Combining pre-treatment strategies for broilers industry waste valorization

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ABSTRACT

aiming at expanding the possibilities for broiler slaughter wastes valorization, composting piles were submitted, at different time points (T_0, T_{10}, T_{20}, T_{30} and Control) representing the treatments, to a solid-liquid fraction separation (FS), after being submerged in water (2:1, water:compost, in the fresh weight). After FS, solid material separated with a strain was again placed in piles for the final stage of composting, being evaluated the organic composts obtained after the stabilization phase (65 days) and maturation (95 days), in the different treatments. Reductions in mass (60–62%) and volume (56–64%) were greater in piles submitted to FS in comparison to control piles (52% and 54%). On the other hand, the FS induced greater losses of C (70.3–71.3%), N (55–62%), P (41.7–54.4%) and K (62.3–72.1%) in comparison to the control (65.2%, 48.0%, 28.1%, and 37.6%, respectively). We conclude that, as a way of integrating bioprocesses, FS does not have negative effects on the composting process. Moreover, compost mixtures from FS-treated piles, when used as substrate, yield better seedlings in comparison to mixtures from control.

1. Introduction

Solid wastes from the broiler industry include those generated during the hatching and grow out phases (bedding materials, hatchery wastes and feed factory waste) and those generated during slaughter and meat processing (floatation sludge and sausage casings). In Brazil, composting represents the main biological process used in the stabilization of such materials (Carneiro et al., 2013; Costa et al., 2016, 2017).

Some of these wastes may provide sources of energy when submitted to anaerobic digestion (AD). Matter et al. (2017) concluded that anaerobic co-digestion of hatchery wastes with a mixture of pig farm and hatchery wastewaters yielded 205 L of biogas per kg of added volatile solids (VS). When evaluating the methane and biogas production potential from co-digestion of flotation sludge, Damaceno et al. (2018) concluded that addition of 20% sweet potatoes (dry matter) increased methane yield (503.2 L kg VS^{-1}).

Although this energy potential exists, in Brazil, the composting process represents the main biological process used for the treatment of these wastes, since they are generated in large quantities (Carneiro et al., 2013). The composting process is effective in stabilizing and reducing the mass and volume of organic solid wastes due to the oxidation of the organic material in CO_2 (Costa et al., 2016). In addition, composting is also a way of valorizing these residues by allowing their agronomic use through the organic compost as a soil conditioner. However, this compost can also be used for higher value purposes as a substrate for seedling production, for example (Costa et al., 2017).

These aspects, coupled with the fact that the wastes from broiler chicken productive chain are rich in nutrients and minerals, resulting in the production of organic compost with high electrical conductivity (EC) by the concentration of the salts. Numerous studies identified problems with the use of organic compost as an alternative substrate when the material displays high EC. Excess salts are a limiting and phytotoxic factor for the development of the root system of the seedling and can cause delay in the germination of the seeds (Zhong et al., 2018).

In this sense, submitting the composting piles to hydrolysis in static tanks followed by the separation of the solid and liquid fractions (FS) could be a strategy capable of making the broiler chicken sector even more sustainable for two reasons: 1) the solid fraction would present

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lower electrical conductivity and could proceed to composting, in order to provide an ideal compost for the production of seedlings; 2) the liquid fraction, because it contains particles with a grain size lower than the mesh of the sieve, solubilized organic molecules and the salts of the wastes, could be submitted to anaerobic processes and promote the conversion of the labile carbon into biogas (Amaral et al., 2016).

The hydrolysis followed by FS applied to the composting piles, depending on the stage in which the composting process occurs, can affect the chemical composition of the solid and liquid phases differently and positively or negatively alter the proposed integration of biological processes. Lin et al. (2018) suggested that composting could be integrated to AD as a pre- or post-treatment method. Composting as a pre-treatment of lignocellulosic biomass may improve the hydrolysis of complex substrates during AD due to the degradation of lignin. The determination of the most suitable phase of the composting process to submit the mass of wastes to the hydrolysis followed by FS can provide the maximum use of the wastes from the broiler chicken productive chain in the solid and liquid phases, adding higher value to the final product. However, no previous work has shed light on this matter.

Considering the above, composting piles were submitted at different times to hydrolysis in static tanks followed by FS. We aimed at evaluating the effects of this strategy on composting performance as well as on the physicochemical and spectroscopic characteristics of the final compost. In addition, we tested the compost as a substrate for the production of tomato seedlings during the stabilization (69d) and maturation (99d) phases.

2. Materials and methods

2.1. Waste characterization

Table 1 displays the physicochemical characteristics of the broiler industry waste, the urban tree trimmings and also the initial characteristics of the piles used in the present study of composting strategies. Trimmings comprised of crushed leaves and branches were used as a primary source of carbon, and as a bulking agent. Costa et al. (2017) provided a complete description of these materials.

2.2. Composting process

The experiment was conducted in the Experimental Center of Agricultural Engineering belonging to Western Parana State University (Cascavel, Paraná, Brazil). Composting piles were initially formed with a trapezoidal format (approximately 1.0 m wide x 1.5 m long x 1.0 m high) within a covered area with cemented floors. The ten piles were divided into five treatment groups, including four FS treatments at days 0, 10, 20 and 30 (T_0, T_{10}, T_{20} and T_{30}, respectively) and a control group not submitted to FS (Control). Initial composition of the ten piles was identical regarding the amount of wastes and consequently the C:N ratio (Table 1).

According to our experimental design (Fig. 1), the two T_0 piles were separately immersed in water, at ambient temperature, in the proportion

of 2:1 (water:compost) in a box that simulated the equalization tank. The water-compost mixture was allowed to rest for 24h, after which, the solid-liquid fractions were separated using a strain with 5 mm mesh. The liquid fraction was collected in a tank, and the solid fraction, retained by the strain, was returned to the composting site. The same procedure was repeated for T_{10}, T_{20} and T_{30}. The two control piles were maintained in the composting site and were not submitted to FS.

During the composting process, piles were turned twice weekly. Moisture was maintained at an optimal level for microbial metabolism (50–60%) with the addition of water after turning the material. Air temperature and the temperature inside the piles were monitored daily with an analogical thermometer. Pile mass and volume were determined every 10 days using, respectively, a digital scale Filizola ID 1500 with capacity for 300 kg and 0.2 kg precision, and a wooden box measuring $1.80 \times 1.20 \times 0.8$ m. After weighing, the waste mass was packed in the wooden box and leveled. The height of the wastes mass was determined with the aid of a scale and then the volume of the piles was calculated.

