboxyl group content (*31*).

The E_4/E_6 ratios were calculated as the ratio of absorbances measured at 465 and 665 nm on solutions of 3.0 mg of each HA in 10 mL of 0.05 M NaHCO$_3$, with the pH adjusted to 8.3 with 0.02 M NaOH (*32*). A Perkin-Elmer (Norwalk, CT) Lambda 15 UV−vis spectrophotometer was used.

The FTIR spectra were obtained in the range from 4000 to 400 cm^{-1} (peak resolution of 2 cm^{-1}) using a Nicolet 5PC FTIR spectrophotometer equipped with Omnic 1.2 software. The samples were prepared as pellets by pressing a mixture of 1 mg of HA and 400 mg of dried KBr (spectrometry grade).

Fluorescence spectra were obtained on aqueous, slightly alkaline solutions of HA at 100 mg/L after overnight equilibration at room temperature and adjustment to pH 8 with 0.05 M NaOH. Three-dimensional spectra in the form of excitation−emission matrix (EEM) plots were recorded using a Hitachi model F-4500 fluorescence spectrophotometer equipped with F-4500 system software for data processing. Emission and excitation slits were set at a 5 nm bandwidth, and a scan speed of 240 nm min^{-1} was selected for the emission monochromator. The wavelength emission range was from 300 to 600 nm, whereas the excitation wavelength was increased sequentially from 250 to 550 nm by 5 nm steps. The overall

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Table 2. Yield, Elemental Composition, and Atomic Ratios (on a Moisture- and Ash-free Basis) of Humic Acid-like Fractions Isolated from Composting Piles C1, C2, and C3 at Different Sampling Times (0, 30, 60, and 90 Days)

	yield (g/kg)	g/kg					atomic ratios		
		C	H	N	S	O	C/N	C/H	O/C
					Composting Pile C1				
0	145	588 ± 4	48 ± 1	51 ± 1	2 ± 0	311 ± 3	13.6	1.0	0.4
30	154	540 ± 1	47 ± 2	52 ± 1	2 ± 1	359 ± 4	12.1	1.0	0.5
60	174	540 ± 6	44 ± 2	63 ± 2	3 ± 1	350 ± 6	10.0	1.0	0.5
90	178	583 ± 3	40 ± 3	63 ± 1	4 ± 0	310 ± 8	10.8	1.2	0.4
					Composting Pile C2				
0	143	591 ± 2	45 ± 2	55 ± 1	2 ± 1	307 ± 3	12.6	1.1	0.4
30	172	566 ± 5	47 ± 1	55 ± 1	2 ± 0	330 ± 4	12.1	1.0	0.4
60	174	533 ± 4	44 ± 3	60 ± 1	2 ± 1	361 ± 3	10.3	1.0	0.5
90	176	577 ± 3	41 ± 1	61 ± 2	2 ± 1	319 ± 5	11.2	1.2	0.4
					Composting Pile C3				
0	149	607 ± 7	44 ± 2	55 ± 1	2 ± 1	292 ± 2	11.2	1.2	0.4
30	152	596 ± 3	46 ± 2	56 ± 2	2 ± 0	300 ± 5	12.4	1.1	0.4
60	172	518 ± 5	46 ± 1	59 ± 1	1 ± 0	376 ± 2	10.3	1.0	0.5
90	174	581 ± 2	43 ± 3	59 ± 0	1 ± 1	316 ± 7	11.5	1.1	0.4
soil[a]		562	47	32	8	355	20.5	1.0	0.5

[a] From Schnitzer (*33*).

relative fluorescence intensity (RFI) was expressed in arbitrary units as the unitless reciprocal to the gain used to normalize each emission spectrum (*12*).

