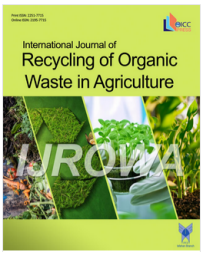

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Grape pomace transformed by specific fungi has the potential as a promising substrate for vermicomposting

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Abstract:
Purpose: It is known that the combination of bioprocesses can contribute to obtaining better results compared to those achieved by applying each process individually. Solid state fermentation of *Vitis labrusca* grape pomace was carried out using different saprotrophic fungi following a vermicomposting process.
Method: A palatability test was performed to evaluate the permanence of *Eisenia foetida* adults on pomace transformed by fungi. Subsequently, pomace treated with *Ulocladium botrytis* LPSc 813 was vermicomposted for 90 days to evaluate comparatively the earthworm population dynamics and different physio-chemical and biological parameters with respect to control treatment.
Results: The pomace treated with *Coriolopsis rigida* LPSc 232 and *U. botrytis* showed a 100% permanence of the earthworms, though only this last fungus was able to modify the acidity of the parent grape pomace (pH 7.66 ± 0.84) without increasing its salinity. The combination of *U. botrytis*-vermicomposting showed a reduction in the adult and juvenile earthworm number at 60 days and presented a higher germination index compared to the control.
Conclusion: Results suggest that grape pomace pretreated with **U. botrytis** could be an optimal starting substrate for vermicomposting, obtaining an organic fertilizer in a short period.

Keywords: *Vitis labrusca*; Solid state fermentation; Saprotrophic fungi; *Eisenia foetida*; Organic fertilizer

1. Introduction

In terms of circular economy, agro-industries produce a lot of waste that is a potential source of products to which value could be added (Nizami et al. 2017). The grape pomace (GP) (seed, skin, and remnant pulp of grapes) is the main derivative of wine elaboration. Each year, $1.05 - 1.31 \times 10^{10}$ kg of this waste is produced worldwide (Dávila et al. 2017). A large amount of GP is produced in a short period; only a small fraction is used to obtain new products and the remaining part is generally incinerated or disposed of on land, causing environ-mental damage (Beres et al. 2017).

Vermicomposting is a biological treatment that stands out as one of the main alternatives that could reduce the large amounts of agro-wastes, allowing to revalue of these remains as organic fertilizers (Bhat et al. 2018). This bioprocess, which is applied on organic substrates, involves the action of microorganisms and earthworms through mainly degradation and stabilization reactions (Nogales et al. 2005; Martínez-Cordeiro et al. 2013; Domínguez et al. 2017). Among earthworm species, *Eisenia foetida* (Annelida, Oligochaeta), which is an epigeic one, is the most

used for the management of organic wastes due to its high voracity, high reproductive capacity, easy handling, and ability to adapt to adverse conditions (Gunadi et al. 2002; Pérez-Godínez et al. 2017).

Recalcitrant agro-wastes, rich in lignin and/or other phenolic compounds that are toxic, are not generally used for composting due to their negative effects on microorganisms and earthworms involved in the process (Arora and Kaur 2019; Gong et al. 2019). Previous studies reported that *Vitis vinifera* GP biomass was reduced in a vermireactor with *E. foetida* (Domínguez et al. 2014) or plastic containers using *Eisenia andrei* (Gómez-Brandón et al. 2011); though in both cases a long time is required to utilize this material as organic fertilizer. Therefore, one of the alternatives that might contribute to overcoming this problem could be the incorporation of a previous treatment on the GP, which could generate a more palatable substrate for earthworms and microorganisms.

In recent times, solid state fermentation (SSF) with fungi has become a frequently utilized strategy to treat agro-wastes, since it is a low-cost technology to obtain a broad spectrum of products (Manan and Webb 2017). The use of saprotrophic fungi as transformation agents of *V. labrusca* GP has been studied and constitutes a promising alternative (Troncozo et al. 2019; Troncozo et al. 2020). The information available on how to obtain organic fertilizers through the synergistic effect of two bioprocesses, such as SSF with saprotrophic fungi followed by vermicomposting, is limited (Singh et al. 2018; Arora and Kaur 2019; Gong et al. 2019). The present work first evaluated the potential of several saprotrophic fungi to increase the palatability of *V. labrusca* GP to *E. foetida* earthworms. Then, the pomace treated with a selected saprotrophic fungus was subjected to vermicomposting, where physico-chemical and biological parameters were evaluated.