At day 65, we stopped revolving the piles, which were maintained on the composting site until they reached a moisture level that was adequate for the sieving, conducted at day 69. At this point the pile stabilization phase (S) was complete. After the S phase, piles were kept on the composting site, for a further 30 days, with adequate moisture correction for the maturation phase (M) of the compost. The physicochemical characteristics of compost materials were assessed after stabilization (S_0, S_{10}, S_{20}, S_{30}, S_C) and after maturation (M_0, M_{10}, M_{20}, M_{30}, M_C).

2.3. Physico-chemical and spectroscopic analysis

Total Kjedahl Nitrogen (TKN), phosphorus (P) and potassium (K) were determined according to the methodology proposed by Malavolta et al. (1997). Total solids (TS), VS and ashes were determined by gravimetric methods, based on sample drying and ignition (APHA, 2005). Total organic carbon (TOC) was obtained by dividing the VS percentage by 1.8 (Carmo and Silva, 2012). The C:N ratio was obtained from the ratio of TOC to TKN. The cation exchange capacity (CEC) was determined according to the methodology proposed by Brasil (2014). The CEC/TOC ratio was calculated according to the same methodology. The organic matter losses (OM) were calculated from the initial (X_1) and final (X_2) ash content according to the Eq. (1) of Paredes et al. (2000):

$$\text{OM loss } (\%) = 100 - 100 \, [X_1(100 - X_2)] \, / \, [(X_2(100 - X_1)] \tag{1}$$

The concentrations of the volatile acids (acetic, propionic, butyric and formic) were determined by High Performance Liquid Chromatography (Shimadzu® 2010), equipped with Aminex® HPX-87H column (300 mm, 7.8 mm), CTO-20A furnace the temperature of 64 °C, CBM-20A controller, UV detector with SPD-20A diode arrangement at wavelength of 208 nm and LC-20AT pump. The mobile phase was composed of ultrapure water Milli-Q (Millipore®) acidified with 0.005 mol L^{-1} H_2SO_4 in a flow of 0.5 mL min^{-1} and injection volume of 20 μL (Penteado et al., 2013). The samples were filtered on a 0.2 μm pore glass vibration membrane and acidified with 2 mol L^{-1} H_2SO_4 solution.

Fourier transform infrared (FTIR) spectra were obtained according to the methodology proposed by Stevenson (1994). The samples were compacted in KBr pellets in the ratio 1:100 (compost: KBr). Afterwards, analyzed from 32 scans in the range of 4000 to 400 cm^{-1} with spectral resolution of 4 cm^{-1}. The FTIR analyzes were performed on the Frontier Perkin-Elmer spectrometer, belonging to the Analysis Center of the Federal Technological University of Paraná, Campus Pato Branco-PR.

2.4. Organic matter extractable in water

Aqueous extraction of OM was done following the methodology proposed by Said-Pullicino et al. (2007) with modifications. To the fresh compost was added deionized water (1:10, m v^{-1}). The material was then stirred for 24 h. After stirring the material was centrifuged at 3000 rpm

Table 1
Physical and chemical characterization of the ten piles and the wastes used in them.

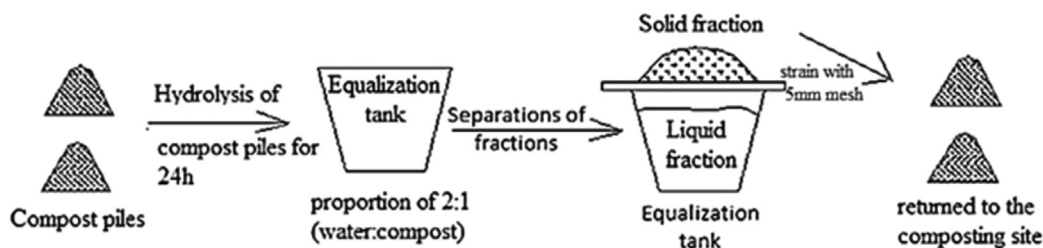| Wastes/piles	TOC (%)	TKN (%)	C:N	P (g kg^{-1})	K (g kg^{-1})	Amount in kg of DM
Tree trimmings	51.4	1.3	40	2.6	6.1	58.3
Hatchery	12.1	3.0	4	4.9	1.2	21.3
Feed	50.9	3.7	14	12.7	6.5	10.2
Sausage skins	37.8	0.5	71	8.2	19.2	18.8
Flotation sludge	49.4	7.8	6	45.9	0.7	16.4
Piles	44.1	2.4	18	8.8	6.8	125

Fig. 1. Scheme of the experimental design.

for 30 min and then vacuum filtered using a 0.45 μm aperture cellulose nitrate filter. Before the centrifugation of the material, immediately after the agitation, pH and EC readings were made in pH meter (TECNAL®, model TEC-3MP) and conductivity meter (MS Tecnopon®, model mCA 150).

The filtered extract was used to determine the soluble carbon (Cw) and the soluble nitrogen (Nw), using the Shimadzu TOC analyzer. The determination of the germination index (GI) adapted from Zucconi et al. (1981) was also performed using the obtained extract. Petri dishes (9.5 cm diameter) with double filter paper, both autoclaved were used 3 mL of the aqueous extract and 10 seeds of watercress (*Lipidium sativum*) were placed on the filter paper. The assembled plates were wrapped in plastic packages to prevent loss of moisture and incubated in a BOD chamber at 26 °C, without photoperiod, for 72 h. After the incubation period, the germinated seeds were counted and their respective rootlets were measured to calculate the GI. Ten plates of each treatment were prepared at the end of the stabilization phase (S_0, S_{10}, S_{20}, S_{30} and S_C) and after the maturation phase (M_0, M_{10}, M_{20}, M_{30} and M_C). As a control, 10 plates moistened with 3 mL of distilled water were made.

The germination index test was calculated as the product of the percentage of viable seeds, which was performed by monitoring the emergence of seedlings, number of germinated seeds (after 24 h) and root growth (after 72 h), using the following Eq. (2):

$$GI\% = (NGext \times LRext) / (NGwater \times LRwater) \times 100 \tag{2}$$

In which NGext and NGwater represent, respectively, the number of seeds germinated in soluble extracts and in distilled water (control); and LRext and LRwater represent the root length in soluble extracts and in distilled water (control), respectively.

2.5. Evaluation of the compost as a substrate for seedling production

The organic composts produced were evaluated for its use as substrate for the production of tomato seedlings (*Solanum lycopersicum*) under greenhouse conditions. The experimental design was a complete randomized complete block design in a factorial scheme, the main factor being represented by the different periods in which the piles was submitted to FS and the secondary factor by the phases in which the organic compost was, that is, stabilization and maturation. Six trays were produced (2 per block containing the 10 treatments), with 20 cells being filled for each treatment. The seed evaluations were performed after observing the first seedling emerged (8 days after sowing), obtained by counting the number of seedlings emerged for 16 days. It was considered emerged the seedlings that presented the cotyledons totally free and normal. The number of emerged seedlings was counted daily over a period of 16 days and the Emergence Speed Index was calculated by Eq. (3) according to Maguire (1962):

$$ESI = N1/D1 + N2/D2 + ... + Nn/Dn \tag{3}$$

in which:

ESI – Emergence speed index;

N – number of seedlings checked on the day of the counting;
D – number of days after the sowing at which the counting was performed.

