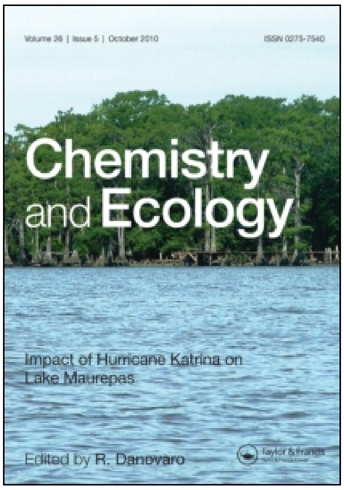

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Characterization of fulvic acids during olive mill waste composting (Elemental, Thermal and fluorescence analyses)

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Elemental analysis, fluorescence spectroscopy and differential scanning calorimetry (DSC) were applied to the study of fulvic acids isolated from different stages during olive mill waste composting. The fulvic extracted acids are characterized by a high nitrogen content and O/C ratio values that may result from the high degree of humification and the synthesis of more condensed humic complexes. This was confirmed by fluorescence spectroscopy in the synchronous-scan mode by the decrease of shoulder intensities at intermediate wavelengths indicating the increase of polycondensation and conjugation of unsaturated structures and the greater uniformity of fluorophores. Fluorescence spectra in the emission, excitation and synchronous modes became simpler with compost maturation. This was confirmed by DSC results which proved the high degree of polycondensation of aromatic nuclei of fulvic acid molecules during olive mill waste composting.

Keywords: Fulvic acids; Olive mill wastes composting; Elemental analysis; Fluorescence spectroscopy; Differential scanning calorimetry

1. Introduction

Olive oil production generates significant amounts of by-products, mainly solid residues, composed of olive husk, and liquid residues, commonly named olive mill waste water. Olive oil industries are interesting from an environmental sustainability viewpoint concerning the

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quantity and the difficulty of the waste treatment. These industries are concentrated in the Mediterranean area, where more than 95% of the world's olives are harvested and consequently up to 30 million tons of residue are generated per year [1]. The average amount of residues produced varies according to the processing technology used. Both Schmidt and Knobloch [2] and Raggi *et al.* [3] have reported that waste production varies from 40% of olive husk and 40% olive mill waste water for pressure extraction to 50% of olive husk and 95% of olive mill waste water for continuous centrifugation. These two types of wastes represent a high microbiological and chemical pollutant load [4] which is 25–80 times greater than the pollution reported for municipal waste waters [3]. Unfortunately in Morocco, no use is made of these wastes. The olive husk is processed in seed oil factories to extract the small percentage of residual oil, following which it is burnt. The most common way to dispose of the olive mill waste waters is soil spreading or rejection with municipal waste water. This causes environmental problems such as water pollution and insect multiplication. In addition to the problems caused by the solid and liquid press residues, some specific features of olive oil production industries, such as seasonal production and widespread scattering of small sized mills, complicate the adoption of environment-compatible approaches for sustainable waste disposal. As a consequence, the need arises for the adoption of solutions suited to this situation, which are more environmentally compatible and economically sustainable. In order to overcome this problem, waste treatment technologies to recycle these wastes generating a useable product, represent an interesting alternative for the sustainable disposal of residues. Composting is one sustainable method, which can transform these wastes into a stabilized product that can be used as a source of nutrients and humic substances. Furthermore, the composted product has the advantage of improving soil structure, increasing soil organic matter, suppressing soil borne plant pathogens, and enhancing plant growth [5, 6]. Consequently, composts used as substrates should have a high degree of maturity, which means the absence of undesirables, such as phytotoxic compounds. The evaluation of compost maturity is still an open question [7]. Indeed, the measure of compost maturity is not always straightforward, and, in fact, researchers across the world are still exploring the best methods for determining different levels of maturity in compost. Thus, 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 [8] proposed the use of the humification index, the humification rate and the degree of humification. Tomati *et al.* [7] suggested monitoring the molecular weight of the humic substances as an index of stability. These parameters may be good indicators of the progression of the humification processes but do not provide sufficient data concerning the composition of the humic substances. In this respect, many publications have tried to characterize compost maturity by several spectroscopic and chromatographic methods [9–16]. A part from these methods, the sensitivity and rapidity of fluorescence and thermal techniques are well suited for studying the properties of humic substances and of composts [17, 18]. In the bibliography, several studies have tried to characterize especially humic acids from different origins. However, studies concerning the characterization of fulvic acids extracted from various composted wastes (sewages [19–21]; straw [22–24]; municipal solid wastes [25]; urban wastes [26, 27]), are very scarce, especially by fluorescence and thermal methods, probably because of the chemical complexity of their constituents.