2. Material and methods

2.1 Pomace, saprotrophic fungi and earthworms

Ten random samples (2 Kg each) of GP from isabella cultivar were collected from "Cooperativa de La Costa" (Berisso, Buenos Aires, Argentina 34°53′22.70″S/57°49′21.11″O) to obtain a composite sample. The lignocell lignocellulosic material was dried in an oven with forced air circulation at 60°C for 48 h and then stored at room temperature and protected from light in a hermetic box until use.

The fungal strains were selected due to their ability to transform and reduce GP toxicity in lettuce and tomato seeds (Troncozo et al. 2019). Five strains were obtained from the Instituto Spegazzini (LPSc) culture collection: *Coriolopsis rigida* LPSc 232, *Gloeophyllum sepiarium* LPSc 735, *Peniophora albobadia* LPSc 285, *Pycnoporus sanguineus* LPSc 163, and *Ulocladium botrytis* LPSc 813; while *Trichoderma harzianum* 18 was obtained from Facultad Ciencias Agrarias y Forestales, UNLP (FALH). Stock cultures of the strains were kept at 4°C on 2% (w/v) malt-agar slants (Saparrat et al. 2000).

Clitelated earthworm specimens of *E. foetida* were obtained from División Zoología Invertebrados, Museo de La Plata, Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata. The earthworm population was maintained according to Schuldt et al. (Schuldt et al. 2005).

2.2 Transformation of GP under SSF with saprotrophic fungi

The fungal transformation with the previous mentioned strains was carried out in autoclavable bags containing 250 g of GP steam-sterilized (121ºC at 1 atm for 30 min) at a humidity level adjusted to 70% and posteriorly inoculated with a mycelial suspension at 20% p/v (Troncozo et al. 2019). Static incubation of inoculated bags was performed at 28°C for 30 days. Control treatments were prepared incubating non-inoculated sterilized GP under the same conditions. The experiment presented a completely random design, and each treatment was replicated four times.

Three physical-chemical parameters were analyzed in the water-soluble fraction (WSF) of GP at 1:5 proportion (Saparrat et al. 2008). The values of EC and pH were measured by conductometry and potentiometry using a Benchtop pH/mv meter (Sper Scientific) (Jackson and Barak 2005). The water-soluble phenol (WSP) content was determined according to the Folin–Ciocalteau method and the absorbance was estimated at 760 nm utilizing a spectrophotometer (Shimazdu, Tokyo, Japan) (Osono and Takeda 2001). A one-way ANOVA and Tukey test ($P < 0.05$) were used to evaluate differences between the fungal species.

2.3 Palatability test

The effect of GP treated with fungi on earthworms was evaluated following the procedure reported by Ferreira et al. with modifications (Ferreira et al. 2021). Briefly, the different substrates obtained with SSF (humidity of 80%) were placed in cylindrical containers (500 cm^3) where four clitellated worms were added for a period of 72 hours in the dark. The experiment presented a completely random design and four replicates per treatment were made. The number of earthworms that remained in the substrate was estimated and expressed as a percentage. A one-way ANOVA and Tukey test ($P < 0.05$) were used to evaluate differences between the activity triggered by each one of the tested fungi. Data obtained were transformed using arc sen $\sqrt{P}$, prior to statistical analysis.

2.4 Vermicomposting of GP transformed by *U. botrytis*

According to results obtained in the palatability test, the GP transformed by SSF with *U. botrytis* was subjected to vermicomposting following the methodology reported by Nogales et al. with modifications (Nogales et al. 2005). The GP treated with the fungus was obtained as was previously described. Perforated plastic containers (5 L) were filled to three quarters of the capacity with the moistened substrate transformed with the fungus (80% humidity). Considering the rate of substrate consumption per day reported by Pérez-Godínez et al., a total of 15 earthworm adults per container were placed on the surface of the substrate (Pérez-Godínez et al. 2017). A control treatment was incorporated that consisted of GP composted for 30 days, prior to the vermicomposting process. All containers were covered with a shade mesh to reduce water evaporation and kept in darkness throughout the whole vermicomposting period. The