In order to calculate the seedlings quality index (SQI) by Eq. (4), five plants of each replicate were used and the following parameters were evaluated: seed height, diameter, dry mass of shoot and root (Dickson et al., 1960).

$$SQI = \frac{TDM}{\frac{H}{D} + \frac{APDM}{RDM}} \tag{4}$$

in which:

TDM – total dry mass (g);
H – plant height (cm);
D – diameter (cm);
APDM – aerial part dry mass (g);
RDM – root dry mass (g).

2.6. Statistical analysis

With the daily thermal profiles obtained from the piles, the square exothermic index accumulation (EXI^2) was calculated as the quadratic sum of the daily difference between the temperature inside the pile and that in the ambient temperature during the bio-oxidative phase of composting.

The losses of OM during the degradation process were adjusted to a first order exponential function (Paredes et al., 2000), using the Eq. (5):

$$Loss\ of\ OM\ (\%) = A\ (1-e^{-kt}) \tag{5}$$

In which A is the maximum degradation of OM (% C), k is the rate constant (d^{-1}) and t is the composting time (days). The values of the mean square of the residual (MSR) and F were calculated to compare the adjustments of different functions and the statistical significance of the adjustment curve.

The variables pH, EC, TOC, TKN, P, K, C_w, N_w, CEC, CEC:TOC were analyzed jointly by the multivariate technique through principal component analysis (PCA). The main component selection criteria were the percentage of explanation of the total variance above 70% (Ferreira, 2011) and eigenvalues higher than 1 ($\lambda \geq 1$) (Kaiser, 1958).

A factorial scheme was used to GI, ESI and SQI, being the main factor represented by the different times in which the piles were submitted to FS (T_0, T_{10}, T_{20}, T_{30} and C) and the secondary factor by the phases in which the organic composts were, that is, S or M. The data presented were individually evaluated using ANOVA and multiple comparison test of Scott Knott averages with 5% significance.

Multivariate analyses were evaluated by PCA using Pirouette software (version 4.0, Infometrix Inc., Woodville, WA, USA). Before the analysis, the physical-chemical data were autoscaling and the spectral data were mean-centered and the pretreatment multiplicative signal correction (MSC) was used.

3. Results and discussion

3.1. Performance of the composting process

Temperature provides an important indicator of the stage of the composting process, regarding organic matter degradation and maturation (Cotta et al., 2015) and also reflects composting efficiency (Zhang and Sun, 2016). Fig. 2 shows that piles submitted to FS had slightly lower temperatures than control piles. The difference may arise from the 24-h interruption of the process, a time during which the material was submersed in water in the equalization tank.

The thermophilic phase of the composting process, when temperatures remain above 40 °C, spanned 46 and 50 days for FS-treated piles and control, respectively (Table 2). In all groups the temperature remained above 55 °C for more than 15 days, which sanitized the compost according to Gavilanes-Téran et al. (2015). The treatments T_0, T_{10}, T_{20} and T_{30} presented a smaller exothermic accumulation when compared to the control, due to the shorter duration of the thermophilic phase caused by the interruption of the composting process to perform the FS. Thus, FS treatment affected the thermal profile of piles, but not the minimum time during which temperatures should remain above 55 °C.

Initial pile mass and volume, as well as the changes that occur during the composting period are key factors in determining the size and optimizing composting sites (Costa et al., 2017).

At the end of the composting process, FS-treated groups displayed similar changes among them, with greater mass reduction than observed in control group (Table 3). The higher losses (60–62% total loss) observed among treatment groups may have resulted from greater moisture – submersion in water for 24h might have hydrated fibrous materials to a larger extent allowing for better degradation. According to Wang et al. (2015), moisture level affects the degradation of soluble organic compounds and the hydrolysis of fibrous substrates. It is also possible that some solid materials were lost through the strain used for FS, which would have affected final pile mass.

All piles had the same dry matter mass at the beginning of the study. However, volumes varied slightly because of the heterogeneity of the material, especially tree trimmings. Volume reduction followed a similar pattern to mass reduction, and control groups decreased less than all treatment groups.

Table 4 shows that, regardless of FS, all groups lost TOC, TKN, P and

Table 2
Control parameters developed during the composting process.

Parameter	T_0	T_{10}	T_{20}	T_{30}	C
Bio-oxidative phase (days)	65	65	65	65	65
Thermophilic phase[a] duration (days)	46	46	46	46	50
Maximum temperature (°C)	70.0	68.1	70.0	69.8	70.9
Maximum temperature (day)	11°	8	11	11	11
Temperature ≥55 °C (days)	17	20	20	24	25
Cumulated EXI[2] [b]	53530	49740	56357	56362	68433
EXI[2]/days of bio-oxidative phase	824	765	867	867	1053

T0-Separation of the solid-liquid fraction at the beginning of the composting; T10-Separation of the solid-liquid fraction at 10 days of composting; T20-Separation of the solid-liquid fraction at the 20 days of composting; T30-Separation of the solid-liquid fraction at 30 days of composting; C-No separation of fractions.

[a] Thermophilic phase: temperature >40 °C.
[b] EXI[2]: Square Exothermic Index accumulation.

Table 3
Weight in dry matter and volume at the beginning and in the end of each treatment.

Treatments	Dry matter weight (kg)		% Red	Windrow volume (m³)		% Red
	Initial	Final		Initial	Final	
T0	125	48.3	61	0.66	0.29	57
T10	125	49.6	60	0.64	0.28	56
T20	125	48.7	61	0.64	0.27	58
T30	125	48.0	62	0.69	0.25	64
C	125	59.5	52	0.68	0.31	54

%Red-percentage reduction; T0-Separation of the solid-liquid fraction at the beginning of the composting; T10-Separation of the solid-liquid fraction at 10 days of composting; T20-Separation of the solid-liquid fraction at the 20 days of composting; T30-Separation of the solid-liquid fraction at 30 days of composting; C-No separation of fractions.