The present study tries to follow fulvic acid evolution during different stages of composting, by using elemental analysis, fluorescence spectroscopy and differential scanning calorimetry (DSC) as complementary analyses.

2. Material and methods

A heap of 280 kg of solid olive mill wastes, 20 kg of cereal straw and 85 litres of liquid olive mill waste was composted on a platform for one year. Over the one-year study period, the heap was forked over every fortnight to aerate the compost. The microbiological activity was assessed by measuring the temperature of the heap in different places. To obtain the representative samples and analyses, a good homogenizing of compost pile was made and sub-samples (500 g) were taken from 10 different points of compost heap (bottom, surface, side, centre) at each stage of composting: raw mixture = 0 day, mixture after 30, 90, 180 days of composting. The main characteristics of product at the start and the end of olive mill wastes composting are shown in table 1.

2.1 *Extraction and purification of fulvic acids*

The samples (60 g) were extracted with 100 mL of NaOH (0.1M) under continuous agitation for 2 hours. The extract was isolated after centrifugation (25 minutes at 5000 rpm). This operation was repeated 40 times. The supernatants were adjusted to pH 2.0 with H_2SO_4 (3M) and left to coagulate for 24 hours at 4 °C to separate fulvic acids from humic acids. The former were neutralized and dialysed with a dialysis membrane (Spectra Por MWCO 1000 Daltons) to eliminate excess salts, then freeze-dried.

2.2 *Elemental analysis*

The ash content of the fulvic acids was determined by ignition at 550 °C in a muffle furnace for 24 hours. Carbon, hydrogen, nitrogen, and sulphur were determined using a CHNS Carlo Erba microanalyser and the oxygen content was calculated as the difference from 100 [28].

2.3 *Fluorescence spectroscopy*

2 mg of the freeze-dried fulvic acid samples were resuspended in 10 ml water adjusted to pH 10 with 0.01 M NaOH. Solutions were put under continuous agitation overnight at room temperature and a slight decrease of pH was observed ($\sim$0.5–1 pH unit) during this stage. Before analyses, the pH of the solution was readjusted to 10 with 0.01M NaOH, solutions diluted to 50 mL and centrifuged for 10 min at 3000 rpm.

Fluorimetric analyses were performed at room temperature (20 °C) on a model LS-55 computer controlled spectrofluorimeter (Perkin-Elmer, Norwalk, CT, USA). Emission spectra were recorded over the 380–550 nm range at an excitation wavelength of 360 nm. Excitation spectra were recorded over the 300–500 nm range by measuring the emission radiation at a fixed

Table 1. The main characteristics of olive mill wastes at the initial mixture and final compost.

Composting time (month)	pH	C/N ratio	D.R. (%)	HA (%) P.S.	FA (%) P.S.	L (%) DW	Ce (%) DW	Hm (%) DW	P (%) FW	F.a. (%) FW
T0	4.62	69.25	–	–	–	33.56	24.45	42.46	0.74	7
T12	7.82	16.50	56.50	7.76	14.24	40.99	22.32	22.17	0.25	0.2

D.R.: Decomposition rate; HA: humic acids; FA: Fulvic acids; L: Lignin; Ce: Cellulose; Hm: Hemi-Cellulose; P: Polyphenols; F.a.: Fatty acids, DW: Dry Weight, FW: Fresh Weight.

wavelength of 520 nm. Synchronous-scan excitation was measured by simultaneously scanning both the excitation, from 290 to 550 nm, and the emission wavelength while maintaining a constant optimised wavelength $\Delta\lambda = \lambda_{em} - \lambda_{exc} = 18$ nm. All spectra were recorded with a 5-nm slit width on both monochromators. No corrections for fluctuation of instrumental factors or for scattering effects were applied to experimental spectra, so a comparative discussion on the spectra is acceptable, at least on a qualitative basis, since all were recorded on the same instrument, the same day, using the same experimental conditions. The spectra were smoothed by a 12-point moving average method before further interpretation.