experimental design was completely randomized, and each treatment was replicated four times. The number of cocoons, juveniles, and adults were monitored monthly for 90 days (Schuldt et al. 2005). Also, samples of vermicomposted GP were randomly removed monthly per container without repositioning. One part of the collected material was stored in a plastic vial at 4°C and microbial activity was estimated by dehydrogenase activity (Thalmann 1968), using 0.5% TTC (2, 3, 5-triphenyl tetrazolium chloride) in 0.1 M Tris-buffer (pH 8) as substrate. The absorbance was estimated at 546 nm in the spectrophotometer (Shimazdu, Tokyo, Japan). The remaining material was utilized to analyze physical-chemical parameters and carry out a phytotoxicity bioassay. The pH, EC, and WSP were determined as previously described. The phenol-hypochlorite reaction was used to determine the NH_4^+-N content and the absorbance was estimated at 635 nm using a spectrophotometer (Shimazdu, Tokyo, Japan) (King et al. 1990). The methodology proposed by Tiquia et al. was followed to carry out the bioassay (Tiquia et al. 1996). For this experiment, lettuce (*Lactuca sativa*) was used as a plant test. The percentage of germination (G), root length, and germination index (GI) were calculated and interpreted according to classification criteria proposed by Zucconi et al. (Zucconi et al. 1985). A two-way nested ANOVA with two main effects "Treatment (T)" and "vermicomposting time (VT)" was applied for all the variables analyzed ($P < 0.05$). Discrete data were transformed using ln prior to statistical analyses.

3. Results and discussion

3.1 Screening of fungi for GP transformation to increase its palatability to *E. foetida* earthworms

The earthworms are very sensitive to changes in the environment, such as ones caused by fungal activity on organic matter; the reason why, it is essential to elucidate the factors that are more influential on the permanence of the annelids in the substrate (Edwards and Fletcher 1988; Ansari and Humphrey 2015; Arora and Kaur 2019).

In this study, all fungi tested colonized the GP and their activity produced changes in the pH, EC, and WSP content, and affected the permanence of *E. foetida* on the substrate (Table 1). A neutral pH and low salt content (≤ 4 dS/m) in the substrate are considered optimal for earthworm activity (Pellegrini et al. 2014). Only *U. botrytis* LPSc 813 produced an increase of $127.37 \pm 24.8\%$ in the pH, neutralizing the characteristic acidity of pomace. As regards salinity, all fungi showed values below the reported limiting value. Cova et al. suggested that polyphenolic compounds represent earthworm anti-nutritional factors and have anti-reproductive effects that interfere with their biology (Cova et al. 2007). In this work, *P. albobadia* LPSc 285 presented the greatest reducing effect on the WSP content of GP ($80.7 \pm 2.2\%$). Furthermore, it was observed that a particulate material ($1 - 2$ cm) was generated by fungal action on GP which would favor ingestion and assimilation by earthworms (Curry and Schmidt 2007; Palaniappan et al. 2018). Only *C. rigida* LPSc 232 did not produce this material size, showing an agglutination of all the GP particles.

Although, the SSF with the fungal isolate *C. rigida* LPSc 232, *P. albobadia* LPSc 285, *T. harzianum* FALH 18, and *U. botrytis* LPSc 813 increased the persistence of the earthworms to invade GP, only *U. botrytis* and *C. rigida* proved to develop the best habitat for earthworms (100% permanence). So, what is it that favors the permanence of the worms on the pomace transformed by these last two fungi? The "Dematiaceous" fungus *U. botrytis* stood out since it kept an optimal particle size similar to the parent substrate, reduced the soluble phenol content, and mainly because it altered the initial GP pH, which could be due to the fact that the enzymatic system is optimal in the range of $7 - 8$ pH (Saparrat et al. 2007). On the other hand, the lignocellulolytic fungus *C. rigida* was one of the most efficient in removing the phenol content but under acidic conditions and agglomerating the GP particles.

To consider only the physio-chemical parameters may not be enough to determine whether the earthworms have a preference to stay in the pomace, in this sense it is possible to formulate other hypotheses. It has been reported that worms in natural environments incorporate fungi in their diet and it is quite possible that *E. foetida* is selective about the organisms it digests (Bonkowski et al. 2000). Californian earthworms might have a strong preference for dark pigmented fungi (hyphae and/or conidia) since these contain more nitrogen compounds and other important elements for animal nutrition, such is the case of *U. botrytis* (Maraun et al. 2003; Saparrat et al. 2007). On the other hand, some fungi might have a greater enzyme ability to degrade/detoxify recalcitrant polymers of plant residues, providing better quality food for the earthworm, as reported by *U. botrytis* and *C. rigida* (Moody et al. 1995; Maraun et al. 2003; Saparrat et al. 2014; Troncozo et al. 2019). All this suggests that it would be necessary to include in future studies the examination of the alimentary tract content of *E. foetida* earthworms after vermicomposting with GP treated with saprotrophic fungi, to establish which is the role in the fungus-earthworm interaction and its influence in the vermicomposting process. Overall, considering the physicochemical changes produced in the GP by *U. botrytis* and the potential nutritional input for the earthworms, this fungal treatment was selected to evaluate further the combination fungus-vermicomposting.

