

# Evaluation of Organic Matter Stability During the Composting Process of Agroindustrial Wastes

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## Abstract

Composting of wastes from citrus industrial processing (pastazzo and sludge) was studied in order to evaluate the evolution of organic matter during the process and to individuate chemical and/or biochemical techniques able to set the stability of the final product. Composts from two open-air piles of different composition were sampled every month during the whole period of composting (5 months) and the organic matter of each sample was characterised by chemical and biochemical techniques. Humification rate (HR%) and humification index (HI) were determined. Extracted organic matter of the six samples collected for each compost was investigated by isoelectric-focusing technique (IEF). The biochemical analysis were based on the study of C-mineralisation after the addition of each collected sample to soil. Results obtained clearly demonstrated organic matter evolution during composting processes. Humification rates increased and humification indexes decreased over time, while extracted organic matter showed electrophoretic behaviour typical of stabilised organic compounds. Moreover, mineralisation patterns confirmed the increased level of organic matter stability during the composting process.

## Introduction

During the last years the citrus-processing industry, in Italy, has been increasing its economical importance. Nowadays, about 800 000 tons year<sup>-1</sup> of citrus fruits are processed, with a production of 500 000 tons year<sup>-1</sup> of pastazzo, a mixture of citrus pulp and skins, which represents the main residue of citrus-processing squeezing treatment. In addition to pastazzo, citrus-processing industry produces also a high amount of effluents, mainly constituted by fruits and factory plant-washing waters, which undergo a purification treatment with the production of sludge. The use of these residues as matrices for the production of a quality compost could constitute

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a typical example of transformation of a waste into a resource with a market value (Sequi and Tittarelli 1998).

One of the key issues in defining compost quality is the evaluation of its organic matter stability. For this reason, it is necessary to individuate and set up analytical methods able to follow the organic matter transformation during the composting process.

During composting, for a combination of biological and chemical transformations, total organic carbon content decreases, but the relative content in humic (or humic-like) compounds increases. Determination of the humification rate (HR%) and the humification index (HI%) demonstrated to be effective indicators of the formation of humic-like substances during compost maturation, being able to establish with accuracy the moment of complete stabilisation (Ciavatta et al. 1990).

Another chemical technique able to characterise organic matter of composted amendments is isoelectric focusing (IEF) (Ciavatta et al. 1993). This technique allows one to fractionate the organic compounds on the basis of their isoelectric point and their electrophoretic mobility. IEF was usefully utilised in order to obtain information on the qualitative characteristics of organic matter in soils (Ciavatta and Govi 1993), amendments and organic fertilisers (Ciavatta et al. 1997; Canali et al. 1998).

An effective biochemical method able to define, for a compost, the level of organic matter stability is the study of organic carbon mineralisation after addition of compost to soil (Kirchmann 1991; Tittarelli et al. 1998). Since organic matter resistance to soil microbial degradation is directly correlated to its chemical stabilisation, it is possible to follow organic matter evolution, during a composting process, by detecting evolved CO<sub>2</sub> after soil amendments with compost sampled from the heap at prefixed time.

The aim of this work is to study organic matter evolution of two composts from agroindustrial wastes (the first obtained mixing pastazzo, citrus-processing sludge and green residues, the second mixing only pastazzo and green residues), by means of chemical and biochemical techniques able to set the different degrees of organic matter stability during the composting process.

Composts considered in this work were further investigated by thermal analysis in Dell'Abate and Tittarelli (in press).

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## **Materials and Methods**

Composting trials were performed in Azienda Sperimentale Palazzelli (Lentini) of the Istituto Sperimentale per l'Agrumicoltura, Acireale (CT, Italy).

Two piles were prepared, the first by mixing pastazzo, coming from citrus-processing industry, with green residues (Cp) and the second by adding sludge from the citrus-processing industry to the matrices mentioned above (Cps).

Input matrices were mixed taking into account the chemical analysis of pastazzo and sludge (Table 1), and the C/N ratio of green residues (which was 77). The weight percentages of the used matrices are reported in Table 2.

Temperatures of the two piles were detected during the whole composting period and moisture maintained at the optimum values (50÷60%). Piles were turned more frequently during the thermophilic phase and less frequently during the mesophilic composting phase. At mixing time (T0) and after 29 (T1), 67 (T2), 89 (T3), 130 (T4) and 165 (T5) days, compost samples were collected from each pile. Each sample was constituted by six subsamples, taken after turning the heap, then mixed until homogenisation. The samples were oven-dried at 50 °C, ground and sieved at 1 mm and finally stored for subsequent analysis. All analysis were performed in three replicates.

