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ORIGINAL ARTICLE

Recycling and valorization of distilled grape marc through vermicomposting: a pilot-scale study

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Abstract
The present study sought to evaluate the effectiveness of vermicomposting for the valorization of distilled grape marc, one of the main solid by-products of the winery sector during a 56-day pilot-scale trial. The increase in the density and biomass of earthworms (*Eisenia andrei*) during the earlier stages of the process reflected the suitability of the distilled marc (*Vitis vinifera* L. cv. Mencía) as feedstock in order to sustain large earthworm populations on a pilot-scale level. Supporting this, from 14 days onwards the pH of Mencía distilled marc fell within weak-alkaline levels and the electrical conductivity was between 0.21 and 0.11 mS cm^{-2} providing optimum conditions for earthworm growth. A rapid decrease in microbial activity as well as in the content of total polyphenols, both indicative of stabilized materials was also recorded after 14 days of vermicomposting. Moreover, the content of macro- and micronutrients in the end product matched with those considered to have the quality criteria of a good vermicompost with respect to plant health and safe agricultural use. Altogether, it underlines the feasibility of vermicomposting as an environment-friendly approach for the biological stabilization of distilled grape marc fulfilling both environmental protection and fertilizer production.

Keywords Winery industry · Distillation · Earthworms · *Eisenia andrei* · Vermicompost · Organic amendment

Abbreviations

NW	Northwestern
EC	Electrical conductivity
FW	Fresh weight
DW	Dry weight
ICP	Inductively coupled plasma
OES	Optical emission spectroscopy
USEPA	U.S. Environmental Protection Agency
ISO	International Organization for Standardization
TPC	Total polyphenol content
GAE	Gallic acid
UV	Ultraviolet
ANOVA	Analysis of variance
Tukey HSD test	Tukey Honestly Significant Difference test
LCA	Life cycle assessment

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Introduction

Vermicomposting as a means of organic waste treatment fulfils the dual purpose of environmental protection and fertilizer production [1]. In line with the circular economy principles [2, 3], the bio-conversion of biomass waste through vermicomposting contributes not only to overcome the liner "take-make-dispose" model but also to generate value-added products with the potential to improve soil health and crop yields [4, 5]. This is of particular interest for the winemaking industry whose ever increasing activity goes hand in hand with the generation of an ample variety of liquid and solid by-products that need to be treated, disposed of and reused in a sustainable manner [6].

A growing body of literature has demonstrated the feasibility of vermicomposting for processing raw grape marc derived from white and red winemaking processes [7–19; Table 1], yielding a nutrient-rich, biologically active and polyphenol-free end product. Grape marc accounts for

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Table 1 Overview of vermicomposting research studies dealing with raw and distilled grape marc

Starting material	Grape variety	Set-up conditions	Duration (days)	References
Raw marc	White grapevine cultivar (*Vitis vinifera* L. cv. Albariño)	Pilot scale	112	[9]
Raw marc	White grapevine cultivar (*Vitis vinifera* L. cv. Albariño)	Pilot scale*	720	[12]
Raw marc	White grapevine cultivar (*Vitis vinifera* L. cv. Albariño)	Pilot scale	91	[13]
Raw marc	Red grapevine cultivar (*Vitis vinifera* L. cv. Mencía)	Pilot scale	91	[14]
Raw marc	Red grapevine cultivar (*Vitis vinifera* L. cv. Mencía)	Pilot scale	112	[15]
Raw marc	White and red grapevine cultivars *(Vitis vinifera* L. cv. Albariño and Mencía)	Pilot scale	63	[18]
Raw marc	Not specified	Lab scale*	240	[11]
Raw marc	White and red grapevine cultivars *(Vitis vinifera* L. cv. Albariño and Mencía)	Lab scale*	294	[16]
Raw marc	Red grapevine cultivar (*Vitis vinifera* L. cv. Mencía)	Lab scale	15	[19]
Distilled marc	Not specified	Lab scale	112	[23]
Distilled marc	White grapevine cultivar (*Vitis vinifera* L. cv. Albariño)	Pilot scale	42	[24]
Distilled marc	Not specified	Pilot scale	180	[25]

*Refer to those studies in which a continuous-feeding vermicomposting system was used

roughly 10–30% of the crushed mass of grapes and on the whole, 10–13 Mtons are produced annually worldwide [17]. If properly treated, this winery by-product represents an important source of organic matter, macronutrients and polyphenols for its potential use as a soil amendment [8, 17]. Nonetheless, certain phenolic compounds can exert a phytotoxic activity and its presence in soil may cause inhibition problems for seed germination and disruption of root development [20].