3.2 Vermicomposting the GP treated with *U. botrytis*

Fig. 1 shows the population dynamics of adult and juvenile earthworms, as well as the number of cocoons of *E. foetida* during the vermicomposting process. The two-way nested ANOVA showed that the adult earthworms and cocoon number were different among the vermicomposting time and different treatments (composting or SSF with *U. botrytis*) ($P < 0.05$). The reduction in the number of adult earthworms ($88.9 \pm 3.1\%$) in the GP treated with the fungus at day 60 could be due to a lack of availability of nutrients in the substrate (Fig. 1a) (Usmani et al. 2019). The juvenile population was only affected by vermicomposting time ($P < 0.05$) (Fig. 1b). In addition to the scarce presence of nutrients, the reduction in the juvenile earthworms could be due to intraspecific competition with adult earthworms (Ansari and Humphrey 2015). The highest production of

Table 1. GP untreated and treated with fungi: physico-chemical parameters and permanence percentage of *Eisenia foetida*.

Registered parameters	Control	*C. rigida*	*G. sepiarium*	*P. albobadia*	*P. sanguineus*	*T. harzianum*	*U. botrytis*
pH	3.37 ± 0.02 b	4.07 ± 0.04 b	3.84 ± 0.17 b	3.90 ± 0.03 b	3.75 ± 0.06 b	3.78 ± 0.27 b	7.66 ± 0.84 a
EC (dS/m)	2.49 ± 0.15 bc	3.98 ± 0.15 a	2.91 ± 0.25 b	2.02 ± 0.20 c	2.83 ± 0.11 b	2.50 ± 0.14 bc	2.64 ± 0.50 b
WSP (mg/100 ml)	12.21 ± 0.25 a	3.67 ± 0.49 cd	12.63 ± 0.35 a	2.35 ± 0.26 d	4.43 ± 0.93 c	6.52 ± 0.94 b	7.69 ± 1.02 b
E. foetida permanence (%)	16.65 ± 16.65 c	100.00 ± 0.00 a	0.00 ± 0.00 c	66.70 ± 0.00 b	8.33 ± 14.42 c	77.80 ± 13.60 ab	100.00 ± 0.00 a

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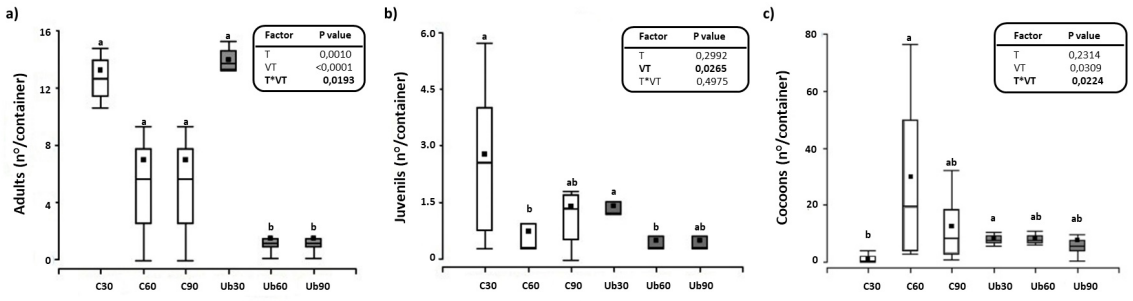

Figure 1. Changes in the number of adult (a), juvenile (b), and cocoon (c) number of *Eisenia foetida* at different times of vermicomposting (VT: 30, 60 and 90 days) under two conditions of treatment of grape pomace (T): Composted control (C) and treated by solid state fermentation with *Ulocladium botrytis* (Ub).
Different letters among boxplots indicate significant differences (Tukey test, $P < 0.05$).

cocoons was observed in the composted GP at day 60 but with a high statistical dispersion in the data (Fig. 1c). This dispersion could be related to antinutritional/toxic factors in the food substrate that may reverse sexual maturity in earthworms (loss of clitellum) or causing sterility (Nogales et al. 1999; Das et al. 2015).