2.4 *Differential scanning calorimetry (DSC) analysis*

Dynamic thermogravimetric analyses were carried out in a Perkin-Elmer TGS-1 thermal balance with Perkin-Elmer UV:1 temperature program control on intact lyophilized humic acid samples not subjected to any treatment or extraction. 5 mg of sample were placed in a platinum sample holder. An empty pan scaled in the same way was used as reference.

Thermal degradation measurements were carried out between 20 and 700 °C at a rate of 5 °C min^{-1} under air atmosphere. Indium was used as a standard for temperature calibration.

3. Results and discussion

3.1 *Elemental analysis*

The elemental content and atomic ratios of the fulvic acids (table 2) are typical of this humic fraction reported by [24]. The nitrogen content of fulvic acids increased significantly during composting and varied from 1.61 at T1 (1st month) to 4.21% at T12 (12th month), while contents of sulphur, carbon and hydrogen showed only a slight increase and reached 2.00, 50.23, 7.18% respectively after the 12th month of composting. In contrast, the oxygen content decreased from 41.75 at T1 to 36.39% at T12. Carbon concentrations of fulvic acids reached 50.23% at T12. This value was found to be in the range recommended by Rice and MacCarthy [29]: 46.9–60.8%. The C/H ratio was very stable during composting, suggesting few changes in the molecular structure. Riffaldi *et al.* [21] reported that the carbon and hydrogen content of fulvic acids extracted from various organic sources at different stages of maturity remained almost unchanged during composting. As the C/H ratios remained stable, they cannot be related to the degree of maturity.

Kalbitz *et al.* [15], reported that O/C of fulvic acids extracted from topsoil and ground and surface water are also high, compared to those from soil reported by Senesi *et al.* [24] and

Table 2. Elemental composition of the fulvic acids extracted at various steps of olive mill waste-straw composting (moisture and ash-free basis).

Composting time (months)	C (%)	N (%)	H (%)	S (%)	O (%)	Ashes (%)	Atomic ratios		
							C/N	O/C	C/H
1	48.20	1.61	6.94	1.51	41.75	13.96	34.92	0.65	0.58
3	50.03	3.47	6.99	3.93	35.60	23.43	16.81	0.53	0.59
6	48.56	3.53	6.85	2.36	38.70	19.85	16.06	0.59	0.59
9	48.82	3.70	6.90	2.30	38.30	18.46	15.41	0.59	0.59
12	50.23	4.21	7.18	2.00	36.39	17.92	13.95	0.54	0.58
Soil fulvic acid values*	45.70	2.10	5.40	1.90	44.80	–	25.50	0.70	0.70

*M. Schnitzer [30].

they related this to the young age of the fulvic acids. In our case, the O/C ratios measured are in the lower range of fulvic acids reported by Senesi *et al.* [24], which can explained the high degree of humification of these acids.

A comparison of carbon, hydrogen, and sulphur contents of fulvic acids of composted olive mill wastes with those of soil fulvic acids reported by Schnitzer [30] showed that our values were very high, although the C/N ratio was low. This low value could probably be attributed to the high levels of products arising from protein decomposition [24] and confirms the greater level of humification [23]. While in comparison to fulvic acid parameters reported for other composts of various origins such as sludge, straw, or municipal waste [24], those of mature olive mill wastes compost were very similar.