Distillation also constitutes a common way to economically valorize grape marc for the recovery of ethanol for its further use in the elaboration of alcoholic beverages [21]. In a simplified way, the sugars present in the raw marc are subjected to alcoholic fermentation under anaerobic conditions, followed by the subsequent distillation and the release of vinasses and distilled grape marc as the main waste streams [22]. During distillation, the fluctuations in temperature and the selective pressures exerted by the reduced levels of pH and oxygen are likely to influence the chemical and microbiological properties of the resulting waste streams [23]. The plausible differences between the raw and distilled marcs' initial composition are expected to distinctly affect the dynamics of the vermicomposting process and in turn, the properties of the final vermicompost. Indeed, Gómez-Brandón et al. [24] found that distilled grape marc-derived vermicompost was characterized by a reduced bacterial communities' functional diversity, assessed as a proxy of their metabolic capacity when compared to the respective raw marc [13]. Taken together, this highlights the clear need for more comprehensive studies on distilled grape marc to further evaluate the effectiveness of vermicomposting for the biological stabilization of this winery by-product on a pilot-scale level given the limited evidence to date in comparison with raw marc [23–26] (Table 1).

A main feature that determines the polyphenol content and the microbiome composition of a wine, and consequently of the raw and the distilled grape marc, is the winemaking process [27]. In contrast to red winemaking, the fermentation of the grape juice takes place with minimal or no contact with the grape marc in the case of white wine vinification. Bearing such differences in mind, knowledge on this matter cannot simply be overlooked as the evidence we do have on raw marc [16, 18] suggests we cannot expect the same changes in response to earthworms´ activity during the vermicomposting of winery by-products derived from red and white grapevine cultivars. In the present study we sought to evaluate the effectiveness and viability of vermicomposting for the valorization of distilled grape marc obtained from *Vitis vinifera* L. cv. Mencía, which represents 95% of the annual red grape harvest in northwestern Spain, in order to yield a value-added end product for its further use as a soil amendment. First, we monitored the population dynamics of the earthworm species *Eisenia andrei* by tracking the changes in the density of cocoons, juveniles and mature individuals as well as in earthworm biomass in a pilot-scale vermireactor over a period of 56 days. Second, we evaluated the changes in the physico-chemical properties and the nutrient dynamics of the distilled grape marc, as well as in the basal respiration as a proxy of microbial activity and the total content of polyphenols throughout the 56-day vermicomposting trial.

Material and methods

Vermicomposting set-up and sampling design

The distilled grape marc (*Vitis vinifera* L. cv. Mencía) was provided by Destilerías Compostela, a distillery located in A Coruña in northern Galicia (NW Spain). The distilled marc was stored at 4 ºC until needed, and turned and moistened with water for two days prior to the vermicomposting trial.

Vermicomposting of Mencía distilled grape marc was performed in a rectangular metal pilot-scale vermireactor ($4 \times 1.5 \times 1$ m; 6 m^3; Domínguez et al. [9]) housed in a greenhouse with no temperature control. The vermireactor comprised a 10 cm layer of mature vermicompost used as a bed for the earthworms (*Eisenia andrei*) before adding the grape marc. A 5-cm layer containing the fresh distilled marc (150 kg fresh weight, fw) was placed over a plastic mesh (5 cm mesh size) to prevent the mixing of the processed grape marc and the vermicompost bedding. The density and biomass of the earthworm population were determined every 14 days during the 56-day trial by collecting 10 samples (five from above and five from below the plastic mesh) of the material in the vermireactor. The moisture content of the substrate was maintained around 85% throughout the study period and the reactor was covered with a shade cloth to prevent moisture loss.

For the characterization of the physico-chemical, chemical and microbiological properties, the distilled grape marc layer was divided into five sections and two samples (10 g) were taken at random from each section at the beginning of the experiment and after 14, 28, 42 and 56 days. The two samples from each section were combined and stored at 4 ºC prior to analysis.

Physico-chemical and nutrient analyses

The moisture content was assessed drying the samples for 24 h at 105 °C. The volatile solids content was determined from the weight loss after ignition in a Carbolite CWF 1000 muffle furnace at 550 °C for 5 h. The electrical conductivity (EC) and pH were measured in aqueous extracts (1:10 weight to volume) using a Crison CM35 conductivity meter and a Crison MicropH 2000 pH meter, respectively. Total C and N contents were determined in oven-dried (60 °C) samples in a Carlo Erba EA 1108 CHNS-O 1500 C/N analyser. The total content of the macro- and micronutrients (P, K, Ca, Mg, S, Fe, Mn, B and Mo) were assessed in oven-dried (60 °C) samples, previously subjected to nitric-perchloric digestion by optical emission spectrometry with inductively coupled plasma (ICP-OES) following the USEPA 3050 B method [28]. The cellulose, hemicellulose and lignin contents were assessed at the beginning and at the end of the vermicomposting trial (0 and 56 days, respectively) by the detergent fiber method [29] using the FibreBag System (Gerhardt, Königswinter, Germany).