Several physio-chemical properties of composted GP and SSF with *U. botrytis* during the vermicomposting process were evaluated (Fig. 2a-d). A two-way nested ANOVA showed differences in WSP and NH_4^+-N content, as well as in E.C. among the vermicomposting time and the type of bioprocess combination ($P < 0.05$). The combination fungus-earthworm was more efficient in maintaining lower WSP levels than the control treatment during the first 30 days (Fig. 2a). The highest content of free NH_4^+-N was observed at day 30 in both bioprocesses; however, the NH_4^+-N level was low in the GP treated with the fungus at initial time (Fig. 2b). Troncozo et al. showed that *U. botrytis* effectively reduced the availability of toxic monoaromatic compounds and did not participate actively in ammonium liberation in the GP, supporting the results obtained in this work (Troncozo et al. 2019). Variations in pH values during the vermicomposting time were significant ($P < 0.05$), with a slight tendency from alkaline to neutral (Fig. 2c). The degradation of organic compounds by microorganisms, with the subsequent liberation of NH_4^+-N, could be the cause of the slight alkaline pH observed at the initial stages of vermicomposting (Gong et al. 2019). Although a high E.C. was registered at the initial time of vermicomposting with the GP treated with the fungus, optimal values of salinity were observed after vermicomposting which would favor the utilization as organic fertilizer (< 4 dS/m) (Fig. 2d). This reduction in the E.C. could be attributed to the action of microorganisms, including the fungus inoculated, which immobilize the ions in their biomass or due to the loss of salts by leaching (Bhat et al. 2018).

Usmani et al. mentioned that the excavation of the substrate by the worms, in combination with the secretion of several digestive exoenzymes from their intestines that cleave macromolecules, stimulates the proliferation of microorganisms (Usmani et al. 2019). While a reduction in microbial activity could be due to the fact that the same microorganisms are incorporated into the earthworms' diet (Suthar and Singh 2008), significant differences were observed in DH-ase activity levels (biological indicator of microbial activity) among the vermicomposting time, and among the different treatments ($P < 0.05$), showing a similar reduction in enzymatic activity after 60 days in both treatments (Fig. 2e). These results suggest that the reduction in activity of microbial population in the substrate could be attributed to a depletion in nutrients availability caused by the reduction in the number of adult and juvenile earthworms from the substrate observed at this time (Fig. 1a, b).

The phytotoxicity bioassay is commonly used to evaluate the vermicompost maturity, being the germination index the most efficient parameter to elucidate the absence or presence of toxic substances (Gong et al. 2019). The two-way nested ANOVA showed differences in germination and root length, as well as in the germination index of lettuce due to interaction among initial treatments of GP and vermicomposting time ($P < 0.05$) (Fig. 2f, g, h). High germination values ($\geq 80\%$) were obtained in this study (Fig. 2f), suggesting that the treated substrates did not affect this stage. The seeds were able to come out of the dormant state and this could be due to the absence in the GP of organic and/or inorganic substances (Paradelo et al. 2009). Although at the beginning of the experiment, an inhibitory activity on root length was observed in the fungus-treated GP, this effect was posteriorly reversed during the vermicomposting process (Fig. 2g). The increase in root length could be due to the biological activity of earthworms on the substrate and also to the ability of *U. botrytis* to reduce monomeric phenolic compounds with inhibitory activity, as previously discussed. Finally, the GP transformed with the fungus increased the germination index in a short time compared with the control (Fig. 2h), nevertheless, the values obtained ($< 50\%$) suggest the presence of moderate amounts of phytotoxic substances in the GP, indicating that maturity has not yet been reached in the system (Zucconi et al. 1985).

The integration of bioprocesses allows combining the best attributes of each one to improve the general process and the quality of the desired product (Ndegwa and Thompson

