ASSESSMENT OF THE PESTICIDE PROFILE DURING VERMICOMPOSTING OF GRAPE MARC

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Abstract

Grape marc, the main by-product of the wine industry, is a biomass rich in bioactive compounds and has been used as a raw material for soil remediation, among its many applications. However, indiscriminate management could lead to a combination of phytochemical overdose and the presence of xenobiotic compounds, mainly pesticides, from upstream cultivation. A sustainable alternative for its recovery is biotransformation by earthworms, resulting in vermicompost. Although vermicomposting is known to produce a substrate that can be applied directly as a soil amendment, the effect of the starting material on the potential pesticide pool remains uncertain, raising questions about its safety. Thus, the objective of this study is to detect and quantify pesticide content in Albariño white grape marc and to track its biotransformation into vermicompost by the red earthworm *Eisenia andrei*. The analysis combined a green extraction technique, ultrasound-assisted extraction, with a large set of marc samples and the different batches of vermicompost generated from them. Ultra-high-performance liquid chromatography coupled with quadrupole-time-of-flight mass spectrometry (UHPLC–Q-TOF) was used for pesticide detection, employing a pseudotarget approach based on a library optimized for detecting more than 2000 pesticides. The pseudotarget analysis identified 41 pesticides, including acaricides, fungicides, and insecticides, with higher responses in marc than in vermicompost. Targeted quantification of six selected pesticides revealed levels in grape marc of up to 12 $mg·kg^{-1}$, with heterogeneity between sampling batches, whereas in vermicompost, concentrations were homogeneous and reduced by more than 98% to less than 0.25 $mg·kg^{-1}$, indicating the suppressive and homogenizing effect of the vermicomposting process. Taken together, the findings highlight the potential of vermicomposting to generate a nutrient-rich, bioactive, and safe organic amendment, while also providing a detailed pesticide profile relevant to the regulation and safe reuse of grape marc in agriculture.

Introduction

Grape marc, also known as grape bagasse or pomace, is the primary by-product of the wine industry. This residual material, generated after the winemaking process, consists of skins, seeds, and stems, which are separated from the must before fermentation in white wine production. This transformation, which is mainly physical in the case of white grape marc, allows it to largely retain its original chemical profile, maintaining a rich and diverse composition that includes carbohydrates, lipids, and a high concentration of its main bioactive compounds, phenolic compounds. These metabolites have been associated with multiple biological functions, including antioxidant and antibacterial activity (Castillo *et al* 2022), which has led to the reevaluation of grape marc across various industrial sectors, such as food (Castillo *et al* 2025), cosmetics (Alvarez-Casas *et al* 2016), and agriculture, where it is used as an organic amendment to improve soil quality and plant development (Domínguez *et al* 2014). In contrast, the indiscriminate disposal of grape marc in soil can lead to high concentrations of these phytochemicals, resulting in overdose of these bioactive compounds, which, combined with their low pH, can have biocidal effects (Buchmann *et al* 2025). Accordingly, marc from wine grapes can contain high levels of xenobiotic agents such as pesticides, which are regulated in both table grapes and wine but not in marc (Buchmann *et al* 2025; Cermeño *et al* 2025). Currently, there is no specific regulation regarding the safety of grape marc, and no known sources comprehensively address its pesticide content (Buchmann *et al* 2025). A single study conducted more than a decade ago on common fungicides used on these agricultural crops detected 11 fungicides, with benalaxyl concentrations exceeding the European maximum residue limits (MRLs) for table grapes (Celeiro *et al* 2014). In contrast, more than 60 pesticides are known to be applied to this crop type, including not only fungicides but also more than 20 types of herbicides and insecticides (Liviz *et al* 2025). To facilitate their regulation and control, incorporating modern high-resolution chromatography technologies coupled with next-generation mass spectrometers enables comprehensive analysis of pesticide and other contaminant profiles in grape marc. Among these detectors, the quadrupole time-of-flight (Q-TOF) is particularly promising, although its use remains limited by its high cost (Syrgabek *et al* 2022). When coupled with gas or liquid chromatography, this technology provides a powerful tool for untargeted screening and quantitative determination of multiple pesticide residues in grapes (Pang *et al* 2020). In addition, it enables the detection of a wide range of compounds at early stages, before their possible spread. This facilitates the identification of both the most commonly used compounds in vineyards and less frequent residues and emerging contaminants, offering detailed tracking from application in the crop to accumulation in the by-product. An effective strategy for reusing the nutrients in grape marc and safely reincorporating them into the substrate is to recover them through vermicomposting. Earthworm species, such as

Eisenia andrei, are used to transform this by-product into high-value vermicompost (Gómez-Brandón *et al* 2023). Vermicompost derived from marc has been shown to provide multiple benefits, including improving soil structure, increasing nutrient availability, and promoting beneficial microbial activity (Gómez-Brandón *et al* 2019). However, it remains unknown how vermicomposting affects the pesticide profile present in the original marc. Therefore, it is of great interest to evaluate whether earthworm activity can attenuate the presence of these toxic residues while avoiding overaccumulation of phytochemicals in the soil, thereby producing a vermicompost that is not only highly bioactive but also safe for field application. Thus, this study focuses on extracting, identifying, and quantifying pesticide residues in grape marc and in vermicompost generated by the earthworm *Eisenia andrei*. This analysis combines a green ultrasound-assisted extraction technique with ultra-high-performance liquid chromatography (UHPLC–Q-TOF) analysis, using a pseudo-target approach based on a database of more than 2000 pesticides and related compounds.