K. According to Wang et al. (2017), loss of nutrients, especially N, is an inevitable problem of the composting process of organic raw materials. Key factors affecting N losses include initial pile composition, C:N ratio, temperature, turning frequency of pile (Bernal et al., 2009). During the present study, high thermophilic phase temperatures of 68.0–70.9 °C and twice weekly turnings of piles may have contributed to N losses. The
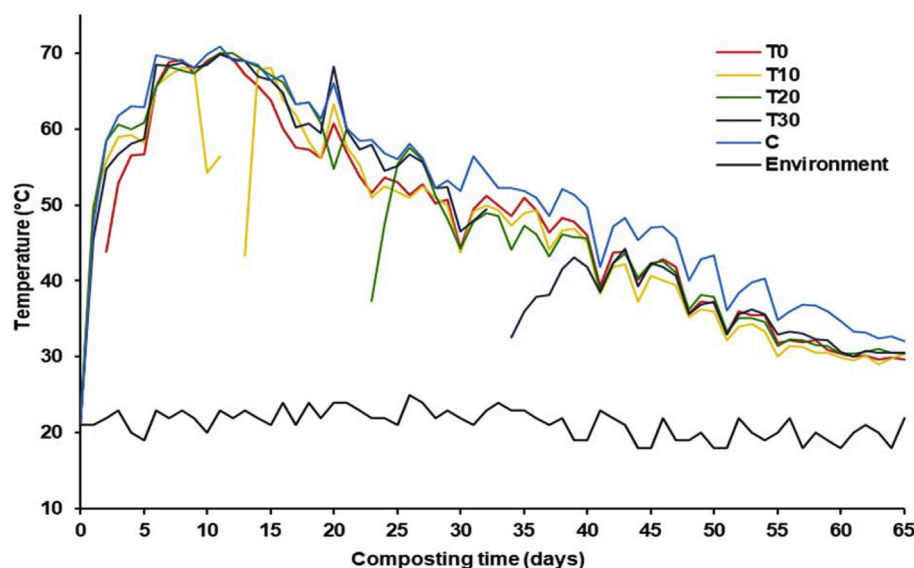

Fig. 2. Temperature profile. T0-Separation of the solid-liquid fraction at the beginning of the composting; T10-Separation of the solid-liquid fraction at 10 days of composting; T20-Separation of the solid-liquid fraction at the 20 days of composting; T30-Separation of the solid-liquid fraction at 30 days of composting; C-No separation of fractions.

Table 4
Mean values of total organic carbon (TOC), total nitrogen (TKN), total phosphorus (P), total potassium (K) and percentage of reduction.

Variables		Treatments				
		T0	T10	T20	T30	C
TOC	Initial (kg)	55.2	55.2	55.2	55.2	55.2
	Final (kg)	16.3	15.9	16.4	16.1	19.2
	Reduction (%)	70.4	71.3	70.3	70.9	65.2
TKN	Initial (kg)	3.0	3.0	3.0	3.0	3.0
	Final (kg)	1.4	1.2	1.2	1.2	1.6
	Reduction (%)	55.0	62.0	60.7	61.0	48.0
P	Initial (kg)	1.1	1.1	1.1	1.1	1.1
	Final (kg)	0.6	0.5	0.5	0.6	0.8
	Reduction (%)	48.0	55.1	54.4	41.7	28.1
K	Initial (kg)	0.9	0.9	0.9	0.9	0.9
	Final (kg)	0.2	0.3	0.3	0.3	0.5
	Reduction (%)	72.1	69.0	66.6	62.3	37.6

T0-Separation of the solid-liquid fraction at the beginning of the composting; T10-Separation of the solid-liquid fraction at 10 days of composting; T20-Separation of the solid-liquid fraction at the 20 days of composting; T30-Separation of the solid-liquid fraction at 30 days of composting; C-No separation of fractions.

greater losses of TKN observed in FS-treated piles could be related to a reduction in the concentration of soluble forms of N, including NH_4^+- N and NO_3 - N. Wang and Zeng (2018) indicated that, during composting, N_{org} is mineralized into aqueous phase ammonia ($NH_3(l)$) after organic matter (OM) degradation. The $NH_3(l)$ incorporates one proton and becomes aqueous phase NH_4^+, which increases substrate pH. Bernal et al. (1998) observed a decrease in NH_4^+ after the thermophilic phase in a majority of evaluated protocols, which led to an increase in NO_3^- concentration due to nitrification. Table 4 shows that the smaller reduction (55%) occurred in T_0 piles. Among the other treatment groups, T_{10} displayed the greatest N losses. The reasons of the differences observed are related to the phase in which the FS occurred. The earlier the FS, the smaller the concentrations of NH_4^+ and NO_3^-, the smaller the loss of N.

The mass of P and K also decreased with the process. The greatest losses were observed in FS-treated piles, probably due to leaching of the inorganic fraction of these nutrients (Costa et al., 2017). Losses of K were lower in groups that went through FS at later time periods. The pattern may result from K adsorption by OM, which, with longer degradation periods, increases in surface area. Altogether, our results suggest that FS promotes the loss of nutrients leading to lower economic value of the final compost.

OM content decreased with time due to composting-induced mineralization. Reductions during the bio-oxidative phase represented 59.6,

62.3, 60.0, 60.8 and 64.2% for groups T_0, T_{10}, T_{20}, T_{30} and C, respectively (Fig. 3).

OM loss during composting was adjusted to fit a first order kinetic equation, whereby OM loss = A ($1-e^{-kt}$). The equation provided parametric values displayed on Table 5, where standard deviation is shown in parenthesis. The values of A were, in general, similar to those obtained by Sáez et al. (2017), who found a range of 56.5–65.5% while analyzing composts of the solid fraction derived from pig farm effluents. However, the same authors found degradation constants (0.0167 and 0.0179) and mineralization rates OM (A *k) (1.01 and 1.09% OM d^{-1}), which were lower than reported here. Our control group displayed the highest A value and the lowest OM mineralization rate (2.79%), in comparison to all treatment groups.

3.2. Effect of composting on the agronomic value of the final compost

Compost can only be safely applied to agriculture as a fertilizer or substrate when it reaches a high degree of stabilization and maturation – both of which translate into a stable level of OM, as well as the absence of phytotoxic compounds and plant or animal pathogens (Bernal et al., 2009).

The C:N ratio reflects the ease and speed with which organic waste will decompose. As OM decomposition takes place the ratio decreases. In the present study, the initial C:N ratio was the same for all groups (Table 6), as all piles had very similar compositions.

The final C:N varied between 13.5 and 14.8 among groups, values that resemble those reported by Kiehl (2010) of 8–12, and indicate that the compost is dry, mature and humified. However, the use of C:N as the

Table 5
Parameter values of the first-order equation describing OM degradation.