RESULTS AND DISCUSSION

Yield and Elemental Composition of HAs. The extraction yield, elemental composition, and atomic ratios of HAs examined are shown in **Table 2**. The extraction yield values were similar for the three HA series and increased with time of composting, which indicated that the humification process progressively transformed the original organic matter to a more stable material enriched in HA fractions (*5*). Also, the C content in the three HA series showed a similar trend with composting time; that is, it decreased slightly until 60 days and then increased, reaching a value slightly lower than the initial one. The O content followed an opposite trend. The H content tended to decrease slightly, and the S content and C/H and O/C ratios showed no relevant variation. In general, the C, H, and O contents and C/H and O/C ratios were similar to the means calculated for several soils (*33*) and in the range of those measured for HAs from different agricultural soils (*18*, *19*, *21*).

The N content tended to increase slightly and the C/N ratio to decrease, indicating the incorporation of N compounds in HAs (*9*). These results agree with those of other authors that reported an enrichment in N compounds in HAs during composting of a variety of wastes and composts (*11*, *13*, *15*, *25*). The highest increase of N content (about 20%) was measured for C1 HAs isolated from the mixture containing the highest proportion of WS, that is, the highest content of lignin. This result is in agreement with humification theories (*9*) that hypothesize N incorporation in HS mainly occurring by condensation reactions of proteins and modified lignin and/or other N-containing compounds and quinones derived from lignin.

In general, the results obtained indicate the chemical stability achieved by HA fractions in the final compost and guarantee an efficient use of the compost as a soil amendment.

Functional Group Composition and E_4/E_6 Ratios. The acidic functional group contents and E_4/E_6 ratios of HAs examined are

shown in **Table 3**. These values fell within the range of soil HAs (*18*, *21*, *33*) with COOH contents being close to the lower soil HA contents and total acidity and phenolic OH group contents slightly higher than those of some soil HAs.

In general, total acidity and phenolic OH groups increased during composting, reaching their maximum values at the end of the process, whereas COOH group content increased up to 60 days, possibly due to the production of organic acids from easily decomposable compounds (*5*), and then decreased. Similar results have been reported for composted materials compared with the original raw organic wastes (*7*, *11*, *13*). The highest total acidity and contents of phenolic OH groups were measured in C1 HAs, the parent mixture of which contained the greatest proportion of WS and, thus, of lignin. These results are consistent with the natural humification process whereby the phenolic groups of HAs are mostly originated by oxidation reactions of methoxyl and alcoholic groups of lignin chains (*9*).

The E_4/E_6 ratio in the three HA series apparently decreased with composting time with an average reduction of about 30% (**Table 3**). A similar trend was ascertained for various composting wastes (*7*, *21*). However, the values measured at the end of composting were slightly higher than those shown by HAs extracted from different soil types (*18*, *33*). Decreasing E_4/E_6 ratios were related to increasing structural complexity and aromatic polycondensation HA macromolecules (*32*). Thus, our results suggest that composting is able to produce a progressive humification of OM in the SMS/WS mixtures, leading to condensation and aromatization processes of the HA molecules. Apparently, this trend was more evident in HAs from piles with a higher proportion of WS (C1 and C2), which confirmed the results obtained for the yield, elemental composition, and functional group contents.

FTIR Spectra of HAs. The FTIR spectra of the HAs examined are shown in **Figures 1**, **2**, and **3**. The assignments of FTIR absorptions are described in the following text according to the current literature (*9*, *34*, *35*).

In general, FTIR bands and peaks show a relative intensity that varied according to the time of composting. The broadband

Table 3. Acidic Functional Group Contents and E_4/E_6 Ratios (on a Moisture- and Ash-free Basis) of Humic Acid-like Fractions Isolated from Composting Piles C1, C2, and C3 at Different Sampling Times (0, 30, 60, and 90 Days)