MATERIALS AND METHODS

White grape marc, vermicomposting and sampling

White grape marc (*Vitis vinifera* L. cv. Albariño) was kindly provided by the winery Terras Gauda (O Rosal, Galicia, northwestern Spain). Vermicomposting was performed in a prototype "industrial-scale" continuous-flow vermicomposting reactor (6.25 m long x 1.7 m wide x 1.6 m high) with a capacity of 3.37 m^3, designed and built by our team. This vermireactor, located in a greenhouse without temperature control, has been operating for the past five years, processing only Albariño grape marc from the winery Terras Gauda, and harbors a very high population density (>20,000 worms/m^2) of *Eisenia andrei* earthworms. Samples of fresh grape marc (n=10) and 6-month-old vermicompost (n=10) were collected from the top and bottom of the vermicomposting reactor, respectively, using a corer sampler and following a regular spatial sampling grid.

Ultrasound-Assisted Extraction

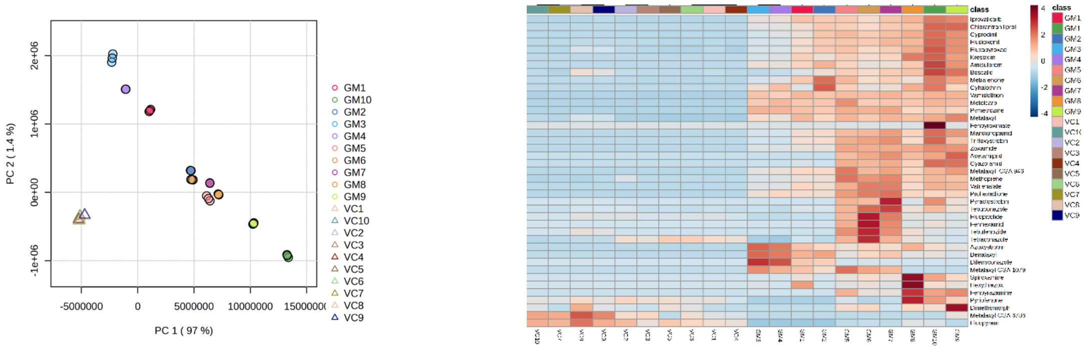
Grape marc and vermicompost samples were extracted using ultrasonic-assisted extraction (UAE). 1.0 g of sample was weighed into a 10 mL glass vial, and 5 mL of a methanol/ethanol mixture (50:50, v/v) was added. The vials were hermetically sealed and sonicated in an ultrasonic bath for 10 min at 40 °C. After extraction, the extracts were recovered by centrifugation at 3500 rpm for 1 min, and the supernatant was filtered through 0.22 µm polytetrafluoroethylene (PTFE) membranes prior to instrumental analysis.

Metabolites detection

Pseudo-targeted analysis was performed using an Elute UHPLC 1300 system coupled to a Compact quadrupole-time-of-flight mass spectrometer (QTOF, Bruker Daltonics). Data acquisition was performed in broadband collision-induced dissociation (bbCID) mode in positive polarity, using electrospray ionization (ESI) as the source. Pseudo-targeted analysis was performed using TASQ® software with the TargetScreener HR 3.1 Pesticides library, which includes more than 2,000 optimized compounds and their corresponding fragmentation transitions. Identification was based on matching retention time, exact mass, and isotopic pattern, with acceptance criteria set at a maximum retention time deviation of ±0.4 min, a mass error of less than 5 mDa, and an mSigma value ≤50. All identifications were confirmed by at least two independent fragmentation transitions. Statistical processing was performed using MetaboAnalyst 6.0, while bar graphs were produced using OriginLab Pro 2026.

RESULTS AND DISCUSSION

To evaluate pesticide migration from initial concentrations in the marc to final concentrations in the vermicompost, ultrasound extracts from the 10 sampling points were analysed. Figure 1 shows the relative responses of 41 pesticides, including fungicides, acaricides, and insecticides. Principal component analysis (PCA) of pesticide distributions across the 10 sampling points indicates a more heterogeneous response in grape marc (GM) than in vermicompost (VC), where a homogenizing effect associated with vermicomposting with *Eisenia andrei* is observed.