	A	k	F	R2a	RMS	SEE	A * k
T0	62.1 (0.9)	0.05 (0.00)	3934	0.990	0.65	0.81	3.28
T10	64.9 (3.6)	0.04 (0.01)	414	0.998	6.43	2.53	2.94
T20	61.9 (1.5)	0.05 (0.00)	1302	0.996	1.96	1.40	3.10
T30	59.4 (1.2)	0.07 (0.01)	1243	0.996	2.06	1.43	4.16
C	69.7 (4.1)	0.04 (0.01)	384	0.987	7.52	2.74	2.79

A = maximum degradation of OM (%C); k = rate constant (d-1); R2a = adjusted coefficient of determination; RMS = residual mean square; SEE = standard error of estimate. T0-Separation of the solid-liquid fraction at the beginning of the composting; T10-Separation of the solid-liquid fraction at 10 days of composting; T20-Separation of the solid-liquid fraction at the 20 days of composting; T30-Separation of the solid-liquid fraction at 30 days of composting; C-No separation of fractions.
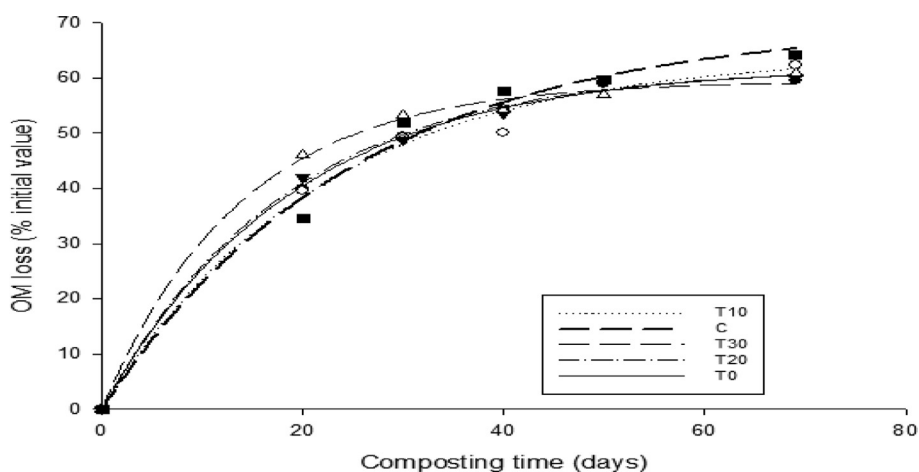

Fig. 3. Organic matter (OM) losses during composting process. Lines represent curve-fitting. T0-Separation of the solid-liquid fraction at the beginning of the composting; T10-Separation of the solid-liquid fraction at 10 days of composting; T20-Separation of the solid-liquid fraction at the 20 days of composting; T30-Separation of the solid-liquid fraction at 30 days of composting; C-No separation of fractions.

Table 6
Mean values of initial and final C and N ratio.

Variable	Treatments				
	T_0	T_{10}	T_{20}	T_{30}	C
C:N initial	18	18	18	18	18
C:N final	14.8	13.5	14.5	13.5	13.5

T0-Separation of the solid-liquid fraction at the beginning of the composting; T10-Separation of the solid-liquid fraction at 10 days of composting; T20-Separation of the solid-liquid fraction at the 20 days of composting; T30-Separation of the solid-liquid fraction at 30 days of composting; C-No separation of fractions.

sole criteria of stabilization in composting processes has shortcomings, as the ratio varies widely depending on the type of waste used.

No single property indicates the degree of maturation of an organic mixture, which should be assessed through two or more parameters (Cerda et al., 2017). To this end, Bernal et al. (2009) listed a number of indicators, including volatile organic acids (VOA) and the GI. Such indicators correlate with the phytotoxicity of organic composts.

We conducted VOA analyses of all groups at the end of the stabilization and of the maturation phases – in neither time point could we find signs of VOA. Melo et al. (2008) and Pinheiro et al. (2013) compared compost material to the initial raw material and found very low concentrations of VOA in more humified mixtures. The authors concluded that, as the mixtures become more humified, the concentration and diversity of these acids decrease. Thus, the compost produced here can be considered mature, with no signs of VOA in analyzed samples. Thus, the results obtained from this additional variable allow us to infer that the organic composts produced were mature.

The results of germination index (Table 7) show no differences, with 5% significance, among FS groups analyzed at end of the stabilization phase. All compost mixtures evaluated at the end of stabilization phase had a germination index above 100, which according to Belo (2011), indicated that the mixtures have phyto-stimulating characteristics regarding germination and root growth. These results are associated with the high temperatures ($>55\,°C$) reached during the thermophilic phase of composting eliminating potentially phytotoxic compounds (Godlewska et al., 2017).

At the end of the maturation phase, compost mixtures from C piles had no phytotoxic effects, but also had no phyto-stimulating effects. Among FS groups analyzed, T30 piles displayed the highest phyto-stimulating potential.

Thus, the FS protocol, regardless of when it occurred during the

Table 7
Mean values for the germination index (%).

Fraction separation	Maturity				
	S	Classification (Belo, 2011)	M	Classification (Belo, 2011)	
C	116.9 a A	Phytostimulant	93.7 c B	Non-phytotoxic	
T0	111.0 a A	Phytostimulant	114.8 b A	Phytostimulant	
T10	111.9 a A	Phytostimulant	110.6 b A	Phytostimulant	
T20	106.6 a A	Phytostimulant	118.6 b A	Phytostimulant	
T30	127.7 a A	Phytostimulant	133.0 a A	Phytostimulant	

Lowercase letters represent the mean difference between fractional separation levels. Capital letters represent the mean difference between maturity levels. T0-Separation of the solid-liquid fraction at the beginning of the composting; T10-Separation of the solid-liquid fraction at 10 days of composting; T20-Separation of the solid-liquid fraction at the 20 days of composting; T30-Separation of the solid-liquid fraction at 30 days of composting; C-No separation of fractions.

composting process, did not affect the phyto-stimulating quality of the organic mixture during the stabilization phase. The reduced germination index of control pile material during maturation may have resulted from the increased EC under these conditions.

Table 8 displays the physicochemical characteristics of compost materials after stabilization (S_0, S_{10}, S_{20}, S_{30}, S_C) and after maturation (M_0, M_{10}, M_{20}, M_{30}, M_C).

The pH of organic composts remained within the alkaline range of 8.1–8.6, which is adequate for agricultural use according to the US Composting Council (2001). The pH profile found here would have acid correction characteristics when applied to acidic soils, as observed by Doan et al. (2015). We observed a mild reduction in pH levels after the maturation phase for all groups including controls when compared to levels after the stabilization phase. Nevertheless, all compost mixtures remained within the alkaline range. Reduced pH levels after maturation were also observed by Sánchez-Monedero et al. (2001) who found statistically significant correlation among $N–NO_3^-$ concentration, pH and EC, suggesting that nitrification caused the decrease in pH and increase in EC. A similar pattern was observed here (Table 8). Samples at the end of the M phase, in comparison to the end of the S phase, had reduced pH, as well as increased TKN and EC.