	total acidity (mmol/g)	carboxyl COOH (mmol/g)	phenolic OH (mmol/g)	E_4/E_6 ratio [a]
		Composting Pile C1		
0	7.2 ± 0.1	1.9 ± 0.1	5.3	9.5 ± 0.4
30	7.3 ± 0.1	2.0 ± 0.1	5.3	8.9 ± 0.4
60	7.7 ± 0.2	2.6 ± 0.1	5.1	7.3 ± 0.5
90	8.4 ± 0.2	2.1 ± 0.0	6.3	6.2 ± 0.5
		Composting Pile C2		
0	6.9 ± 0.1	2.1 ± 0.0	4.8	9.2 ± 0.5
30	7.1 ± 0.1	2.2 ± 0.1	4.9	8.8 ± 0.4
60	7.4 ± 0.1	2.5 ± 0.1	4.9	7.7 ± 0.2
90	7.7 ± 0.2	2.2 ± 0.1	5.5	6.4 ± 0.5
		Composting Pile C3		
0	6.7 ± 0.1	2.2 ± 0.1	4.5	9.5 ± 0.5
30	7.2 ± 0.0	2.2 ± 0.1	5.0	8.8 ± 0.8
60	7.4 ± 0.1	2.4 ± 0.2	5.0	7.8 ± 0.5
90	7.6 ± 0.2	2.3 ± 0.1	5.3	6.7 ± 0.4
soil[b]	6.7	3.6	3.9	4.8

[a] Ratio of absorbances at 465 and 665 nm (*32*). [b] From Schnitzer (*33*).
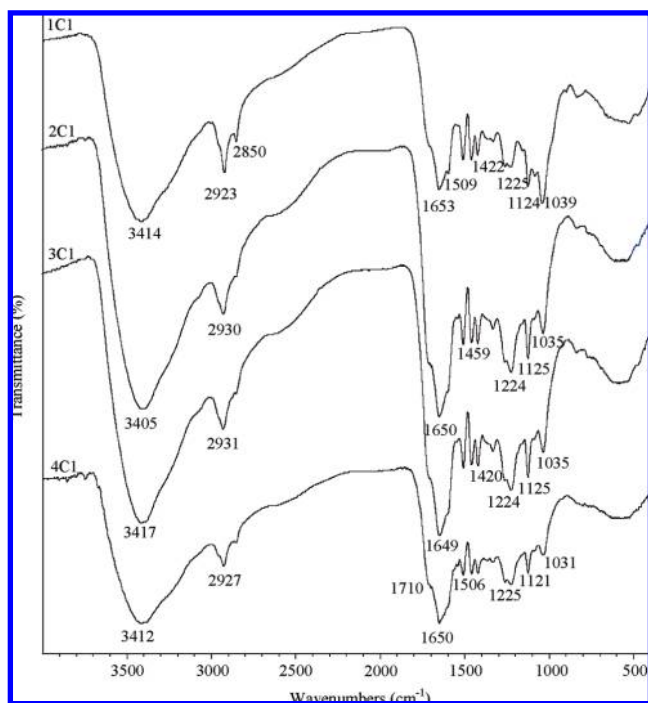

Figure 1. FTIR spectra of humic acid (HA)-like fractions isolated from the C1 pile after 0 (1C1), 30 (2C1), 60 (3C1), and 90 (4C1) days of composting.
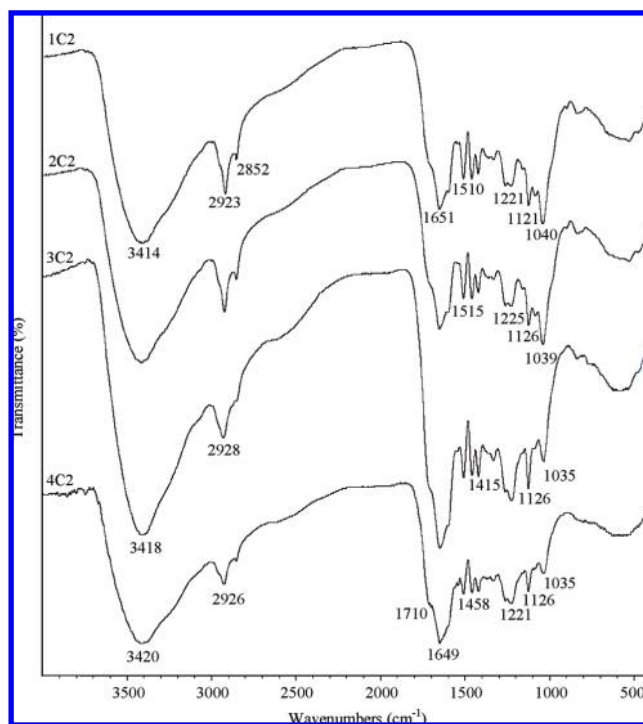