**Table 1.** Main chemical-physical parameters of pastazzo and sludge

Parameter	Unit	Pastazzo	Sludge
Moisture	%	88.6	65.1
pH	-	3.2	8.2
N	%	1.39	3.55
P <sub>2</sub> O <sub>5</sub>	%	0.27	3.66
K <sub>2</sub> O	%	0.90	0.54
C <sub>org</sub>	%	51.4	28.0
C/N	-	37	8
Ca	%	1.06	6.25
Mg	%	0.12	0.72
Cd (total)	mg kg <sup>-1</sup>	<0.5	5.0
Hg (total)	mg kg <sup>-1</sup>	<0.1	0.12
Cu (total)	mg kg <sup>-1</sup>	7.0	105
Zn (total)	mg kg <sup>-1</sup>	12	680
Ni (total)	mg kg <sup>-1</sup>	<0.5	37.5
Pb (total)	mg kg <sup>-1</sup>	<0.5	27.0
Cr(VI)	mg kg <sup>-1</sup>	n.d. <sup>a</sup>	n.d. <sup>a</sup>

<sup>a</sup> n.d. = not detectable

**Table 2.** Weight ratios of the matrices used for the production of composts Cp and Cps

Compost	Pastazzo (%)	Sludge (%)	Green residues (%)
Cps	40	20	40
Cp	60	-	40

## Extraction and Fractionation of Organic Matter, Determination of C/N Ratio

The extraction was carried out on 2 g of each compost sample with 100 ml of a solution NaOH/Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> 0.1 N for 48 h at 65 °C. Samples were centrifuged at 2500 rpm and supernatant solution filtered through a 0.45 µm Millipore filter. Extracts were stored at 4 °C under nitrogen atmosphere.

Humic and fulvic acids were fractionated by acidification of 25 ml of the extract with H<sub>2</sub>SO<sub>4</sub> 50 %, separating humic-like acids (HA) (precipitated) from fulvic-like acids (FA) (in solution). The last ones were purified on a polyvinylpyrrolidone (PVP) column, resolubilised with NaOH 0.5 N and then joined to the humic portion. Combined fractions (HA+FA) were quantitatively transferred into a calibrated 50-ml flask, brought to volume with NaOH 0.5 N and stored at 4 °C under nitrogen atmosphere. Total organic carbon (C<sub>org</sub>) in compost samples was determined according to Springer and Klee (1954), while total extractable carbon (C<sub>extr</sub>) and humic and fulvic acids carbon (C<sub>HA+FA</sub>) were determined following the procedure proposed by Ciavatta et al. (1990). Humification parameters for assessment of organic matter stabilisation in compost were so calculated:

$$\text{Humification rate (HR) \%} = (C_{\text{HA+FA}} \times 100) / C_{\text{org}}$$
$$\text{Humification index (HI)} = C_{\text{not humified}} / C_{\text{HA+FA}} = (C_{\text{extr}} - C_{\text{HA+FA}}) / C_{\text{HA+FA}}$$

Total nitrogen content (%) of each compost sample was determined by the dry combustion method, using a LECO FP 228 nitrogen determinator, and the C/N ratio was then calculated.

## Isoelectric Focusing (IEF)

Isoelectric focusing separations for T0÷T5 samples of Cps and Cp were carried out in a Multiphore II, LKB electrophoretic cell, according to Govi et al. (1994). Ten mL of NaOH/Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> extract were dialysed in 6000÷8000-Da membranes, lyophilised and then separated on a 5.06% T and 3.33% C polyacrylamide slab gel, in which a pH range 3.5÷8.0 was created using a mixture of carrier ampholytes (Pharmacia Biotech): 25 units of ampholine pH 3.5÷5.0; 10 units of ampholine pH 5.0÷7.0; 5 units of ampholine pH 6.0÷8.0.

A prerun (2 h; 1200 V; 1 °C) was performed and the pH gradient formed in the slab was checked by a specific surface electrode. The electrophoretic run (2 h 30'; 1200 V; 1 °C) was carried out loading the water-resolubilised extracts (1 mg C 50 µL<sup>-1</sup> sample<sup>-1</sup>). The bands obtained were stained with an aqueous solution of Basic Blue 3 (30%) and scanned by an Ultrascan-XL Densitometer. In order to compare isoelectric focusing data of Cps and Cp samples, the same operative conditions were applied for both IEF separations.

