Characterization of humic acids produced during composting of olive mill wastes: elemental and spectroscopic analyses (FTIR and ^{13}C-NMR)

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Agronomie 23 (2003) 661–666

DOI: 10.1051/agro:2003042

Original article

Characterization of humic acids produced during composting of olive mill wastes: elemental and spectroscopic analyses (FTIR and ^{13}C-NMR)

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(Received 1st October 2002; accepted 16 May 2003)

Abstract – The purpose of this study is to follow the evolution of the humic acids generated during olive mill wastes composting, by elemental analysis and spectroscopic methods (Fourier Transform Infra-Red: FTIR and Nuclear Magnetic Resonance: ^{13}C-NMR), in order to determine the stability and the maturity of the final compost. During composting, the humic acids presented a decrease of the peptidic and aliphatic structures and an increase of the aromatic structures. This confirms the stability of the final product after 12 months of composting. It results from this study that the quality of the humic substances generated could be an useful indicator to evaluate the maturity of the final product and to assess its agronomic value.

humic acid / olive mill waste / composting / elemental analysis / spectroscopic characterization

Résumé – Caractérisation des acides humiques lors du compostage des déchets d'huileries d'olive : analyse élémentaire et spectroscopique (IRTF et RMN-^{13}C). Cette étude a pour but de suivre l'évolution des acides humiques lors du compostage des déchets d'huileries d'olive par l'analyse élémentaire et par les techniques spectroscopiques (Infrarouge à Transformée de Fourier : IRTF et Résonance Magnétique Nucléaire du carbone : RMN-^{13}C) en vue de déterminer la stabilité et la maturité du compost final. Ces résultats mettent en évidence, qu'au cours du compostage, les acides humiques sont caractérisés par une réduction des structures peptidiques et aliphatiques qui s'accompagne d'une augmentation des structures aromatiques. Ceci confirme la stabilité du compost final après 12 mois de compostage. Il résulte de cette étude que la connaissance de la qualité des substances humiques pourrait être un indicateur supplémentaire pour évaluer la maturité du produit final et pour déterminer sa valeur agronomique.

acide humique / déchet d'huileries / compostage / analyse élémentaire / caractérisation spectroscopique

1. INTRODUCTION

The disposal of olive mill wastes (solid cake: marc and liquid effluent: olive mill wastewaters (OMWW)) leads to environmental problems in olive oil producing areas. In Morocco, the problem is particularly important owing to the scale of production: 500 000 ha are covered with olive groves, representing 55% of the area used for commercial orchards and groves [7]. Olive oil production reaches 48 000 tonnes per year [7]. There is a corresponding quantity of OMWW and olive marc: the pressing of 100 kg of olives (with added water during process extraction) produces on average 60 kg of olive marc and 100 to 240 kg of OMWW [2]. These wastes are an important source of pollution owing to its relatively high content of organic matter in the form of sugars, tannins, polyphenols, polyalcohols, pectins and lipids [12] in addition to lignin, cellulose and the pectin remaining in the olive marc.

Recycling of the wastes by composting is a promising solution [1]. It enables the nutrients to be returned to the soil and avoids the problems involved with spreading the wastes directly on the land [5]. But, evaluating the maturity of the composts is still an open question [26]. Numerous biological, microbiological and physico-chemical techniques have been developed to characterize the agrochemical properties and the maturity of compost. Recently, compost maturity has been investigated via the humification characteristics. Sequi and Benedetti [23] proposed the use of the humification index, the humification rate and the degree of humification. Tomati et al. [26], suggested monitoring the molecular weight of the humic substances as an index of stability. These parameters may be

Communicated by Gérard Guyot (Avignon, France)

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Table I. Elemental composition, atomic ratios, ash and E_4/E_6 ratio of the humic acids extracted from the olive marc-straw-OMWW compost at various stages of maturation.