Microbial activity

Microbial activity was determined by measuring the oxygen consumption using a WTW OxiTop Control System (Weilheim, Germany) according to ISO 16072 [30].

Determination of total polyphenols

The total polyphenol content (TPC) in the distilled grape marc extracts was assessed according to the Folin–Ciocalteu colorimetric method [31] and the absorbance values were measured by spectrophotometry at 760 nm (UV mini-1240, Shimadzu, Tokyo, Japan). The quantification of total phenols was performed based on an external calibration with gallic acid standard solutions as reference compound. TPC was expressed as mg gallic acid (GAE) g^{-1} dry mass.

Statistical analyses

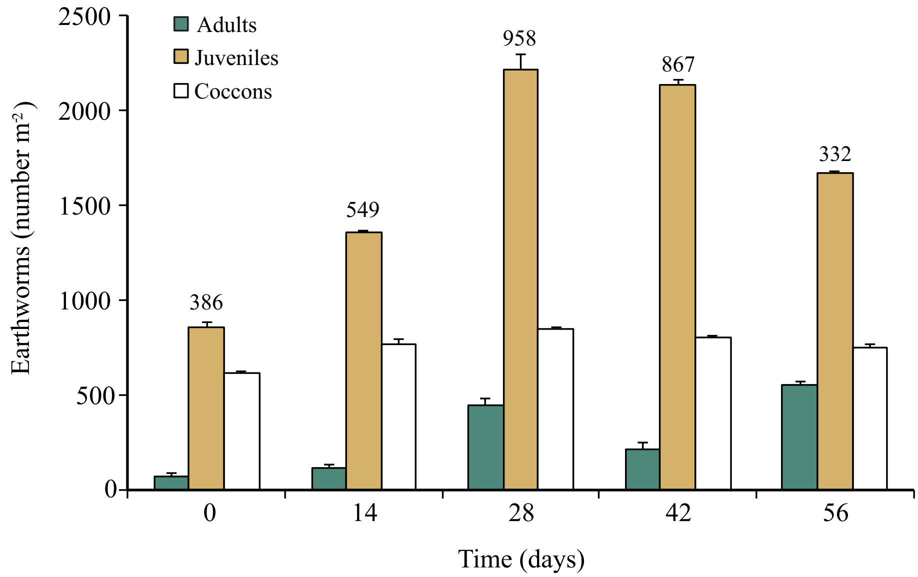
A one-way analysis of variance (ANOVA) was chosen to evaluate the changes in the earthworm population, as well as on the physico-chemical, chemical and microbiological properties throughout the vermicomposting process of the distilled grape marc. Whenever it was necessary, data were transformed to meet the normality assumptions, followed by pairwise comparison tests (Tukey HSD test) when differences were significant. A t-student was used to evaluate the changes in the fiber content (cellulose, hemicellulose and lignin) at the beginning and at the end of the vermicomposting trial (0 and 56 days, respectively). The physico-chemical, chemical and microbiological variables were also subjected to a discriminant function analysis, using the Wilks' lambda statistic and the standardized coefficients as multivariate measures of separability. All statistical analyses were performed with TIBCO Statistica programme 14.0.1.

Results and discussion

Earthworm population dynamics during vermicomposting of distilled marc

The initial earthworm population density in the vermireactor was 920 ± 15 individuals m^{-2}, including 65 ± 21 mature individuals m^{-2}, 855 ± 53 juveniles m^{-2} and 609 ± 12 cocoons m^{-2}, with a mean biomass of 386 ± 16 g m^{-2}

Fig. 1 Earthworm density (number of adults, juveniles and cocoons per square meter) and earthworm biomass (g m^{-2} fresh weight, numbers on top of the bars) throughout the vermicomposting process of distilled grape marc (*Vitis vinifera* L. cv. Mencía). Values are mean ± SE values ($n = 5$)