## **Organic carbon mineralisation**

Organic carbon mineralisation of samples taken during composting processes (T0÷T5) from Cps and Cp piles was followed after their addition to soil, according to Kirchmann (1991), partially modified by Tittarelli et al. (1998). After incubation at constant temperature (30 °C) and moisture (60% WHC), determination of evolved C-CO<sub>2</sub> was performed at prefixed time (1, 2, 4, 7, 9, 11, 14, 16, 18, 22, 28, 35, 42, 49, 56 and 63 days). Soil respiration was used as control. The amount of organic carbon mineralised from the samples taken during the composting process was calculated as difference between the C-CO<sub>2</sub> evolved by the system soil + compost and the C-CO<sub>2</sub> evolved by the not amended soil, assuming that added material does not have priming effect on soil organic matter decomposition (Kirchmann 1991).

## **Results and Discussion**

### **Composting Process**

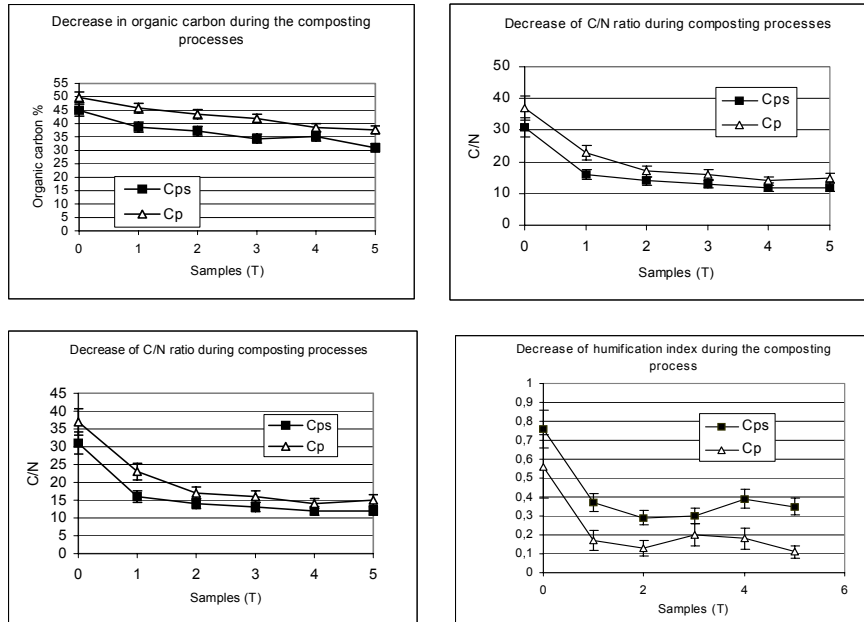
For both piles, after a first thermophilic phase, during which 60 °C were exceeded, the temperature was maintained over 50 °C for about 60 days. During the following period, a slow decrement of this parameter was detected in both piles, until reaching 40 °C in the curing phase. Cps and Cp composts, at the end of the processes, did not contain visible residues of the raw starting materials and appeared brown-coloured and homogeneous.

### **Organic Carbon, C/N Ratio and Humification Parameters**

In Fig. 1, total organic carbon content (%), C/N ratio, humification rate (HR%) and humification index (HI) for each sample of Cps and Cp are reported.

For both the piles, C/N ratio decreased constantly, confirming the correct trend of organic matter transformation. Humification rates sensibly increased during the composting process, for both Cps and Cp, reaching quite high values. The final humification rate was the same for the two composts (about 46%), while a difference was noted in the final value of the humification index, that was 0.35 for Cps and 0.1 for Cp. The difference in the asymptotic value of the HI for the two composts could be determined by the different qualitative characteristics of the organic matter in the raw starting materials, since a difference in this humification

parameter was detected also between the mixtures at T0 (0.75 for Cps against 0.58 for Cp).