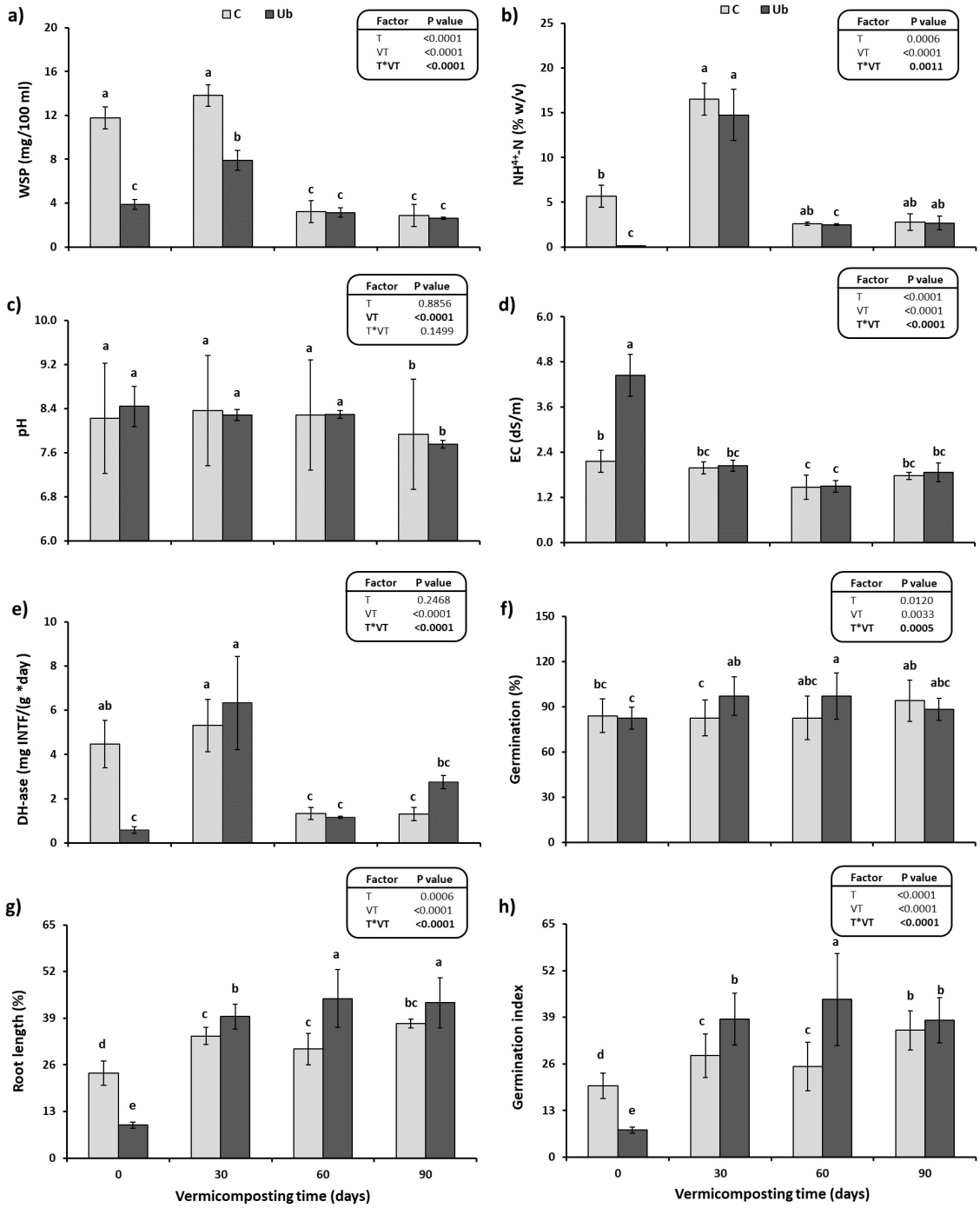

Figure 2. Changes in the physio-chemical and biological parameters evaluated on grape pomace treated (T) by solid state fermentation with *Ulocladium botrytis* (Ub) and composting (C; control group) during the vermicomposting time (VT: 0, 30, 60 and 90 days).
Different letters among boxplots indicate significant differences (Tukey test, $P < 0.05$).

2001; Pérez-Godínez et al. 2017; Esmaeili et al. 2020). In the composting-vermicomposting combination, it is known that composting might provide a partial degradation and disinfection of the food material for earthworms, likewise, the required period of pretreatment is essential to avoid mortality of earthworms during vermicomposting (Gunadi et al. 2002).

Several studies establish that the main attribute of the combination of bioprocesses is that the transformation of lignocellulosic substrates with fungi under SSF constitutes a promising strategy to increase the availability of nutrients that are incorporated by earthworms and microorganisms in the vermicompost in association with the removal of toxic or inhibiting compounds in the system (Fataei et al. 2011; Azizi et al. 2014; Gong et al. 2019). However, another advantage of this combination is that, as the substrate is

sterilized before the incubation of fungi, the absence of pathogens that may be harmful to the earthworms is guaranteed (Gowda and Manvi 2019). According to the results obtained in this study, it could be concluded that the fungal action in the substrate would allow the vermicomposting to develop as a more stable process over time. Further studies are needed to elucidate if there might be other benefits of SSF with a fungi-vermicomposting system that have not been revealed.

4. Conclusion

The novelty of this work is that specific saprotrophic fungi that utilize different metabolic strategies to enhance the earthworm's tolerance to GP were tested. The best result was found with the dematiaceous fungus *U. botrytis*, which conditioned the substrate for vermicomposting to an optimal pH and salinity level and reduced the WSP content. In consequence, GP pretreatment with *U. botrytis* reduced the active phase and phytotoxicity in the vermicomposting process. These results suggest that the treatment of *V. labrusca* GP with *U. botrytis* following vermicomposting is potentially suitable to be used as an organic fertilizer, though more studies are needed to evaluate the benefits of combining both technologies.