3.2 *Fluorescence spectroscopy*

Fluorescence spectroscopy has recently been used as a powerful method for classifying and distinguishing humic substances of various origins and natures [24]. Senesi *et al.* [31] reported that the most efficient fluorophores in humic polymers are variously substituted, condensed aromatic rings, and/or highly unsaturated aliphatic chains. The fluorescence emission spectra (figure 1) of the fulvic acids studied here show two bands. The first is centered at 410 nm which is, according to Senesi *et al.* [31], due to a Raman band of water and the second at around 457 nm. Ouatmane *et al.* [16] have reported the presence of the latter band in domestic organic wastes, sawdust, used coffee grounds and farmyard manure humic acids. In addition,

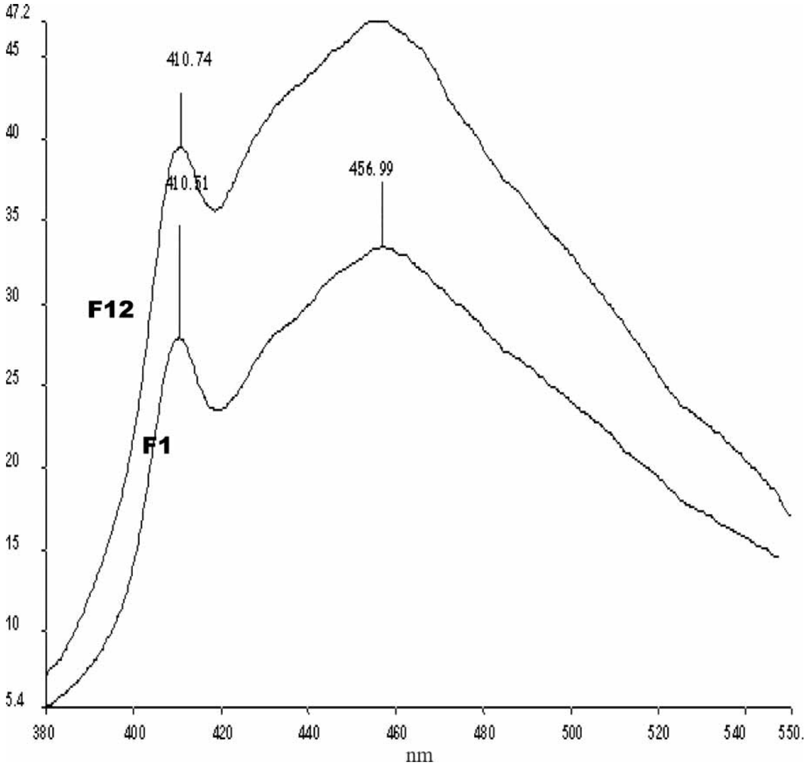

Figure 1. Emission Spectra of fulvic acids of composted olive mill wastes (F1: after 1 month and F12: after 12 month of composting).

Senesi *et al.* [24] have reported that soil fulvic acids feature a unique broad band from 445 to 465 nm.

According to Senesi *et al.* [31], excitation spectra of humic substances are generally better resolved than emission spectra and characterized by a number of peaks and/or shoulders that are generally located around three wavelengths, long (480–440 nm), intermediate (400–380 nm), and short (360–300 nm). The same bands and shoulders are found in excitation spectra of fulvic acids of composted olive mill wastes with a maximum intensity at around 390 nm (figure 2). These spectra are typical of soil fulvic acids [31]. These authors have also reported that fulvic acid samples feature one main excitation in the intermediate region of the spectrum (394–388) with additional peaks and shoulders at longer and/or shorter wavelengths (465–450; 440 and <360 nm) of various relative fluorescence intensities. Furthermore, Bachelier [32] measured a lower wavelength for the main excitation peak of some soil fulvic acids and an additional intense peak in the longer wavelength region. Our results are generally in agreement with those reported in the literature. Additionally, the appearance of the spectra became simpler with compost maturation.

Fluorescence spectroscopy in the synchronous-scan mode should allow better peak resolution than that obtained by conventional fluorescence modes, *i.e.* emission or excitation [31], thus providing more distinct parameters for differentiation between samples and clearer identification of the molecular structures responsible for fluorescence. Common to synchronous