**Fig. 1.** Changes of Cps and Cp chemical parameters during composting process. Data reported are means of three replicates

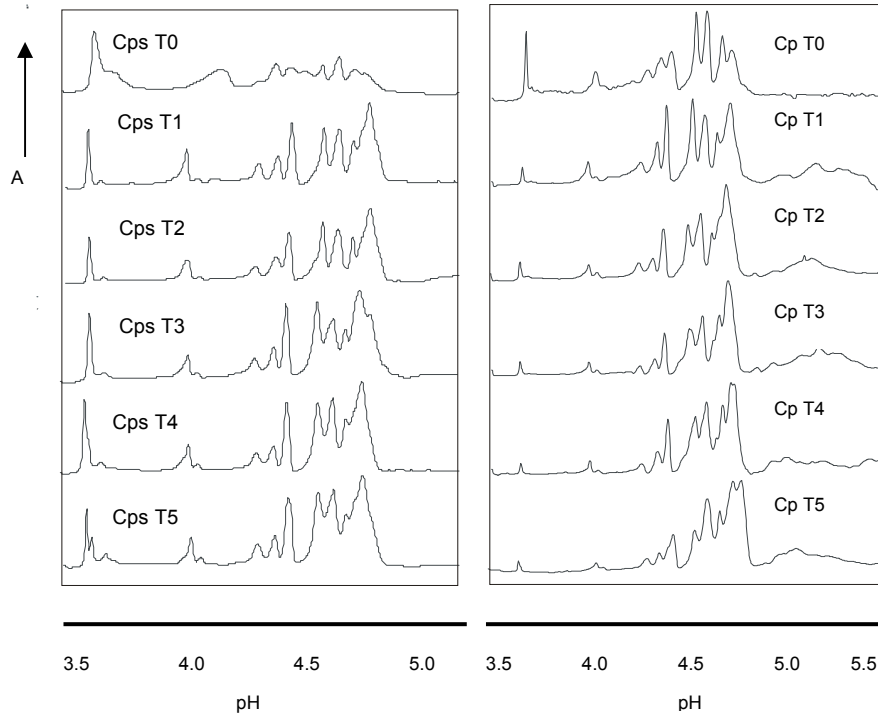
Considered chemical parameters affirm that organic matter of the two composts, from a quantitative point of view, underwent a relevant stabilisation during the composting process, because of the mineralisation of labile fractions (decrease of total organic C and consequent decrease of C/N ratio) and the formation of humified or humic-like compounds, as attested by high values of final HR and low values of final HI (Govi et al. 1994; Sequi 1995; Dell'Abate et al. 1998).

### Isoelectric Focusing (IEF)

In Fig. 1, the IEF profiles of extracted organic matter for Cps and Cp composts from T0 to T5 are reported.

The IEF profile of the extractable organic matter at the mixing phase (sample T0) for Cps compost showed one high peak at pH 3.5 and a group of peaks of lower intensity, not resolved, in the pH range between 4.0 and 4.7. At T1 time, the IEF profile resulted more defined and characterised by a group of bands between pH 4.2 and 4.7. Profiles relative to the subsequent samples (T2÷T5) globally

maintained the same configuration revealed at T1 time, even if at pH 4.7 a little increase of the last peak intensity was detected for the final T5 sample.



**Fig. 1.** IEF profiles of extracted organic matter for Cps and Cp composts (T0-T5); pH ranges below reported are referred to pH values corresponding to the obtained IEF peaks after scansion between pH 3.5 and 8.0

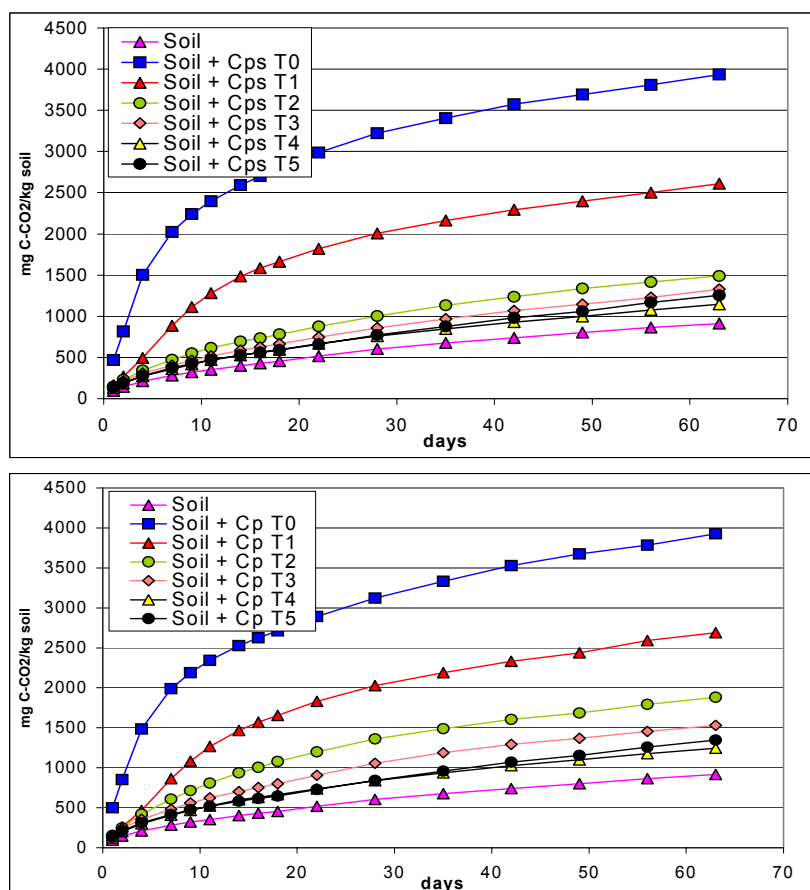
In the case of Cp, the IEF profile of the sample T0 was sufficiently resolved, with one high intensity peak at pH 3.5, one peak of medium intensity at pH 3.9 and a series of peaks well defined in the pH range 4.2-4.7 with decreasing intensity. In T1-T5 profiles, some not-resolved peaks with low intensity appeared at pH >4.8, together with a reduction of peak focused at pH 3.5 and a gradual increase of the band focused at pH 4.7.