Author contributions:
The authors confirm the study conception and design: I. Troncozo, M. Saparrat; data collection: I. Troncozo, M. Escaray; analysis and interpretation of results: I. Troncozo, M. Escaray. Draft manuscript preparation: I. Troncozo, M. Vianna, M. Saparrat. The results were evaluated by all authors, and the final version of the manuscript was approved.

Compliance with ethical standards:
Conflict of interest: The authors declare that there are no conflicts of interest associated with this study.

References

Ansari AA, Humphrey L (2015) An investigation into the preferential palatability of earthworms (Eisenia fetida) on leaves of Terminalia catappa, Blighia sapida and Hibiscus rosasinensis. *Res J Soil Biol,* 1–7. https://doi.org/10.3923/rjsb.2015.21.27

Arora M, Kaur A (2019) Azolla pinnata, Aspergillus terreus, and Eisenia fetida for faster recycling of nutrients from wheat straw. *Environ Sci Pollut Res* 26:32624–32635. https://doi.org/10.1007/s11356-019-06472-5

Azizi AB, Shafiza MS, Noor ZM, Abdullah N (2014) Comparison of bioproduct quality from vermiconversion of spent pleurotus sajor-caju compost and commercial livestock excreta. *Sains Malaysiana* 43 (11): 1699–1705.

Beres C, Costa GNS, Cabezudo I, Silva-James NK da, Teles ASC, Cruz APG, Mellinger-Silva C, Tonon RV, Cabra LM, Freitas SP (2017) Towards integral utilization of grape pomace from winemaking process: A review. *Waste Manag* 68:581–594. https://doi.org/10.1016/j.wasman.2017.07.017

Bhat SA, Singh J, Vig AP (2018) Earthworms as organic waste managers and biofertilizer producers. *Waste Biomass Valor* 9 (7): 1073–1086. https://doi.org/10.1007/s12649-017-9899-8

Bonkowski M, Griffiths BS, Ritz K (2000) Food preferences of earthworms for soil fungi. *Pedobiologia* 44:666–676. https://doi.org/10.1078/S0031-4056(04)70080-3

Cova LJ, García DE, Castro AR, Medina MG (2007) Efecto perjudicial de Moringa oleifera (Lam.) combinada con otros desechos agrícolas como sustratos para la lombriz roja (Eisenia spp.). *Interciencia* 32 (11): 769–774.

Curry JP, Schmidt O (2007) The feeding ecology of earthworms - A review. *Pedobiologia* 50 (6): 463–477. https://doi.org/10.1016/j.pedobi.2006.09.001

Das D, Bhattacharyya P, Ghosh BC, Banik P (2015) Bioconversion and biodynamics of Eisenia foetida in different organic wastes through microbially enriched vermiconversion technologies. *Ecol Eng* 27 (2): 405–423. https://doi.org/10.1016/j.ecoleng.2015.11.012

Domínguez J, Martínez-Cordeiro H, Casas M Álvarez, Lores M (2014) Vermicomposting grape marc yields high quality organic biofertiliser and bioactive polyphenols. *Waste Manag Res* 32 (12): 1235–1240. https://doi.org/10.1177/0734242X14555805

Domínguez J, Sanchez-Hernandez JC, Lores M (2017) Vermicomposting of winemaking by-products. *Handbook of Grape Processing By-Products: Sustainable Solutions,* 55–78. https://doi.org/10.1016/B978-0-12-809870-7.00003-X

Dávila I, Robles E, Egüés I, Labidi J, Gullón P (2017) The biorefinery concept for the industrial valorization of grape processing by-products. *Handbook of Grape Processing By-Products. Elsevier Inc.,* 29–53. https://doi.org/10.1016/B978-0-12-809870-7.00002-8

Edwards CA, Fletcher KE (1988) Interactions between earthworms and microorganisms in organic-matter breakdown. *Agric Ecosys Environ* 24 (1-3): 235–247. https://doi.org/10.1016/0167-8809(88)90069-2

Esmaeili A, Khoram MR, Gholami M, Eslami H (2020) Pistachio waste management using combined composting-vermicomposting technique: Physico-chemical changes and worm growth analysis. *J Clean Prod* 242:118523. https://doi.org/10.1016/j.jclepro.2019.118523

Fataei E, Majd KH, Zakeri F, Jeddi EA (2011) An experimental study of vermicomposting with earthworm (Eisenia foetida) growth in edible mushrooms wastes. *Int J Bio-Resour Stress Manag* 2 (1): 66–68.