Composting stage	% C	% N	% H	% O	% Ash	Atomic ratios C/N	C/H	O/C	E_4/E_6
T_1	49.32	2.91	6.53	30.27	10.97	19.80	0.63	0.47	3
T_3	44.51	3.53	5.46	36.11	10.35	14.70	0.68	0.61	6
T_6	43.00	3.37	5.22	37.27	11.24	14.82	0.68	0.65	6
T_9	43.27	3.52	5.11	37.53	10.57	14.35	0.71	0.65	6
T_{12}	43.12	3.60	5.15	36.18	11.94	13.96	0.70	0.63	8
Mean values of reference humic acids [21]	56.20	3.20	4.80	35.50	-	20.50	1.00	0.50	5

good indicators of the progression of the humification processes but do not provide sufficient data concerning the composition of the humic substances.

The aim of the present study was to follow the variations occurring in the humic acids during the composting of olive mill wastes by elemental analysis, the E_4/E_6 ratio and by FTIR and ^{13}C-NMR spectroscopy.

2. MATERIALS AND METHODS

A mixture of 280 kg of olive marc, 20 kg of straw and 85 kg of OMWW was composted in a heap on a platform. Over the one-year study period, the heap was forked over every fortnight to aerate the mixture. The microbiological activity was assessed by measuring the temperature of the heap.

Elemental and spectroscopic analyses were carried out on samples of humic acids extracted from the olive mill wastes-straw mixture after composting for one month (T1), three months (T3), six months (T6), nine months (T9) and twelve months (T12).

2.1. Extraction of the humic substances

Samples of 60 g of compost were treated with 100 mL 0.1M NaOH under stirring for 2 h and centrifuged for 25 min at 5000 rpm. This operation was repeated until the supernatant was obtained clear. To separate the humic acids, the pooled supernatants were then treated with 3M H_2SO_4 (pH 2) for 24 h at 4 °C. The humic acids were recovered after being dissolved in 0.1M NaOH, dialysed using a Spectra Por MWCO (7500 daltons) membrane to eliminate excess salts and freeze dried for 72 h in a LABCONCO apparatus.

2.2. Elemental analysis (CHON)

Elemental analysis was performed on freeze-dried samples of humic acids (0.4 to 1 mg) using a Fisson E. A 1.04 Carbograph Auto-analyser. The data were recorded with Eager 200 software. The oxygen content was given directly by the apparatus and not calculated by deduction from the total of the other elements.

2.3. E_4/E_6 ratio

The E_4/E_6 ratio, i.e. the ratio between absorbance at 465 and 665 nm was determined from the optical density of 2 mg samples of humic acids dissolved in 25 mL 0.025M $NaHCO_3$ [6].

2.4. Spectroscopy

2.4.1. Fourier Transform Infra-Red (FTIR)

Samples of freeze dried humic acid were further dried at 105 °C for 72 h. A portion of 1.5 mg of each sample was ground with 300 mg KBr and compressed under vacuum for 10 min. The pellets were analysed with a PERKIN-ELMER 1600 series FTIR spectrophotometer from 4000 to 400 cm^{-1}.

2.4.2. ^{13}C Nuclear Magnetic Resonance (^{13}C-NMR)

Freeze-dried humic acid was taken up in 3 mL of 0.5 M NaOD. The ^{13}C-NMR spectra were recorded on a BRUKER WB-AM 300 spectrometer at 75 MHz in 10 mm diameter tubes. The chemical shift (δ) is expressed in ppm with reference to an external standard, 3-(trimethylsilyl)-propane sulfonic acid (TMPS). To suppress increases in signal intensity due to the nuclear Overhauser effect, an impulse program with "gated" decoupling was used. The spectra were recorded in the following conditions: impulse duration 5 µs (35°), repeat time between two impulses 2.8 s, spectral bandwidth 16 700 Hz, peak broadening due to exponential multiplication 50–100 Hz, number of accumulations 50 000 to 60 000 i.e. a total acquisition time of about 48 h. Integration of the spectra was performed with Win NMR software (Bruker).