The increase in EC observed during the maturation period may have also partly resulted from the loss of carbon. Residual TOC degradation by microorganisms during this stage (Table 8) caused an increase in salts and, consequently, an increase in EC as observed by other authors (Costa et al., 2016). Under other conditions the same increase in EC was not observed. Carneiro et al. (2013) evaluated the composting process in open air, and observed that the rain reduced the amount of salts in the piles, reducing this effect. Similarly, FS-treated piles had lower EC values than controls (Table 8). The EC values observed in treated piles suggest the material may have limited use as substrate for seedlings (Costa et al., 2017).

The TOC reduction between initial conditions, end of S phase and end of M phase translated into an increase in the concentration of other analyzed nutrients (N, P and K), even though their absolute mass also decreased. Water-soluble carbon (C_w) has been used as an indicator of organic compost maturation (Bernal et al., 2009). The values we observed at the end of the S phase, below $10\,g\,kg^{-1}$, lie within the limits for stable composts, as determined by Hue and Liu (1995). During the M phase further reduction of these values were observed. The variation was small in the control treatment, which presented the higher values of C_w. Submersion in water followed by FS certainly promoted C_w leaching into the liquid fraction. Nevertheless, this energy would be recovered upon AD of the liquid fraction.

Table 8 shows that N_w values increased in almost all groups during maturation. Bernal et al. (1998) indicated that large amounts of $NO_3–N$ remain in composting samples by the end of the active phase, although maximum values are reached after maturation due to nitrification. Bernal et al. (2009) suggested that nitrification, detected through the formation of $NO_3–N$, occurs when pile temperature falls below thermophilic levels ($40\,°C$). The authors also pointed out that the intensity of the process depends on the concentration of $NH_4–N$ available to nitrifying bacteria. We observed higher concentrations and stronger increase of N_w during maturation in control piles – indicating another effect of the FS protocol.

Parameters such as CEC and the ratio CEC to TOC have been widely used as indicators of humification or maturation of organic compost mixtures (Paiva et al., 2015). Harada and Inoko (1980) working with composts from urban solid waste, suggested that a mixture with CEC above $60\,cmol_c\,kg^{-1}$ (DM) would be appropriate for agricultural use. However, Bernal et al. (2009) raised reservations against this type of waste and animal waste. Here, with the exception of M_{10}, we observed CEC values above $60\,cmol_c\,kg^{-1}$ (Table 8). The CEC:TOC ratios in the present study, except for M_{10}, were above required to indicate good humification (≥ 1.7; Roig et al., 1988) and good maturation (≥ 1.9, Iglesias-Jimenez and Perez Garcia, 1992).

A PCA was used to analyze results obtained from physical-chemical

Table 8
Physical-chemical parameters evaluated in the initial and at the end of stabilization (S) and maturation (M) phase.

Treatments	pH	EC (mS cm^{-1})	TOC (%)	TKN (%)	P (g kg^{-1})	K (g kg^{-1})	Cw (g kg^{-1})	Nw (g kg^{-1})	CEC (cmol kg^{-1})	CEC:TOC ratio
Initial	7.24 ± 0.2	2.34 ± 0.09	44.11 ± 0.6	2.42 ± 0.1	8.78 ± 1.3	6.8 ± 0.4	19.47 ± 1	2.14 ± 0.1	59.05 ± 13	13.39 ± 0.3
S_0	8.5 ± 0.08	1.37 ± 0.04	34.9 ± 0.6	2.35 ± 0.08	10.8 ± 0.3	7.5 ± 0.06	5.7 ± 0.4	0.64 ± 0.03	65.0 ± 2	1.9 ± 0.1
M_0	8.2 ± 0.2	1.44 ± 0.01	32.0 ± 0.3	2.6 ± 0.1	10.2 ± 0.5	8.7 ± 0.1	4.0 ± 0.4	0.73 ± 0.03	66.0 ± 5	2.1 ± 0.2
S_{10}	8.4 ± 0.1	1.18 ± 0.08	32.4 ± 0.4	2.4 ± 0.1	9.9 ± 0.1	6.7 ± 0.6	3.9 ± 0.5	0.44 ± 0.03	61.4 ± 0.8	1.9 ± 0.05
M_{10}	8.1 ± 0.4	1.31 ± 0.00	31.9 ± 0.2	2.51 ± 0.07	9.3 ± 0.1	8.2 ± 0.5	3.5 ± 0.4	0.42 ± 0.00	53.0 ± 2	1.7 ± 0.1
S_{20}	8.6 ± 0.02	1.31 ± 0.00	35.2 ± 0.03	2.43 ± 0.00	10.2 ± 0.6	7.9 ± 0.2	5.0 ± 0.2	0.44 ± 0.00	69.0 ± 4	2.0 ± 0.1
M_{20}	8.4 ± 0.1	1.32 ± 0.00	32.1 ± 0.2	2.45 ± 0.08	9.7 ± 0.7	10.2 ± 0.5	3.9 ± 0.05	0.41 ± 0.00	73.4 ± 0.6	2.3 ± 0.03
S_{30}	8.3 ± 0.2	1.28 ± 0.04	33.9 ± 0.4	2.51 ± 0.02	11.8 ± 0.4	8.8 ± 0.9	5.8 ± 0.04	0.50 ± 0.00	70.0 ± 3	2.1 ± 0.10
M_{30}	8.2 ± 0.00	1.34 ± 0.01	29.2 ± 0.7	2.76 ± 0.04	10.74 ± 0.09	11.1 ± 0.5	4.9 ± 0.2	0.58 ± 0.02	68.0 ± 3	2.3 ± 0.06
S_C	8.5 ± 0.1	2.30 ± 0.3	35.0 ± 2	2.6 ± 0.1	11.78 ± 0.09	12.8 ± 0.2	7.9 ± 0.08	0.88 ± 0.06	80.0 ± 5	2.3 ± 0.01
M_C	8.4 ± 0.04	2.73 ± 0.02	31.3 ± 0.3	2.9 ± 0.1	10.93 ± 0.09	16.0 ± 0.1	7.8 ± 0.00	1.03 ± 0.00	65.1 ± 0.1	2.1 ± 0.02

caracterization of composts after stabilization (S_0, S_{10}, S_{20}, S_{30} and S_C) and after maturation (M_0, M_{10}, M_{20}, M_{30} and M_C) stages (Fig. 4).