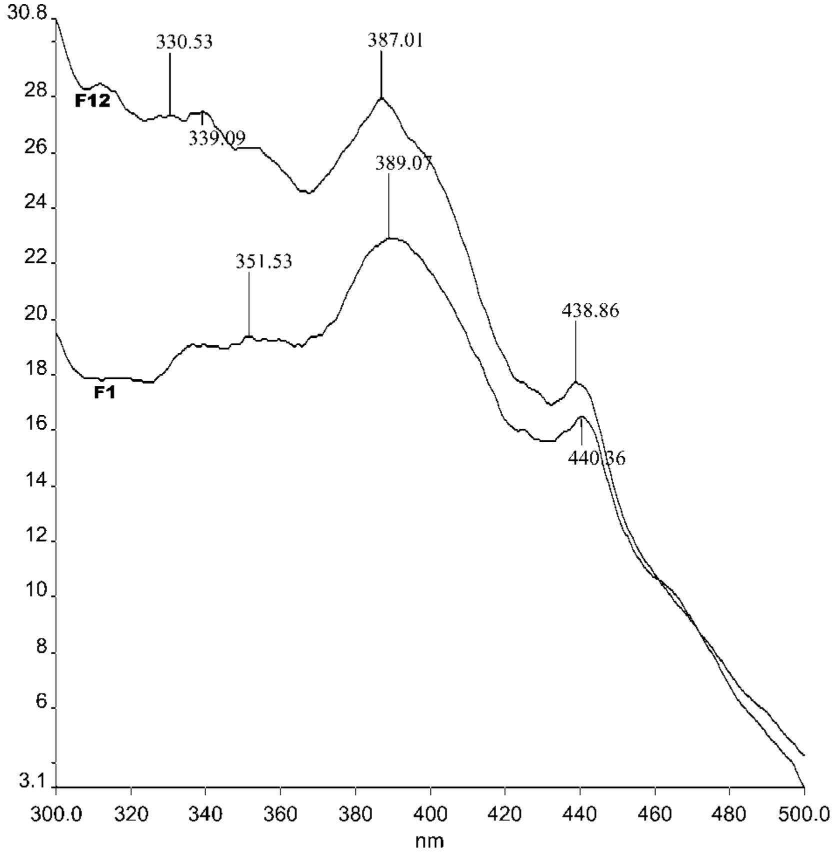

Figure 2. Excitation spectra of fulvic acids of composted olive mill wastes (F1: after 1 month and F12: after 12 month of composting).

fluorescence spectra of the fulvic acids are bands and shoulders at around 357–360; 392–400; 456–457 and 492–493 nm. These bands and shoulders are present in synchronous-scan spectra of composted olive mill wastes (figure 3). Kalbitz *et al.* [15] encouraged the use of the 400/360 nm ratio as a humification index for fulvic acids. Effectively, 400/360 nm ratio passes from 1.42 in the first month of composting to 1.8 at the end, indicating the higher degree of humification and polycondensation of fulvic acids from composted olive mill waste.

Hypotheses may be put forward concerning the molecular structures acting as potential contributors to fluorescence. Senesi *et al.* [31] associated some emission and excitation wavelengths to certain fluorescing compounds (table 3).

In the synchronous spectra of composted olive mill wastes, the intensity of shoulders at intermediate wavelengths decreased with time of composting. This can be explained, according to Miikki *et al.* [27], by the increasing polycondensation and conjugation of unsaturated structures and the greater uniformity of fluorophores. Senesi *et al.* [31] explained that the increase in the extent of the π-electron system and the presence of various oxygen and nitrogen-containing functional groups has the effect of lowering the energy difference between the ground state and the excited state, thus shifting fluorescence to longer wavelengths, whereas the presence of carbonyl and carboxyl substituents largely reduces relative fluorescence intensity. The long wavelengths and low intensities measured for the principal fluorescence peak can be associated with the presence of linearly-condensed aromatic ring systems bearing electron-withdrawing substituents such as carbonyl and carboxyl groups and/or to other unsaturated