3. RESULTS AND DISCUSSION

3.1. Elemental analysis and E_4/E_6 ratio

The elemental composition, the atomic ratios, the amount of ash and the E_4/E_6 ratios of the humic acids extracted from the olive mill wastes compost at different stages of maturity are given in Table I. The level of ash remained very similar throughout composting and close to that of reference humic acids (Tab. I). Note however, that the amount of ash is dependent on the conditions of extraction and purification [4]. The

levels of C and H decreased as composting progressed while the level of O increased and N remained more or less constant. So, the C/N ratio decreased and the C/H and O/C ratios increased.

All the changes in these parameters occurred during the first three months, which correspond to the end of the phase of stabilisation and the start of maturation. During maturation, the molecules which had been degraded by the intense microbiological activity of the first phase, become reorganised to form more condensed compounds, richer in aromatic components. Effectively, Senesi and Brunetti [24] have reported, for humic acids extracted from other types of wastes (sludge, vermicompost), that with increasing composting time and compost maturity, there is loss of aliphatic groups as more stable humic acids are formed. This was supported by the studies of Garcia et al. [9] which attribute the increase of the C/H ratio to the higher levels of aromatic compounds formed by composting.

The E_4/E_6 ratio is related to the molecular weight and the oxygen content of the humic substances [6]. The results reported in Table I are close to those found in the literature for vermicompost humic acids [25]. Our results indicate that the humic acids extracted from olive mill wastes have a low molecular weight and a high proportion of carboxylic acids. This can be accounted for, according to Garcia et al. [9], by the occurrence of an oxidation process characteristic of humification.

Comparing humic acids from the mature olive mill wastes compost (at T12) to reference soil humic acids [21] (Tab. I), shows that olive mill wastes humic acids present lower levels of C, while the level of O is similar and the levels of N and H are slightly higher. The olive mill wastes compost therefore presented humic acids with lower C/N and C/H ratios while the O/C and E_4/E_6 ratios were higher than the reference soil humic acids (Tab. I).

In comparison to humic acid parameters reported for other composts of various origins such as sludge, manure or municipal waste [24], those of mature olive mill wastes compost were very similar.

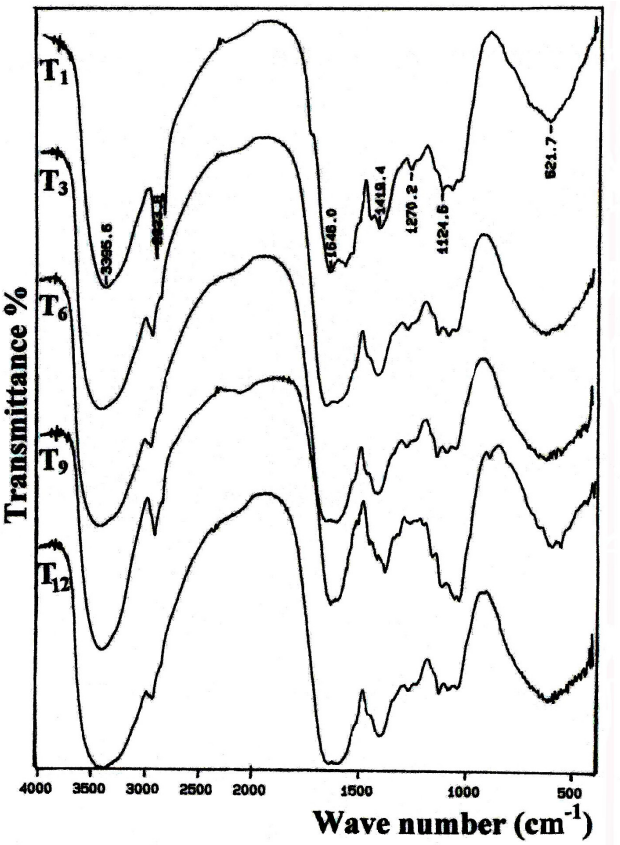

Figure 1. FTIR spectra of the humic acids extracted from the olive marc-straw-OMWW compost at various stages of maturation.