(A) **(B)**

Figure 1. Principal component analysis (PCA) diagram (**A**) and heat map (**B**) of pesticide distribution in GM and VC samples.

Likewise, the most notable feature is the marked decrease in most pesticides in VC compared to GM, as highlighted in the heat map. Only fluopyram, dimethomorph, tetraconazole, and metalaxyl derivatives show responses in VC that are comparable to or even higher than those in GM.Based on the pseudotarget analysis, six pesticides were selected for quantification using external calibration. Figure 2 shows the individual concentrations of each compound at the different sampling points. In general, concentrations near 8 ppm (mg·kg DW^{-1}) are observed in the GM samples, whereas in the VC the maximum concentration does not exceed 0.25 ppm (mg·kg DW^{-1}).

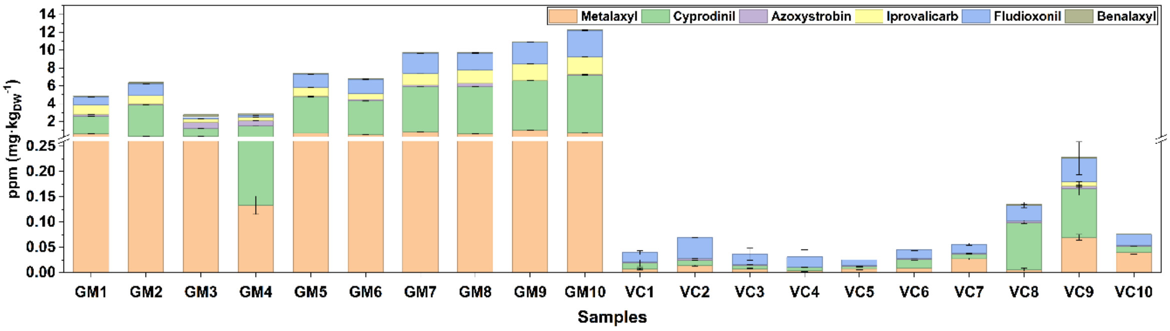


Figure 2. Individual pesticide concentration (ppm) per sampling point.

The pesticides present at higher concentrations in GM are cyprodinil and fludioxonil. These fungicides are commonly marketed as mixtures and used in commercial vineyard treatments to combat diseases such as downy mildew (*Plasmopara viticola*), powdery mildew (*Erysiphe necator*), and gray rot (*Botrytis cinerea*). Next are azoxystrobin and metalaxyl, which are broad-spectrum pesticides. Due to the emergence of resistance, these are applied alternately to mitigate this problem. In vermicompost, the concentrations of these pesticides were significantly reduced, with the highest concentration of metalaxyl below 0.1 mg·$Kgdw^{-1}$. These results reinforce the effectiveness of vermiremediation in improving the safety of wine by-products and their use as a soil amendment, while reducing phytochemical residues and providing nutrients through the action of *Eisenia andrei*.

Conclusions

This study is the first to propose monitoring pesticides in white grape marc, the main by-product of the wine industry, through vermicomposting mediated by the red earthworm *Eisenia andrei*. Using a pseudotarget approach with high-resolution UHPLC–Q-TOF chromatography enabled the exhaustive characterization of the pesticide profile. This demonstrated that vermicomposting can produce high-value organic amendments and mitigate the risks associated with agro-industrial by-products. Vermiremediation reduced the presence of over 30 pesticides in the initial material, achieving a 98% reduction in the quantified compounds: metalaxyl, cyprodinil, azoxystrobin, iprovalicarb, fludioxonil, and benalaxyl. This study demonstrates the effectiveness of vermicomposting grape marc and lays the groundwork for systematically controlling and monitoring the pesticide profile in wine by-products. Currently, this area lacks specific regulation, so this study contributes to developing safer and more sustainable strategies within the circular economy framework.

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References

Alvarez-Casas, M. *et al*. 2016. Eur. Food Res. Technol. 242, 655–665.
Buchmann, C. *et al*. 2025. Sci. Total Environ. 982, 179611.
Castillo, A. *et al*. 2025. PLoS One 20, e0325079.
Castillo, A. *et al*. 2022. Front. Nutr. 9, 1008457.
Celeiro, M. *et al*. 2014. J. Chromatogr. A 1343, 18–25.
Cermeño, S. *et al*. 2025. Fermentation 11, 318.
Domínguez, J. *et al*. 2014. Waste Manag. Res. 32, 1235–1240.
Gómez-Brandón, M. *et al*. 2023. J. Mater. Cycles Waste Manag. 25, 1509–1518.
Gómez-Brandón, M. *et al*. 2019. Crit. Rev. Biotechnol. 39, 437–450.
Liviz, C. do A. M. *et al*. 2025. Food Res. Int. 203, 115771.
Pang, G. *et al*. 2020. Engineering 6, 432–441.
Syrgabek, Y. *et al*. 2022. Foods 11, 1623.