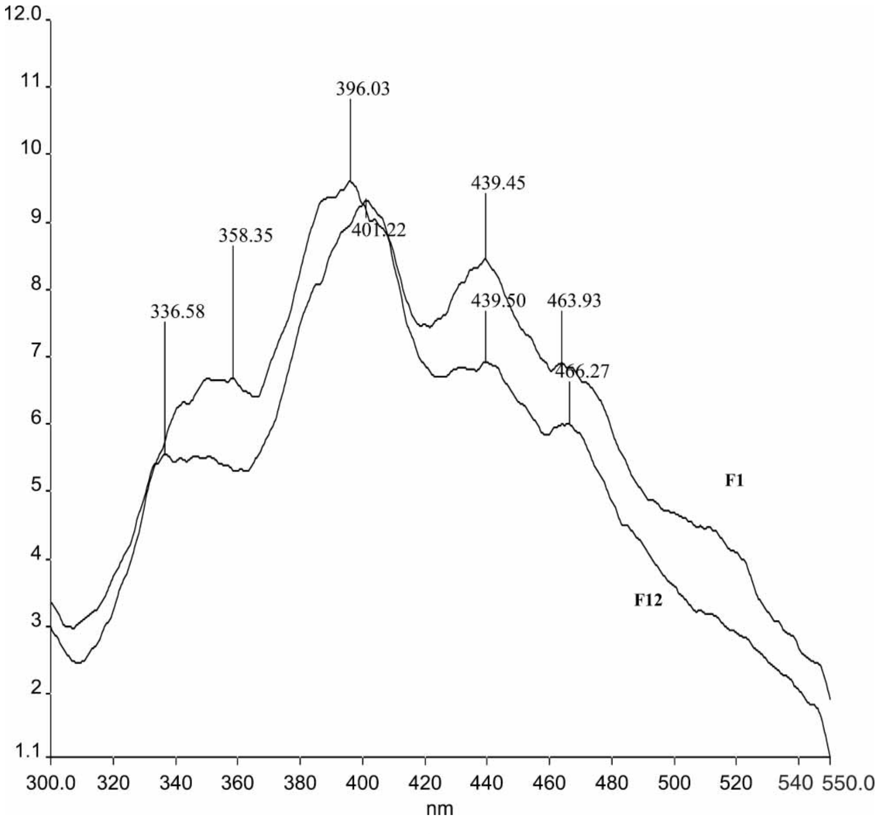

Figure 3. Synchronous-scan spectra of fulvic acids of composted olive mill wastes (F1: after 1 month and F12: after 12 month of composting).

Table 3. Wavelengths of emission and excitation maxima of compounds that are potential contributors to fluorescence spectra of fulvic acids [31].

Fluorescing compound	Emission wavelength (nm)	Excitation wavelength (nm)
Salicylic acid	410	314
Methyl(2 hydroxybenzoate)	448	302, 366
3 hydroxybenzoic acid	423	314
Protocatechuic acid (ionized)	455	340–370
3 hydroxycinnamic acid	407	310
Caffeic acid	450	365
Ferulic acid	440	350
Cumarins		
Unsubstitued	454	376
Hydroxy and methoxy	400–475	320–343
Esculetin	475	390
Scopoletin	460	390
Other disubstituted	430–462	350–419
Chromene and its derivatives	409–490	320–346
Xanthone and hydroxyxanthones	456 and 465	410 and 343, 365

bond systems capable of a high degree of conjugation as well as to the presence of high molecular weight fractions. The higher fluorescence intensities and shorter wavelengths typical of soil fulvic acids have been ascribed to the presence of simpler structural components of low molecular weight bearing electron donating substituents such as hydroxyl, methoxyl and amino groups, a low degree of aromatic polycondensation and low levels of conjugated chromophores. However, these authors reported that a number of environmental factors such as internal conversion and intersystem crossing and/or environment effects, such as inner-filter effects related to factors including concentration, pH, and ionic strength of the sample solution can influence relative fluorescence intensity and wavelengths to various extents.

3.3 *Thermal analysis*

The technique of differential scanning calorimetry has recently been applied to the characterization of humic and fulvic acids of various origins and natures, as well as composts of different organic wastes [33, 34]. Differential scanning calorimetry plots of fulvic acid samples in different stages of olive mill waste composting are represented by figures 4 (a, b, c) and 5 (a, b, c). Thermograms of fulvic acid samples show a very simple and similar pattern. At the beginning of composting, thermograms are characterized by several thermal effects due to the high heterogeneity of the initial substrate. DSC curves are characterized by a low-temperature endotherm (around 140 °C) and an exotherm at around 500 °C. These thermal effects have been attributed respectively to dehydration reactions and/or to the loss of peripheral polysaccharide chains and to oxidation and polycondensation of the aromatic nuclei of the molecule. The same results was obtained by Guignard *et al.* [35] for humic acids of peat which the characterization by using thermochemolysis techniques showed that these acids contained high amounts of polar macromolecular components and aromatic products.