Figure 2. FTIR spectra of humic acid (HA)-like fractions isolated from the C2 pile after 0 (1C2), 30 (2C2), 60 (3C2), and 90 (4C2) days of composting.

at about 3410 cm^{-1}, ascribed to stretching of O−H groups and, secondarily, N−H groups of various functional groups, decreased with the time of composting. This may suggest the continuous and gradual loss of alcoholic functional groups through oxidation of methoxyl and alcoholic groups of lignin, as also suggested from results of functional groups analysis. The two peaks at about 2920−2930 and 2850 cm^{-1}, attributed to the symmetric and asymmetric stretching of aliphatic C−H groups, tended to become less intense with composting, which suggests the reduction of the aliphatic character of HAs. The slight increase in the relative intensity of the strong band at about 1650 cm^{-1}, ascribed to vibrations of aromatic C=C, C=O

stretching of amide groups (amide I band), quinonic C=O, and/or C=O of H-bonded conjugated ketone, confirms the increasing aromaticity and polycondensation of HAs, as also suggested by functional groups and E_4/E_6 ratio results.

Slight variations of intensity with composting time were observed (a) for the shoulder at about 1710 cm^{-1}, ascribed to COOH groups; (b) for the peak at about 1510 cm^{-1}, due to N−H deformation and C=N stretching of amides (amide II band); (c) for the peak at 1450 ascribed to the deformations of aliphatic C−H; (d) for the peak at 1420 cm^{-1}, attributable to O−H deformation and C−O stretching of phenolic OH; and (e) for the band at
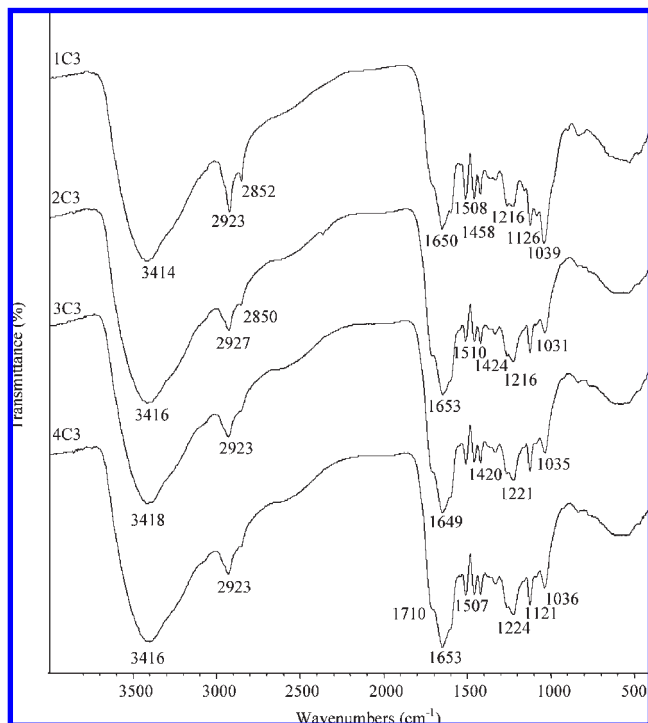
Figure 3. FTIR spectra of humic acid (HA)-like fractions isolated from the C3 pile after 0 (1C3), 30 (2C3), 60 (3C3), and 90 (4C3) days of composting.

Figure 4. Fluorescence excitation−emission spectra of humic acid (HA)-like fractions isolated from the C1 pile after 0 (1C1), 30 (2C1), 60 (3C1), and 90 (4C1) days of composting.

about 1225 cm^{-1} due to the stretching of C─O and O─H deformation of COOH groups. The relative intensity of the peak at about 1125 cm^{-1}, generally attributed to aliphatic CH$_2$, OH, or C─O groups, and that at about 1040−1030 cm^{-1}, ascribed to the stretching of C─O of polysaccharides or polysaccharide-like substances, tended to decrease during the composting time. These results would confirm those of chemical analyses suggesting the progressive degradation of easily decomposable compounds with the relative increase of more complex structures in HAs as composting proceeds.