The combined analysis of score (Fig. 4A) and loading (Fig. 4B) plots allows finding out physical-chemical characteristics related to sample groups. Scores provide the principal component (PC) structure related to the samples, while loadings provide this same structure related to the variables.

The scores plot, in which the first (PC_1) and the second principal components (PC_2) explain 50.1 and 21.1% of data variability respectively (Fig. 4A), suggest the formation of two groups: (1) composts after stabilization and (2) composts after maturation stages. Thus, there is a variation in the physical-chemical characteristics of composts regarding the composting time. It is also possible to observe dispersion among samples associated with the FS time, suggesting heterogeneity of the composts.

The loadings plot showed the influence that each measured variable (physical-chemical parameters) had in each sample (Fig. 4B). The loadings plot allows the inference that the TOC and pH are the most important physical-chemical characteristics which separate the composts (S) from the composts (M). CEC has a highly positive correlation with the control samples of stabilization phase (Sc). The variables Nw, K and EC are linked to M_C samples.

3.3. Fourier transform infra-red - FTIR

The FTIR spectra of composts after stabilization and maturation stages are given in Fig. 5.

The chemical structure characteristics of the composts were observed from FTIR spectra. The attribution and interpretation of absorbance signals in the region of 4000 to 400 cm^{-1} were performed according to Fang et al. (2016). In general, the spectra showed similarity, indicating no major qualitative changes. The main absorbance signals were at 3406, 2924, 1644, 1423 and 1035 cm^{-1}. The broad band at 3406 cm^{-1} can be attributed to the O–H stretching vibration of phenolic compounds. The signal at 2924 cm^{-1} is characteristic to the C–H vibrations of aliphatic groups, the weaker the signal intensity, the greater the degree of the material humification. The signal at 1644 cm^{-1} is associated to C=C in aromatic structure and C=O, in this case, unlike the aliphatic chains, the greater the signal intensity of the aromatic structures, the greater the degree of humification of the material. The signals for O–H of phenols, COO^{-}, –CH$_3$ and amide II are observed at 1423 cm^{-1}. The signal observed at 1035 cm^{-1} is associated to –C–O–C of carbohydrates, aromatic ethers, Si–O–C groupings. Santos (2016) studying the final compost from broiler chicken agro-industrial wastes and urban tree trimmings also observed the presence of functional groups associated with OH stretching (3425 cm^{-1}), CH stretching of low intensity aliphatic chains (2943 cm^{-1}) and stretching of aromatic rings (1441 cm^{-1}).

Despite the qualitative similarity among the spectra, the absorption intensity of composts varied. The spectral data interpretation was supported by PCA. The use of FTIR coupled with PCA can be employed to improve data interpretation, due the amount of information provided by spectroscopy method. Previous research has reported PCA to be an adequate technique for correlating analytical data (Costa et al., 2017).

The PCA, in which PC_1 and PC_2 explain 69.5 and 16.3% of the total variance respectively, shows two groups of samples (Fig. 6) and suggests there is a difference in the structure between the composts after stabilization and after maturation stages. The first group comprised by samples after stabilization stage (S_0, S_{10}, S_{30} and S_C) and the second group by samples after maturation stage (M_0, M_{10}, M_{20}, M_C) and the S_{20} sample.

Therefore, it can be inferred that the greatest effects related to the quality of the organic composts obtained refer to the maturation phase and not to the submission of the composting mass to the FS.
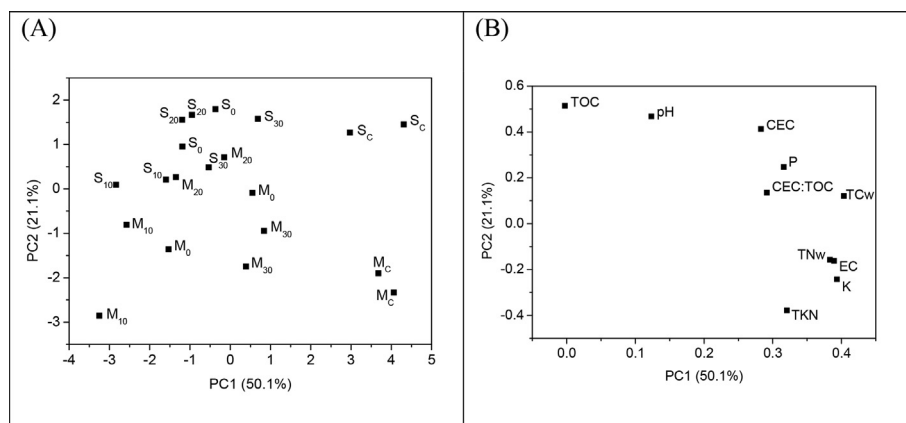

Fig. 4. (A) Scores plot of the samples after 65 days (S_0, S_{10}, S_{20}, S_{30} and S_C) and after maturation stage (M_0, M_{10}, M_{20}, M_{30} and M_C) and (B) loading plot from physical-chemical results of the composts after stabilization and maturation stages.
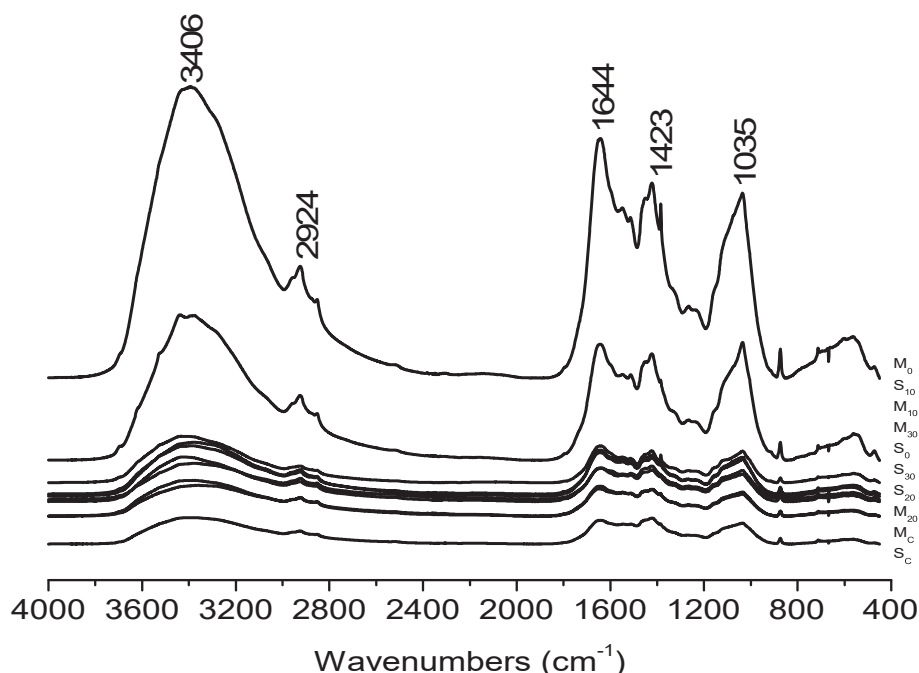
Fig. 5. FTIR spectra of the composts after stabilization (S$_0$, S$_{10}$, S$_{20}$, S$_{30}$ and S$_C$) and maturation stages (M$_0$, M$_{10}$, M$_{20}$, M$_{30}$ and M$_C$).
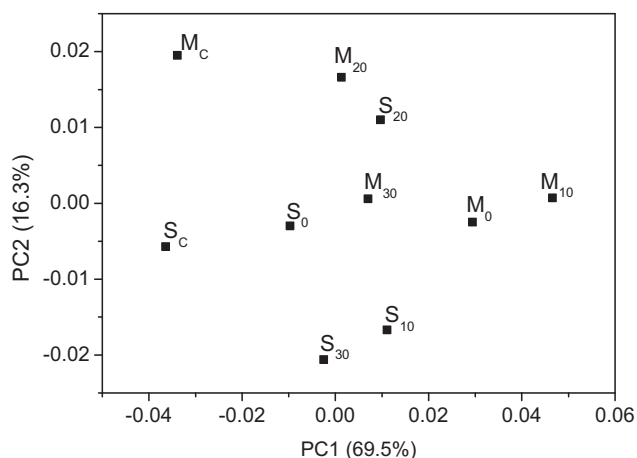