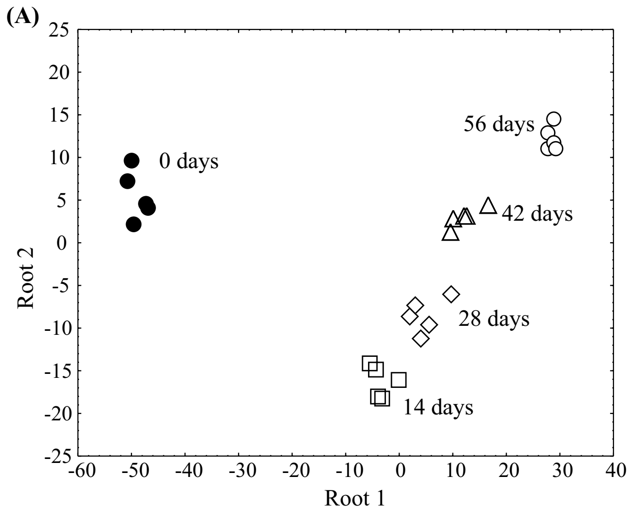


(Fig. 1). From the beginning of the trial until day 28, there was a progressive and significant increase in the earthworm biomass ($F_{4,20} = 157.9$, $p < 0.0001$), as well as in the number of mature individuals ($F_{4,20} = 20.1$, $p < 0.0001$), juveniles ($F_{4,20} = 188.7$, $p < 0.0001$) and cocoons ($F_{4,20} = 32.2$, $p < 0.0001$). A total density of 1465 ± 22 individuals m^{-2} and 2655 ± 80 individuals m^{-2} was recorded on days 14 and 28, accounting for a biomass value of 549 ± 23 and 958 ± 24 g m^{-2} respectively (Fig. 1). This increase in earthworm density and biomass during the earlier stages of vermicomposting reflects the suitability of the Mencía distilled grape marc as feedstock to sustain large populations in vermicomposting systems at a pilot-scale level. These findings are in line with those from previous vermicomposting trials performed on the raw grape marc from the same cultivar [15, 16, 18] and on other plant-derived materials (Scotch broom: Domínguez et al. [32]; Silver Wattle: Rosado et al. [33]).

Compared to day 28, the number of cocoons and juveniles reached lower values on day 56 (750 cocoons m^{-2}; 1666 immature individuals m^{-2}) (Fig. 1); whilst the number of mature earthworms was higher at this latter time point (548 individuals m^{-2}; Fig. 1). In spite of this, from day 28 onwards the total density of earthworms followed a decreasing trend having a mean value of 2214 ± 9 individuals m^{-2} and an average biomass of 332 ± 16 g m^{-2} at the end of the trial (day 56; Fig. 1). The overall decrease in the total density and biomass of earthworms at the later stages of the process is likely driven by the lower input and availability of organic matter from the distilled marc as the vermicomposting progresses. Supporting this, a positive correlation was found between the earthworms´ density and the basal respiration used as a proxy of microbial activity ($R^2 = 0.675$).

Nutrient dynamics and microbial activity during vermicomposting of distilled marc

As can be seen in the discriminant function analysis (Fig. 2a), the fresh distilled grape marc (day 0, negative side) grouped separately from the earthworm-processed samples along the first axis (days 14–56, positive side). Phosphorus, C to N ratio, and pH appeared to be the major variables responsible for this differentiation with the highest standardized coefficients (Fig. 2b). The clear separation between days 0 and 14 provides further evidence about how quickly vermicomposting modulated the physico-chemical, chemical and biological properties of the Mencía distilled marc within the first two weeks of vermicomposting. This goes hand in hand with the rapid compositional and functional changes reported by Gómez-Brandón et al. [14, 24] and Kolbe et al. [13] on raw and distilled grape marc´s bacterial communities during the first stages of the process on a pilot-scale level. Gómez-Brandón et al. [19] also found, on a mesocosm scale, that earthworms' activity (*Eisenia andrei*) promoted the stabilization of grape marc after only two weeks of vermicomposting. This was accompanied by a quick decrease in the labile carbon pool and a reduced microbial biomass and activity in the presence than in the absence of earthworms. The changes that occurred in the short term are primarily ascribed to processes of ingestion, digestion and fragmentation of the organic matter in the earthworm gut and then casting [1]. Later on, we observed that the samples collected on days 42 and 56 were grouped on the positive side of the second axis and clearly apart from the 14- and 28-day samples (Fig. 2a). The C to N ratio along with P, Fe, Mg contents were the major determinants for the clustering of the samples along the second axis (Fig. 2B). Cast ageing processes are likely to play a role as vermicomposting



(B)