3.2. Spectroscopic analysis

3.2.1. FTIR spectra

The analysis of the FTIR spectra of the humic acids extracted from the olive mill wastes compost at different stages (T_1, T_3, T_6, T_9 and T_{12}) (Fig. 1) was based on the studies of Senesi and Brunetti [24], Galli et al. [8], Senesi et al. [25] and Ouatmane [17], relating to humic acids extracted from various organic wastes. This led to the attribution of the main absorption bands presented in Table II. A comparison of the infra-red spectra of the humic acids from the different stages of composting shows that the spectra are qualitatively similar, presenting the same overall pattern. The changes that did occur during composting, concern only the intensity in the absorption bands. This was certainly due to the chemical modifications occurring during the composting process [17]. The bands at 3420–3400 cm^{-1} increased while those at 2925, 2868–2850, 1460–1440 and 1400–1380 cm^{-1} were strongly attenuated during composting indicating a reduction of the aliphatic content. Similar results were reported by Senesi and Brunetti [24]. Meanwhile, the aromatic character became enhanced. The changes observed mainly occurred during the first three months, after which the general pattern of the spectra changed little. Overall, the changes that occurred indicate that there was loss of nitrogen-containing functions, of alkyl chains and of carbohydrates, which are used by the microorganisms during the first phase of composting to generate more condensed molecules. These results support the elemental analysis findings. Similar variations have been reported by Garcia et al. [10] and Gerasimowiez and Byler [11] for the infra-red spectra of the humic acids in composted sludges.

3.2.2. ^{13}C-NMR spectra

The ^{13}C-NMR spectra of the humic acids from the various stages of the olive marc-straw-OMWW compost are presented in Figure 2. These spectra show numerous signals in the region of aliphatic carbons (0–105 ppm), aromatic carbons (105–160 ppm), and carbons in carboxyl and amide functions (160–200 ppm).

In the aliphatic carbon region, the signal at 16.5 ppm is characteristic of a terminal methyl group. The signals at 20 to 32 ppm are attributed to methylene groups in long-chain fatty acids [18] while the signal at 37 ppm is originated from $(-CH_2)_n$

Table II. Attributions of the infra-red peaks of the humic acids extracted from composted olive mill wastes.

Wavelength	Attribution
3300–3500 cm^{-1}	O-H vibrations of the hydroxyl groups of phenols, alcohols and carboxyl functions and N-H vibrations from amides and amines.
2925 cm^{-1}	Symmetric CH stretching in $-CH_3$ and $-CH_2-$ of aliphatic chains.
2840 cm^{-1}	Asymmetric CH stretching in $-CH_3$ and $-CH_2-$ of aliphatic chains.
1725–1710 cm^{-1}	C = O stretching in carboxylic acids and/or in carbonyls, ketones and aldehydes.
1654–1640 cm^{-1}	C = O stretching in quinones and/or in ketonic acids and primary amides.
1540–1510 cm^{-1}	Aromatic C = C stretching and/or N-H deformation and C = N stretching in secondary amides.
1460–1440 cm^{-1}	Aliphatic C-H deformation of structures such as fatty acids and waxes occurring in composts.
1400–1380 cm^{-1}	O-H deformation, C = O stretching of phenols, anti-symmetric COO- stretching and aliphatic C-H deformation.
1260–1200 cm^{-1}	C-OH stretching of aromatic groups and C-O-C stretching of aryl ethers and phenols.
1170 cm^{-1}	Alcohol function vibrations.
1080–1030 cm^{-1}	C-O-C stretching of carbohydrates.
800–817 cm^{-1}	C-H deformation of substituted aromatic groups.