The evolution with composting time of the FTIR spectra of HAs from C1 and C2 mixtures was characterized by slightly higher variations in the relative intensity of absorbances with respect to those of C3. These results suggest that HAs in the former mixture experienced a greater evolution during composting, leading to more advanced humification and stabilization. Results obtained confirm the suitability of FTIR spectroscopy in monitoring the humification process during composting as previously shown for other types of wastes (*13*−*15*, *21*, *22*, *26*, *27*).

Fluorescence Spectra of HAs. The EEM spectra of the HAs examined (**Figures 4**, **5**, and **6**; **Table 4**) show that the excitation/emission wavelength pairs (EEWPs) tended to increase toward the end of composting, whereas the RFI of the main EEM peak tended to increase. Recent works on HA fractions from various composts (*13*, *14*, *20*), organic wastes, and soils (*17*, *21*) provided evidence of the relationship between high wavelength values with elevated fluorescence intensities and the presence of linearly condensed aromatic systems in the HA macromolecules. On the contrary, relatively low wavelength values may be ascribed to the presence of simple structural components with low aromatic polycondensation degree and limited content of conjugated chromophore groups (*12*). On these bases, results obtained here confirm those of chemical analyses and FTIR spectroscopy in suggesting the greater molecular complexity and stability
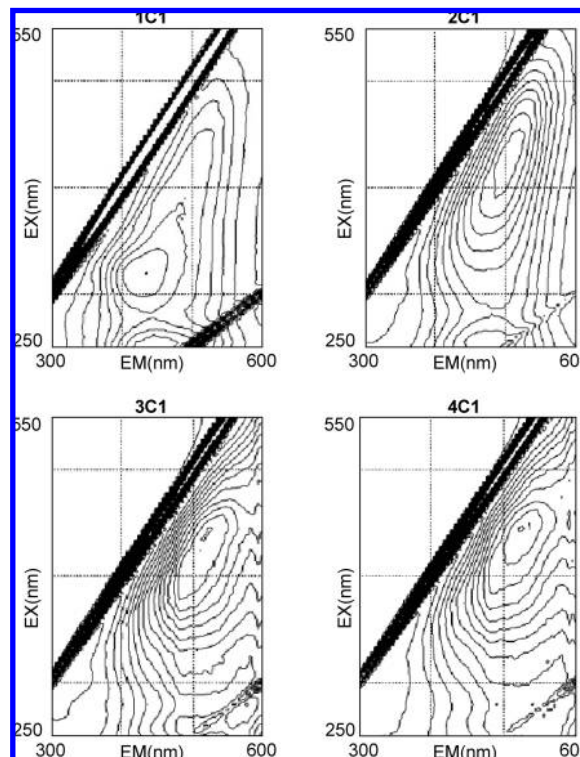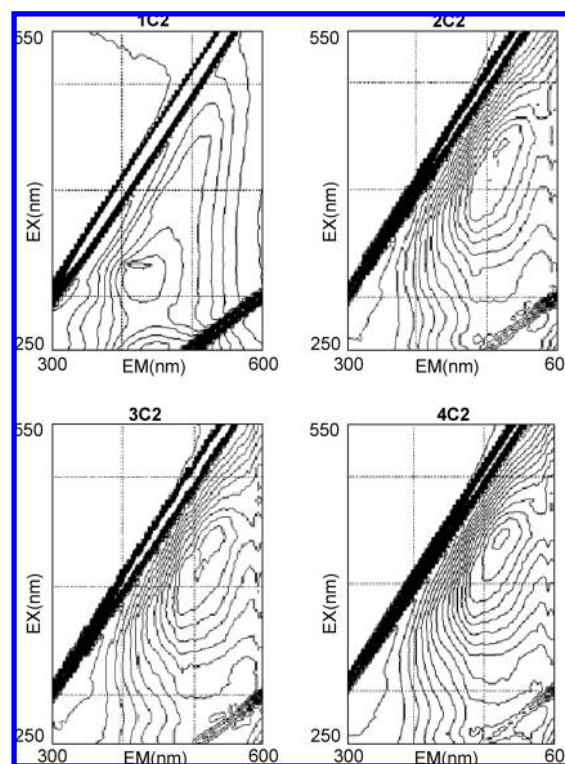