Variables	Root1	Root2
Respiration	-0.7309	-0.8665
Ca	-0.5258	0.0232
K	0.0611	0.6682
P	-2.0736	-1.4879
Mg	0.8824	1.8080
S	0.4370	0.1255
Fe	0.5733	1.5033
Mn	0.2504	-0.9702
Mo	0.0200	0.1091
B	0.4817	0.4138
pH	1.1391	-0.6291
EC	-0.3703	0.8840
total C	-0.3023	-0.6137
total N	0.6687	0.4142
CN ratio	1.7685	2.3894
Eigenvalue	906.9713	160.4891
Cumulative Proportion	0.8274	0.9738

Fig. 2 Discriminant function analysis **a** and standardized coefficients **b** based on the physico-chemical properties, nutrient contents and basal respiration throughout the vermicomposting process of distilled grape marc (*Vitis vinifera* L. cv. Mencía). Full circles refer to the fresh distilled marc (day 0); and empty symbols to the earthworm-processed materials on days 14, 28, 42 and 56

progresses, leading to significative changes in the nutrient dynamics and microbiological properties of the earthworms´ egested materials in the later stages of the process [12, 16]. In this regard, Nogales et al. [23] reported a depletion of hydrolytic enzymes (i.e., ß-glucosidase) during the final stages of a 16-week laboratory vermicomposting trial with different winery feedstocks including exhausted grape marc, probably due to the depletion of readily available organic substrates throughout the process.

As occurred for the raw marc from Mencía cultivar [14, 15], the vermicomposting of the distilled grape marc also followed the normal pattern of an accelerated decomposition process, and was characterized by a rapid reduction in microbial activity from day 14 onwards (Fig. 3A). The basal respiration was 861 ± 14 mg O_2 kg^{-1} OM^{-1} in the starting material and steadily decreased over time reaching a final value of 193 ± 10 mg O_2 kg^{-1} OM^{-1} on day 56 ($F_{4,20} = 157.7$, $p < 0.0001$; Fig. 3a). The accelerated mineralization of the organic matter was also reflected by the reduced contents in cellulose and hemicellulose at the end of the process (*cellulose:* 18.07% ± 0.92; *hemicellulose*: 1.55% ± 0.20) compared to the beginning of the trial (*cellulose*: 23.98% ± 2.30; *hemicellulose*: 3.65% ± 1.00). Conversely, and due to its slower degradation, the lignin content was 1.3-times higher on day 56 (75.01% ± 0.70) than on day 0 (55.84% ± 1.88), supporting the findings from Domínguez et al. [32] on a vermicomposting system with Scotch broom as the starting material. Moreover, when compared to cellulose and hemicellulose, the lignin fraction is more resistant to be digested by earthworms and microorganisms [10]. Lower levels of total C and N were found towards the end of the process, on days 42 and 56 (total C: $F_{4,20} = 31.2$, $p < 0.0001$*;* total N: $F_{4,20} = 22.4$, $p < 0.0001$; Table 2). Nonetheless, the C to N ratio of the distilled grape marc significantly increased towards the later vermicomposting stages ($F_{4,20} = 18.7$, $p < 0.0001$), with a final value of 35 on day 56 (Table 2). In this regard, Goyal et al. [34] recommended a C/N ratio lower than 25 for quality compost production, bearing in mind that the addition of high C/N ratio amendments onto soil might lead to the so-called "nitrogen starvation" and promote the competition between microbial populations and plants for soil N [35].

The use of feedstocks with an acidic pH can also present a risk for not only the survival and reproduction of earthworms during vermicomposting [36], but also for the potential use of the resulting vermicomposts as an amendment for soil. Cataldo et al. [37] reported a more favourable response of crops to organic amendments when soil pH ranges from weak-acidic to weak-alkaline levels. In support of previous findings on raw grape marc [15], processing distilled marc via vermicomposting was effective in neutralizing its initial acidity, as values close to neutrality were reached after 14 days and maintained until the end of the process ($F_{4,20} = 603.5$, $p = 0.001$; Table 2). Together with pH, the changes in EC throughout vermicomposting may influence the dynamics of the process with consequences on microbial activity, the bioavailability of nutrients and, ultimately the suitability of vermicomposts as organic amendments [38]. Recommended EC limits are around 2 dS m^{-1} with respect to plant health and safe agricultural use [39]. In our study, the EC levels did not surpass this threshold level at any of the time points (Table 2) with an average value of 0.90 ± 0.03 mS cm^{-2} in the fresh distilled grape mar, followed by a four-fold decrease within the first 14 days of vermicomposting ($F_{4,20} = 469.2$, $p = 0.001$; Table 2). From