Fulvic acid DSC curves display an additional exotherm in the medium temperature region (around 350 °C), which can be attributed to the loss of peptidic structures which are integrated to humic matter [28]. Later the exotherm (350 °C) disappear as composting time progresses indicating, according to Provenzano *et al.* [28], the incorporation and stabilization of the peptidic structures in the humic macromolecules and consequently the increase of humification degree. This trend is confirmed by the presence of a sharp low-temperature endotherm at around 140 °C, which can be used to evaluate the maturity of composted wastes [28]. After 12 months of composting, fulvic acid DSC thermograms approach those of more homogeneous materials [24]. It also appears from the TGA thermograms (figures 4a and 5a) that every exothermic reaction is associated with weight loss. The presence of an exotherm at around 500 °C is characteristic of humic acids and is explained by polycondensation of aromatic nuclei of the molecules [21]. It appears from the whole results that the stabilization process that occurs during composting is accompanied with the formation of more stabilized products with spectroscopic and thermal characteristics similar to those of the mature ones reported in the literature [24].

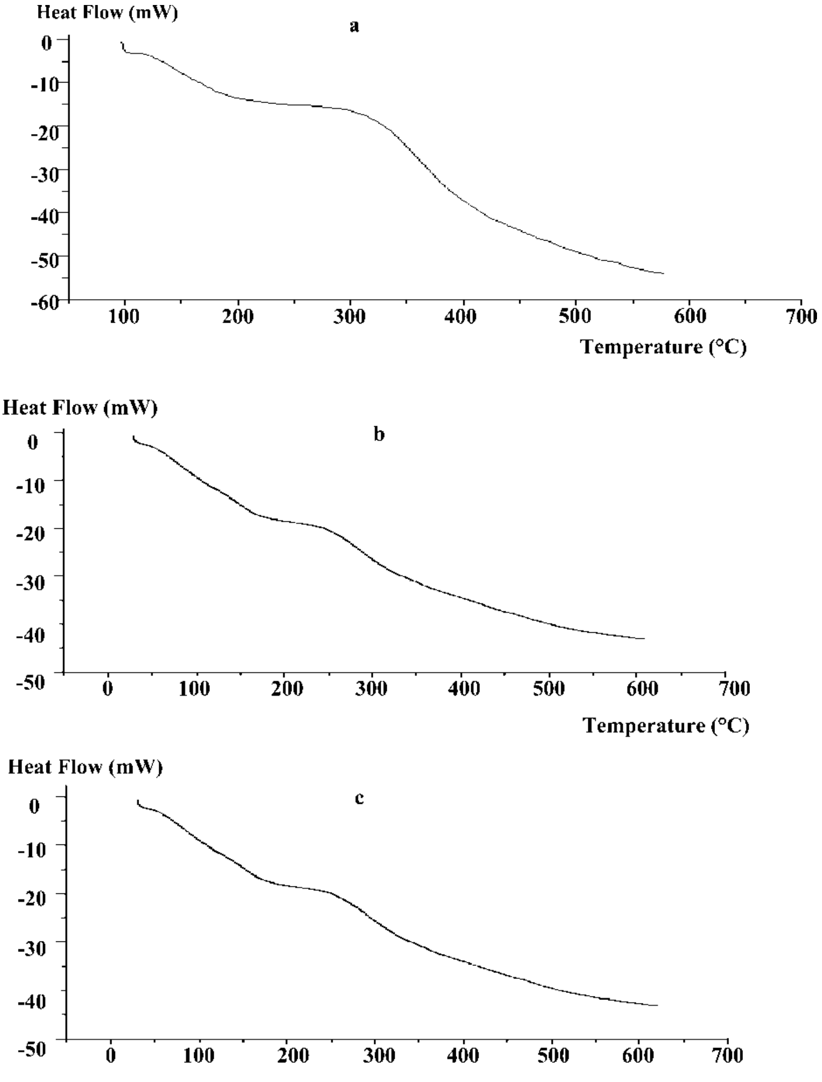

Figure 4. Thermograms (thermogravimetry) of fulvic acids of olive mill wastes after 1 month (a), 3 months (b) and 12 months (c) of composting.