Figure 5. Fluorescence excitation−emission spectra of humic acid (HA)-like fractions isolated from the C2 pile after 0 (1C2), 30 (2C2), 60 (3C2), and 90 (4C2) days of composting.

achieved by HAs in the final compost. Furthermore, fluorescence features of HAs in SMS/WS composts resemble those typical of native soil HAs (*16*, *17*, *33*).
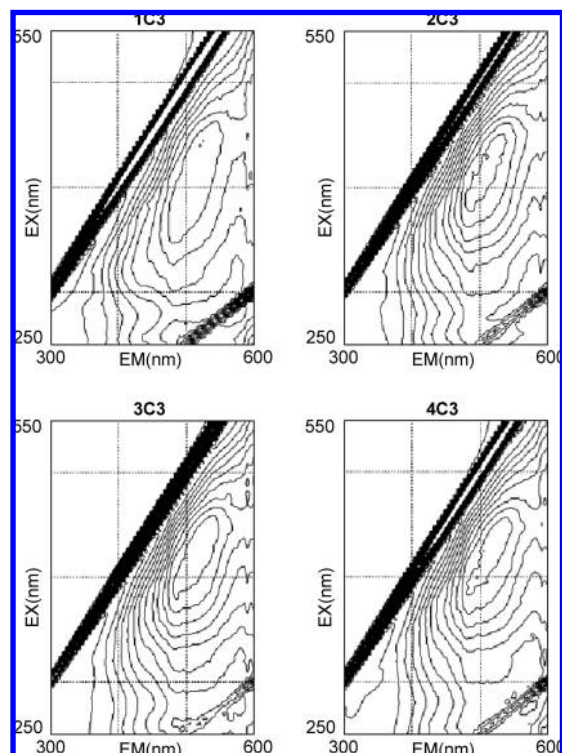
Figure 6. Fluorescence excitation−emission spectra of humic acid (HA)-like fractions isolated from the C3 pile after 0 (1C3), 30 (2C3), 60 (3C3), and 90 (4C3) days of composting.

Table 4. Excitation/Emission Wavelength Pairs (EEWPs) and Relative Fluorescence Intensity (RFI) Values of Humic Acid-like Fractions Isolated from Composting Piles C1, C2, and C3 at Different Sampling Times (0, 30, 60, And 90 Days)

	EEWP $\lambda_{ex}/\lambda_{em}$ (nm)	RFI (arbitrary units)
	Composting Pile C1	
0	320/435	40.1
30	430/505	60.0
60	425/435	80.0
90	445/525	71.0
	Composting Pile C2	
0	330/415	43.1
30	445/525	76.0
60	445/525	72.7
90	445/525	87.5
	Composting Pile C3	
0	440/520	55.4
30	395/500	66.9
60	425/515	68.0
90	445/530	68.4

Conclusions. Various chemical and spectroscopic analyses of the HA-like fraction are proposed as helpful indicators to monitor the evolution and transformation of OM during the composting process of different SMS/WS mixtures. The development of analytical techniques, such as quality/maturity evaluators for composted materials like those used in the present study, will stimulate their demand and acceptability.

The results obtained show that the time of composting determines an increase of the molecular complexity of HAs by decreasing the aliphatic and polysaccharide components while increasing the oxygenated group content and the aromatic poly-condensation degree. A higher maturity degree was achieved in the pile with a lower content of SMS and a 90 day co-composting period, which appears to be satisfactory to stabilize the OM in SMS/WS mixtures and to obtain a mature compost.

The humification degree achieved by the HA-like fractions in the composted materials resembles that of native soil HA, which increases the value of the compost as a soil organic amendment, minimizing possible environmental hazards. These findings will permit EU and other international guidelines to be established for the safe recycling of the large amounts of SMS that are continuously and increasingly produced in the edible mushroom industry.

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