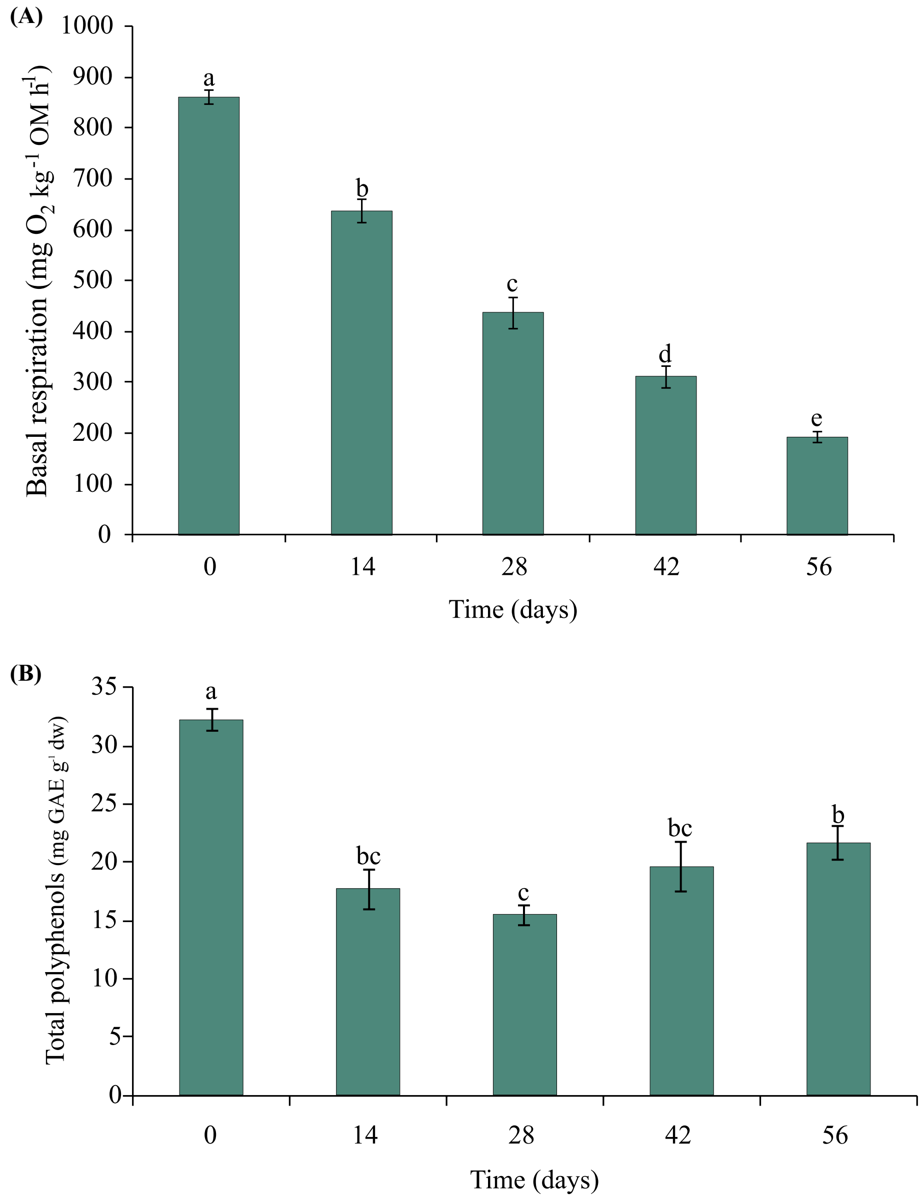


Fig. 3 Changes in **A** basal respiration as a proxy of microbial activity (mg O_2 kg^{-1} OM h^{-1}); and **B** total polyphenol content (mg GAE g^{-1} dry weight) throughout the vermicomposting process of distilled grape marc (*Vitis vinifera* L. cv. Mencía). Values are mean ± SE ($n = 5$). Different letters indicate significant differences among time points (Tukey HSD)

day 14, there was a progressive, albeit slower, reduction in the EC until the end of the trial, achieving a final value of 0.11 ± 0.004 mS cm^{-2} on day 56 (Table 2).