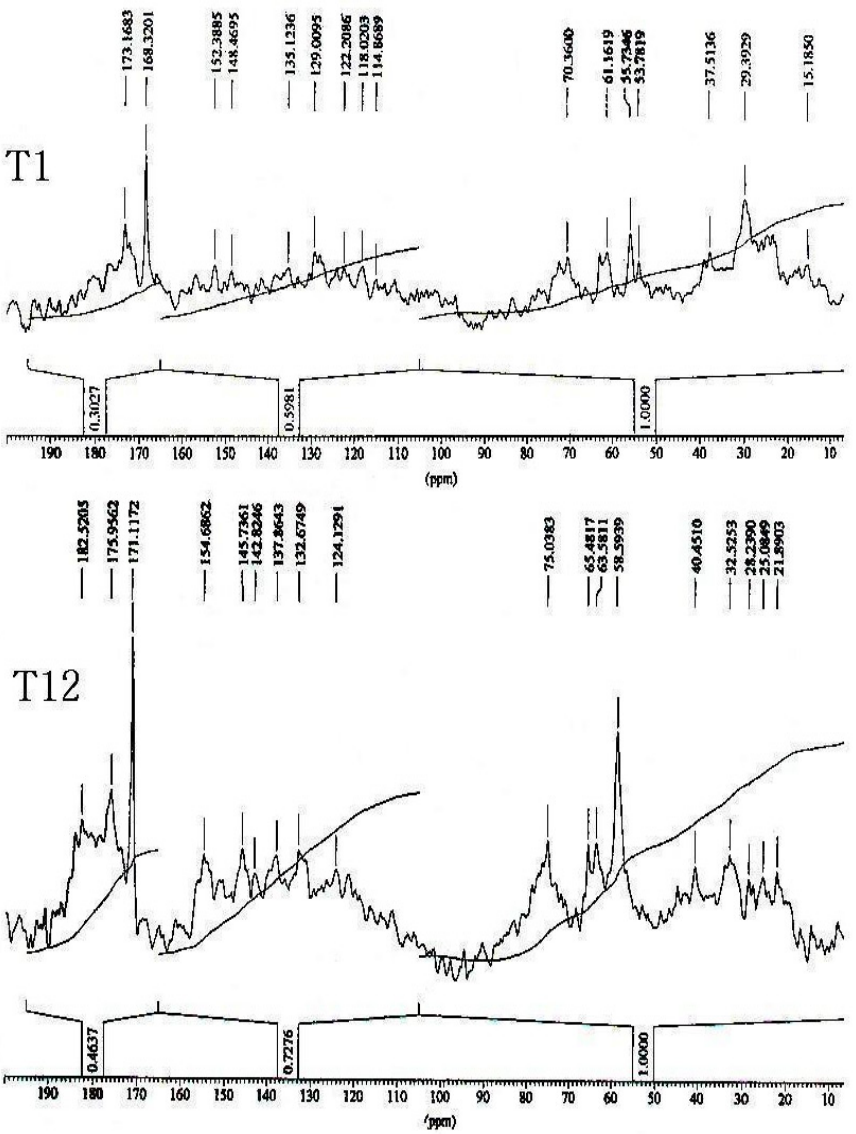

Figure 2. ^{13}C-NMR spectra of the humic acids extracted from the olive marc-straw-OMWW compost (T_1 = 1 month and T_{12} = 12 months).

in long alkyl chains [14]. Ouatmane [17], attributed the occurrence of the latter peak to humic acids containing branched or long-chain aliphatic structures. In the present case of olive mill wastes treatment, the aliphatic structures were much more abundant in the first month of composting. The resonances observed between 40 and 105 ppm are from carbohydrates with oxygen-containing substituents (quinines, ketones, aldehydes and alcohols) and amino acid chains containing glycine (α 42.6 ppm), arginine (δ 41.5 ppm) and lysine (ε 40.0 ppm) [3]. However, the signals at 66, 70 to 80, 89 and 105 ppm are due to the presence of the β-D glucopyranose unit of cellulose [13]. In this region, the signal at 55 ppm is due to aromatic methoxy groups (-O-CH_3) [20]. This peak was particularly intense during the first month of composting.

In the region of aromatic carbons, the spectral range covered by the resonances indicates the variety of the substituents on the aromatic rings. Signals between 115 and 130 ppm are from strongly protonated aromatic structures. Resonances between 130 and 147 ppm suggest carbon substitution of aromatic rings [22]. Thus, the signals at 118 to 130 ppm are due to non-substituted aromatic carbons [19] while the peak at 150 ppm is attributed to phenols and other aromatic rings substituted with carbon, oxygen or nitrogen. Similarly, the signals at 150–160 ppm are attributed to phenols but also to nitrobenzenes and aromatic ethers. Carboxyls, amides and esters give an intense signal at 175 ppm [17]. The strength of this peak reached a maximum at the 12th month of composting which could [17] be related to an increase in the levels of nitrogen of the humic acids from olive mill wastes.