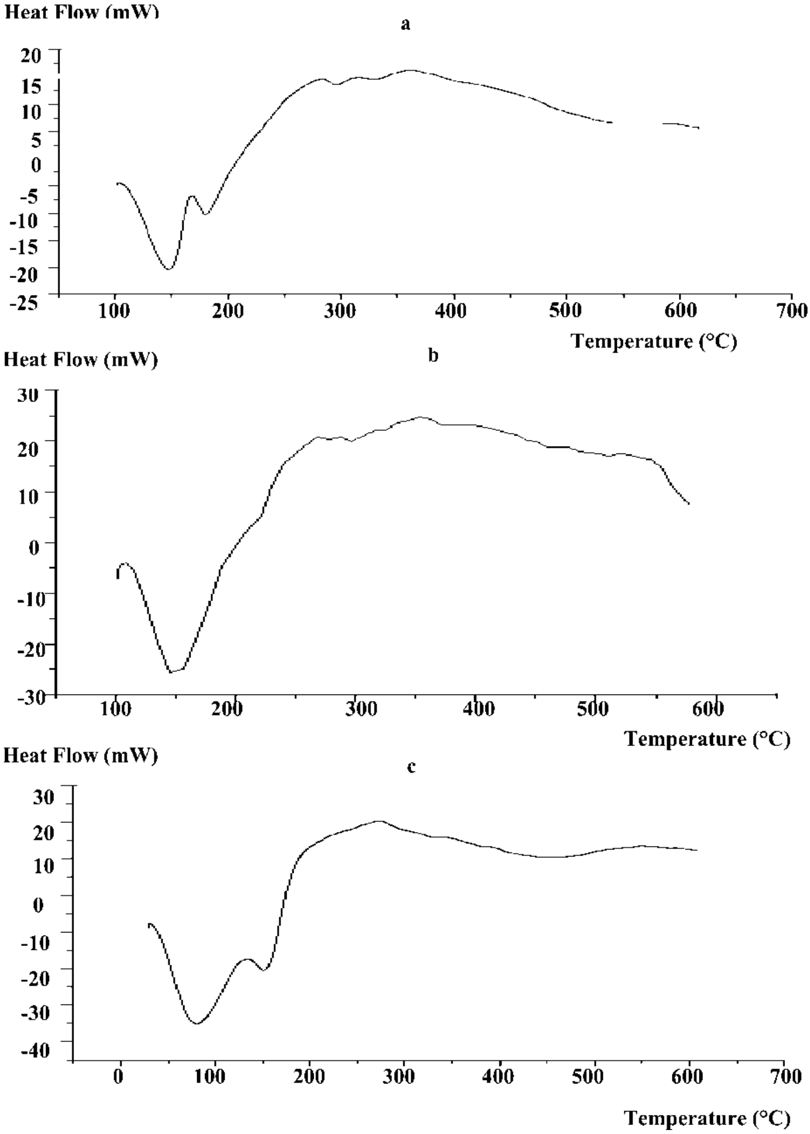

Figure 5. Thermograms (DSC) of fulvic acids of olive mill wastes after 1 month (a), 3 months (b) and 12 months (c) of composting.

4. Conclusion

The application of fluorescence spectroscopy to the study of fulvic acids in composted olive mill wastes confirmed the results obtained from elemental analysis which indicated a higher level of polycondensation and humification of these acids during composting. Fluorescence emission spectra showed peaks and shoulders typical of soil fulvic acids. Additionally, the synchronous-scan spectra confirmed the increasing polycondensation and conjugation of unsaturated structures and the greater uniformity of the fluorophores. Moreover, DSC curves of fulvic acids are characterized by particular thermal effects attributed to the oxidation and polycondensation of the aromatic nuclei of the fulvic acid molecules during olive mill waste composting. Thus, the whole results confirm that the stabilization process which occurs during composting is accompanied with the formation of more stabilized products. 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.

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