In terms of nutrients, the total Ca content of the distilled grape marc significantly increased within the first 28 days of vermicomposting ($F_{4,20} = 25.54$, $p < 0.0001$; Table 2); while K and P underwent a decreasing trend from the beginning of the trial until days 28 and 42 respectively (K: $F_{4,20} = 33.05$, $p < 0.0001$; P: $F_{4,20} = 104.26$, $p < 0.0001$; Table 2). Later on, no further changes were observed for Ca, K and P until the end of the trial (day 56; Table 2). In the cases of Mg, Mn and Fe a significant increase in their content was recorded on day 56 compared to the earlier time points (Mg: $F_{4,20} = 14.58$, $p < 0.0001$; Mn: $F_{4,20} = 18.46$, $p < 0.0001$; Fe: $F_{4,20} = 7.95$, $p = 0.005$; Table 2). However, the contents of S, Mo and B remained without significant changes throughout the duration of the trial (Table 2). Overall, after 56 days of vermicomposting the nutrient concentration in the distilled grape marc-derived vermicompost was similar to that reported for the raw marc vermicompost from Mencía cultivar [15]. In both cases, the end products generally met the required international standards and guidelines for quality organic fertilizers in the United States, Canada and the European Union [40].

Total polyphenol content during vermicomposting of distilled marc

During distillation process, high temperatures and certain abiotic factors such as the low levels of oxygen and pH may

Table 2 Changes in the physico-chemical properties and nutrient content of the distilled grape marc (*Vitis vinifera* L. cv. Mencía) throughout the process of vermicomposting

	Distilled grape marc	Vermicompost			
	Day 0	Day 14	Day 28	Day 42	Day 56
Moisture (%)	75.77 ± 1.06a	73.56 ± 0.80a	69.85 ± 0.49b	70.94 ± 0.64b	73.44 ± 1.02a
Organic matter (%)	97.67 ± 0.23a	97.96 ± 0.35a	95.16 ± 0.60b	92.03 ± 0.35c	89.91 ± 0.36c
pH	4.30 ± 0.08a	7.58 ± 0.08b	7.32 ± 0.05b	7.13 ± 0.02b	7.53 ± 0.04b
EC (mS cm^{-2})	0.90 ± 0.03a	0.21 ± 0.01b	0.13 ± 0.005c	0.12 ± 0.002c	0.11 ± 0.004c
Total C (g kg^{-1})	516 ± 1.56a	517 ± 1.07a	512 ± 0.89a	511 ± 0.48a	500 ± 1.72b
Total N (g kg^{-1})	19 ± 0.30a	20 ± 0.68a	18 ± 0.37a	14 ± 0.68b	14 ± 0.56b
C to N ratio	27 ± 0.49a	27 ± 0.88a	29 ± 0.63a	36 ± 1.65b	35 ± 1.45b
Total Ca (g kg^{-1})	4.66 ± 0.14a	5.61 ± 0.20b	6.41 ± 0.17c	6.39 ± 0.14c	6.44 ± 0.14c
Total K (g kg^{-1})	19.4 ± 0.43a	16.2 ± 0.48b	14.4 ± 0.31c	14.6 ± 0.29c	15.7 ± 0.14c
Total P (g kg^{-1})	2.36 ± 0.05a	1.85 ± 0.06b	1.54 ± 0.06c	1.25 ± 0.04d	1.24 ± 0.02d
Total Mg (g kg^{-1})	1.18 ± 0.03a	1.18 ± 0.04a	1.20 ± 0.02a	1.21 ± 0.03a	1.43 ± 0.01b
Total S (g kg^{-1})	1.67 ± 0.02a	1.69 ± 0.04a	1.76 ± 0.05a	1.62 ± 0.04a	1.67 ± 0.01a
Total Mn (mg kg^{-1})	44.6 ± 1.36a	44.4 ± 1.44a	51.0 ± 1.22ab	52.0 ± 1.14b	61.4 ± 2.58c
Total Fe (mg kg^{-1})	343 ± 8.24a	332 ± 18.0a	404 ± 12.3ab	395 ± 41.6a	516 ± 31.7b
Total Mo (mg kg^{-1})	5.39 ± 0.40a	5.05 ± 0.39a	5.06 ± 0.53a	4.29 ± 0.15a	4.08 ± 0.63a
Total B (mg kg^{-1})	37.0 ± 0.71a	38.4 ± 0.68a	38.0 ± 0.71a	36.2 ± 0.58a	35.8 ± 0.58a