Compared to the spectra of the reference humic acids [25], those studied here present more abundant aliphatic C and low levels of aromatic C. The spectra of the humic acids from composted olive mill wastes are very similar to those from manure [25], presenting a similar signal at 56 ppm corresponding to a methoxy carbon, low proportions of aromatic carbon and high levels of carboxyl and alkyl C-O groups. This suggests the presence of carbohydrates incorporated in the tannins or lignins [25].

Differences between the humic acids are assessed by measuring the relative intensities of the resonance signals in the different ranges of chemical shift (aliphatic, aromatic, carboxylic). The distribution of the signal intensities was measured with WinNMR software. The calculation of the area under the peaks in the aliphatic and aromatic regions (Tab. III) confirms the findings of the infrared and elemental analyses and shows that aromatic carbons were most abundant in the humic acids extracted after 12 months of composting.

Table III. Distribution of carbon between aliphatic and aromatic forms and calculation of the degree of aromaticity of the humic acids extracted from composted olive mill wastes after 1 and 12 months of composting. The data come from the integration of the peaks in Figure 2.

Stage of composting	% aliphatic carbon (A) δ = 0–105 ppm	% aromatic carbon (B) δ = 105–165 ppm	Degree of aromaticty B/A+B
T_1 (1st month)	52.6	31.5	0.3
T_{12} (12th month)	45.6	33.2	0.4

Overall, the results obtained from elemental analysis and spectroscopy (FTIR and ^{13}C-NMR) concur to indicate that aliphatic structures diminish and aromatic structures are enhanced in humic acids extracted after 12 months of composting of the olive marc-straw-OMWW. Indeed, the IR data showed a reduction of the peak at 2900 cm^{-1} (aliphatic C-H stretching), corresponding to a decrease in methylenes (-CH_2–) and terminal methyls (-CH_3). This reduction was supported by ^{13}C-NMR data which revealed a drop in the signals at 20–32.5 and 16.5–19 ppm attributed to methylene and terminal methyl groups respectively. Further support is provided by the strong resonance of the peak at 55 ppm and also by the increase of the O/C ratio from the elemental analysis data. Kakezawa et al. [15], attributed the increase of this ratio to oxidation of the phenolic compounds and/or the aliphatic chains. Similarly, elemental analysis also showed an increase in the C/H ratio during composting which has been interpreted as [15] being due to the formation of unsaturated bonds suggesting the start of a condensation and/or polymerisation process.

Likewise, the increase in E_4/E_6 ratio indicates a high proportion of carboxylic functions. A strong ^{13}C-NMR band at 160–190 ppm, characteristic of carboxyl groups confirmed this. In this region, the strong resonance of the peak at 175 ppm could be correlated to the increase in the N/C ratio found by elemental analysis indicating [16] the formation of humic nitrogen from the nitrogen of carbohydrates and alpha-amino groups.

4. CONCLUSION

The humic acids extracted from olive mill wastes compost at various stages of maturation were characterized by elemental analysis, the E_4/E_6 ratio and by FTIR and ^{13}C-NMR spectroscopy. The results indicate the high degree of humification of these acids as composting progresses. After 12 months of composting, the humic acids showed greater abundance of aromatic structures while the levels of aliphatic molecules were strongly decreased. This indicates that the compost had reached a mature and stable stage as confirmed by germination tests carried out in the same product in later studies.

Acknowledgements: This work received financial support form JER 6013 in association with the *Agence Universitaire Francophone* and PRAD 99/10 (*Projet de Recherche Agronomique et Développement franco-marocain*).
We thank Pr. A. Ouadia from *Gueliz Faculté des Sciences et Technique*, University Cadi Ayyad, Marrakech, for his help in freeze drying the samples.

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