IEF traces of the two composts showed that, during the composting process, extractable organic matter underwent a qualitative transformation, determined by the increase or appearance of bands in correspondence of pH >4.5 values. As reported in literature (De Nobili et al. 1989; Govi et al. 1994; Canali et al. 1998), more humified organic compounds focus at high pH values (4.5-7.0). The increase of peaks intensity at pH higher than 4.5 during both the composting processes could correspond to the presence of more stabilised organic compounds. This result confirmed what arose from the determination of humification parameters.

## Organic Carbon Mineralisation

Organic carbon mineralisation of Cps and Cp samples (T0-T5) added to the soil showed similar trends (Fig. 2).

In both cases, during 64 days of trial, about 30% of added organic carbon of composts was mineralised at T0, while about 16% was mineralised at T1. At time T2, mineralised organic matter from Cps compost was around 6% of added organic carbon, reaching the level of stability which was detected in the later samples T3, T4 and T5. In the Cp compost, at T2 the amount of mineralised organic carbon from compost was 10% and reduced to 6% for sample T3, whose level of stability resulted comparable to those of T4 and T5.



**Fig. 2.** Carbon mineralisation curves for Cps and Cp composts added to the soil. Values reported are means of three replicates. Calculated standard deviations are not graphically detectable



For both the typologies of composted amendments, the level of organic matter stability to the mineralising activity of soil microorganisms at T4 and T5 resulted the same (4% of total added carbon was mineralised during the whole period of incubation). The cumulative curves related to the considered composts evidenced that organic matter reached, at the end of the composting process, similar stability in presence or absence of sludge in the starting mixture. Moreover, while in Cps this stabilisation was reached after 67 days (T2), in Cp 90 days (T3) were necessary to obtain comparable level of organic matter evolution.

## **Conclusions**

This study demonstrated that considered techniques were able to detect the organic matter transformation during the composting process. Humification parameters revealed a similar increase in organic matter stability for Cps and Cp composts, also confirmed by the study of organic matter mineralisation and the isoelectric focusing technique. Moreover, IEF traces of the two composts showed some qualitative differences in more stable organic matter fraction, probably determined by the difference in composts starting mixtures. Apparently Cp compost, produced without sludge, seemed to be better humified, as attested by the presence of some bands focused at  $\text{pH} > 4.8$ , not revealed for Cps compost.

Humification rates were in accordance with data obtained by carbon mineralisation tests, while humification indexes corresponded to information arising from the isoelectric focusing technique. These findings were probably due to the different organic matter fractions considered in the analytical procedures. Indeed, mineralisation trials and HR determinations were based on total organic carbon contained in the studied composts, while HI and IEF were referred to total extractable carbon fraction. So, if mineralisation curves together with the HR could furnish quantitative information on the organic matter stability, the HI and the IEF are able to demonstrate changes in compost organic matter quality.

In conclusion, the composting processes developed in a regular way both in presence and absence of sludge in the starting mixture. Moreover, mineralisation patterns indicated that organic matter stability to soil microbial degradation activity was reached in advance in presence of sludge in respect to the compost produced without this starting material. The potential technological and economical consequence of this observation should deserve further investigation.

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