Nutrient data are expressed on a dry weight (dw) basis

EC electrical conductivity

Values are means ± standard error

Different letters within the same row indicate significant differences among sampling times according to Tukey´s HSD test

influence the amount of polyphenols present in the distillery effluent. Indeed, when compared to the Mencía raw marc (97.5 ± 4.5 mg GAE g^{-1} dry mass; Gómez-Brandón et al. [15]), the content of total polyphenols was about three times lower in the distilled by-product on day 0 (32.19 ± 0.92 mg GAE g^{-1} dw; Fig. 3b). During vermicomposting, the polyphenolic content of the distilled grape marc continued to decrease until 17.73 ± 1.67 mg GAE g^{-1} dw in a period of 14 days ($F_{4, 25} = 19.31$, $p < 0.0001$; Fig. 3b). Domínguez et al. [9] and Gómez-Brandón et al. [15] also reported a rapid and significant reduction in the content of polyphenols of raw marc in a pilot-scale vermireactor in the presence of *E. andrei*. Similar findings were found by Nogales et al. [25] on a pilot-scale level. Altogether, these findings indicate that earthworms´ activity enhance the depletion of polyphenols in both raw and distilled grape marc in the short-term. A plausible explanation for the removal of polyphenols during the process of vermicomposting could rely on the increased aeration and organic matter consumption in the presence of earthworms [41, 42], favouring microbial colonization and further decomposition. Furthermore, earthworms have a comprehensive digestive enzyme system, and high levels of enzymes of interest in bioremediation as carboxylesterases have been detected in vermicompost [43]. Nonetheless, the underlying biological mechanisms of how earthworm-microbe interactions and their enzymatic capabilities affect polyphenols degradation still need to be further explored in the context of improved soil health and crop productivity within circular agriculture.

During vermicomposting the recovery of polyphenols from the grape marc and grape seeds will contribute not only in reducing the phytotoxicity of the end-product [9], but also in increasing the income of wine producers. Winery by-products are considered high-quality raw materials owing to their content in polyphenols (circa 60% in seeds; [7]) which, due to their antioxidant and scavenging activities, can be further exploited in cosmetic, food and pharmaceutical sectors [8, 17, 44]. All in all, the green recovery of high-added value compounds from the winery sector through vermicomposting embraces a circular and sustainable bioeconomy, which is in line with biomass valorization via other methodological alternatives like pyrolysis [45].

The abovementioned has also contributed to paving the way for researchers and wine producers to establish the basis to jointly scale up this technology at an industrial level. Nonetheless, to fully achieved this goal, it is crucial to shed light onto the overall sustainability of vermicomposting systems and offer solutions to mitigate the environmental hot spots and energy sinks [46]. In this regard, the integrative use of sustainability assessment tools can help to assess the environmental impacts associated with waste valorization, from raw material acquisition to end-of-life, in order to unravel the stages of the process that contribute the most to environmental burdens. As pointed out by Aghbasahlo et al. [47] each sustainability assessment method has its own merits and demerits, so the optimum method depends on the study objective, process complexity, and desired level of precision. Up to now, only a few studies have analysed the environmental implications of vermicomposting by using a Life Cycle Assessment (LCA) approach [46, 48–50]. Cortés et al. [46] found the energy needs of grape marc distillation as an important hotspot of the vermicomposting process. Moreover, they reported that the performance of the process was not optimal in terms of carbon footprint and normalised impact index, but the environmental impact assessment gave better results when economic revenues were considered within the LCA analysis.

Conclusions and outlook

- The findings of the present study underline the feasibility of vermicomposting as an environment-friendly approach for the biological stabilization of distilled grape marc in pilot-scale systems.
- Overall, reduced values of basal respiration close to 200 mg O_2 kg OM h^{-1} and indicative of stabilized materials were found in the resulting vermicompost. Likewise, the pH and EC levels along with the content of macro- and micronutrients achieved for the end product are in line with those considered to have the quality criteria of a good vermicompost with respect to plant health and safe agricultural use.
- Vermicomposting of distilled grape marc was also found to be effective in reducing the content of polyphenols of the starting material in a period of fourteen days.
- The use of advanced sustainability assessment tools is still needed for an in-depth investigation of the environmental implications, from raw materials acquisition to end-of-life, of the vermicomposting process of distilled grape marc and winery wastes in general. By using these tools, it can help to enhance our understanding of the environmental burdens and resource conversion efficiency of the process, as well as on the potential agronomical uses of the vermicomposts during farm application.
- Future work appears merited towards assessing an uncertainty analysis in order to evaluate the fidelity, repeatability and validity of the experimental data within studies integrating advanced sustainability assessment tools in the field of vermicomposting of distilled grape marc and winery wastes in general.
- By exploiting the potential positive use of the distilled marc vermicompost as a soil amendment it could contribute to alleviate rising environmental concerns about the application of mineral fertilizers and synthetic pesticides. Under this context, pelletizing-drying processes could be regarded as a promising strategy to improve the environmental and agronomical performance of organic biofertilizers during farm application [51]. Further field case studies are also encouraged to assess the optimal amendment rate of the vermicomposts to ensure high nutrient release and synchrony for crop uptake and utilization.

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