Fig. 6. Scores plot from FTIR results of the composts after stabilization (S$_0$, S$_{10}$, S$_{20}$, S$_{30}$ and S$_C$) and maturation stages (M$_0$, M$_{10}$, M$_{20}$, M$_{30}$ and M$_C$).

Table 9
SQI mean values.

Treatments	SQI
T30	0,0064 a
T20	0,0056 a
T10	0,0055 a
T0	0,0045 b
C	0,0031 b

Lowercase letters represent the mean difference between fraction separation levels by the Scott Knott test at 5% significance. T0-Separation of the solid-liquid fraction at the beginning of the composting; T10-Separation of the solid-liquid fraction at 10 days of composting; T20-Separation of the solid-liquid fraction at the 20 days of composting; T30-Separation of the solid-liquid fraction at 30 days of composting; C-No separation of fractions.

3.4. Seedling quality index - SQI and Emergence Speed Index - ESI

Analysis of variance did not reveal interaction (p > 0.05) between the two studied factors: time of FS and compost stage (S or M). Thus, only simple effects were analyzed. No differences between S and M phases were detected, in other words, maturation had no effect on SQI. Differences related to the FS were only observed regarding SQI (Table 9).

The FS protocol at T$_{10}$, T$_{20}$ and T$_{30}$ potentiated seedling quality in comparison to T$_0$ and control groups. The lower SQI means observed in control and T$_0$ groups may result from the higher EC levels detected in these mixtures, 2.34 mS cm^{-1} and 1.37 mS cm^{-1}, respectively. Busta-mante et al. (2008) evaluated the use of organic composts mixed to different proportions of peat as substrates for the production of green-eries and condiments. Increases in the proportion of organic compost elevated EC and, consequently, led to the production of lower-quality seedlings. The experimental group that used 100% organic compost had the highest EC level (2.24 mS cm^{-1}) and the lowest biomass pro-duction. According to Pagliarini et al. (2012) an elevated EC may alter

water absorption, reducing growth potential, and leading to burnt or withered leaves.

Analysis of variance did not reveal interaction (p > 0.05) between the two studied factors to the ESI. Thus, only simple effects were analyzed. Fig. 7A shows the mean ESI values calculated during a period of 16 days for composts in the M and S phases. Mature organic composts displayed statistically higher mean ESI than composts at the end of the S, stabili-zation phase. Fig. 7B shows mean ESI values calculated after 16 days for the different FS time points. FS-treated composts favored seedling growth, probably due to lower EC.

4. Conclusions

The hydrolysis protocol combined with solid-liquid fraction separa-tion as a pretreatment strategy increased the added value of the broiler industry wastes. Among the benefits, it has enhanced the reduction of pile mass and volume, which impacts on a smaller required area in the composting plants; reduced the EC of the organic compost, which makes the material more suitable as a substrate for the production of higher-quality tomato seedlings, as well as allowing the recovery of energy
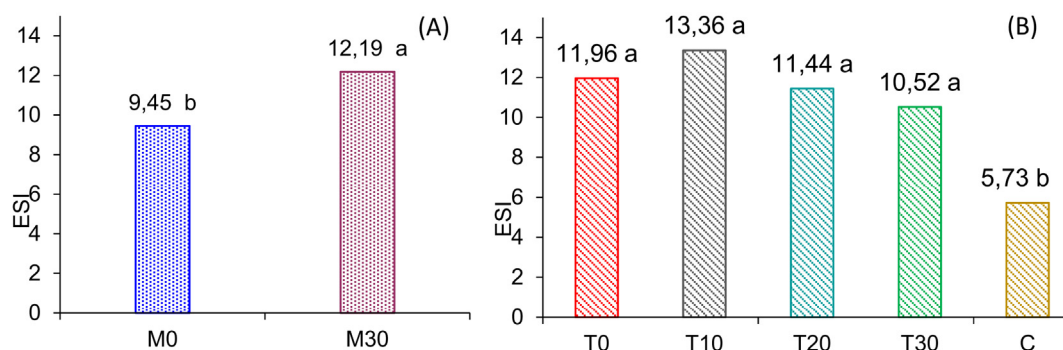
Fig. 7. ESI for the organic compost phase (A) and for the fraction separation (B). T0-Separation of the solid-liquid fraction at the beginning of the composting; T10-Separation of the solid-liquid fraction at 10 days of composting; T20-Separation of the solid-liquid fraction at the 20 days of composting; T30-Separation of the solid-liquid fraction at 30 days of composting; C-No separation of fractions. Mean values followed by different letters are significantly different by the t test ($p \leq 0.05$).

from the remaining liquid fraction of the FS process. Hydrolysis followed by FS affected the thermal profile of compost piles, but had no effect on the minimum time required for material hygiene at temperatures ≥ 55 °C but promoted a greater nutrient loss, which reduces the agricultural value of the final product as fertilizer.

Declarations

Author contribution statement

Rosana Niedzialkoski, Mônica Sarolli, Luiz Antonio Costa, Larissa Tonial, Felippe Damaceno, Higor Eisten Francisconi Lorin, Jakson Bofinger, Maico Chiarelotto: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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