Ferreira T, Hansel FA, Maia CMBF, Guiotoku M, Cunha L, Brown GG (2021) Earthworm-biochar interactions: A laboratory trial using Pontoscolex corethrurus. *Sci Total Environ* 777:146147. https://doi.org/10.1016/j.scitotenv.2021.146147

Gong X, Li S, Carson MA, Chang SX, Wu Q, Wang L, An Z, Sun X (2019) Spent mushroom substrate and cattle manure amendments enhance the transformation of garden waste into vermicomposts using the earthworm Eisenia fetida. *J Environ Manag* 248:109263. https://doi.org/10.1016/j.jenvman.2019.109263

Gowda NAN, Manvi D (2019) Agro-residues disinfection methods for mushroom cultivation: A review. *Agric Rev* 40 (2): 93–103. https://doi.org/10.18805/ag.R-1735

Gunadi B, Blount C, Edwards CA (2002) The growth and fecundity of Eisenia fetida (Savigny) in cattle solids pre-composted for different periods. *Pedobiologia* 46 (1): 15–23. https://doi.org/10.1078/0031-4056-00109

Gómez-Brandón M, Lazcano C, Lores M, Domínguez J (2011) Short-term stabilization of grape marc through earthworms. *J Hazard Mater* 187 (1-3): 291–295. https://doi.org/10.1016/j.jhazmat.2011.01.011

Jackson ML, Barak P (2005) Soil chemical analysis: advanced course: A manual of methods useful for instruction and research in soil chemistry, physical chemistry of soils, soil fertility, and soil genesis. *Parallel Press, University of Wisconsin-Madison Libraries*

King GA, Wollard DC, Irving DE, Borst WM (1990) Physiological changes in aspargus spear tips after harvest. *Physiol Plant* 80:393–400. https://doi.org/10.1111/j.1399-3054.1990.tb00058.x

Manan MA, Webb C (2017) Design Aspects of solid state fermentation as applied to microbial bioprocessing. *J Appl Biotechnol Bioeng* 4 (1): 511–532.

Maraun M, Martens H, Migge S, Theenhaus A, Scheu S (2003) Adding to "the enigma of soil animal diversity": Fungal feeders and saprophagous soil invertebrates prefer similar food substrates. *Eur J Soil Biol* 39 (2): 85–95. https://doi.org/10.1016/S1164-5563(03)00006-2

Martínez-Cordeiro H, Casas M Álvarez, Lores M, Domínguez J (2013) Vermicompostaje del bagazo de uva: Fuente de enmienda orgánica de alta calidad agrícola y de polifenoles bioactivos. *Recursos Rurais* 9 (9): 55–63. https://doi.org/10.15304/rr.id1696

Moody SA, Piearce TG, Dighton J (1995) Selective consumption of decomposing. *Soil Biol Biochem* 27 (9): 1209–1213. https://doi.org/10.1016/0038-0717(95)00024-9

Ndegwa PM, Thompson SA (2001) Integrating composting and vermicomposting in the treatment. *Bioresour Technol* 76:107–112. https://doi.org/10.1016/S0960-8524(00)00104-8

Nizami AS, Rehan M, Waqas M, Naqvi M, Ouda OKM, Shahzad K, Miandad R, et al. (2017) Waste biorefineries: Enabling circular economies in developing countries. *Bioresour Technol* 241:1101–1117. https://doi.org/10.1016/j.biortech.2017.05.097

Nogales R, Cifuentes C, Benítez E (2005) Vermicomposting of winery wastes: A laboratory study. *J Environ Sci Health* 40 (4): 659–673. https://doi.org/10.1081/PFC-200061595

Nogales R, Elvira C, Benítez E, Thompson R, Gomez M (1999) Feasibility of vermicomposting dairy biosolids using a modified system to avoid earthworm mortality. *J Environ Sci Health* 34 (1): 151–169. https://doi.org/10.1080/03601239909373189

Osono T, Takeda H (2001) Organic chemical and nutrient dynamics in decomposing beech leaf litter in relation to fungal ingrowth and succession during 3-year decomposition processes in a cool temperate deciduous forest in Japan. *Ecol Res* 16 (4): 649–670. https://doi.org/10.1046/j.1440-1703.2001.00426.x

Palaniappan S, Alagappan M, Rayar S (2018) Influence of substrate particle size on vermicomposting of pre-processed vegetable waste. *Nat Environ Pollut* 17 (1): 277–286.

Paradelo R, Moldes AB, Barral MT (2009) Properties of slate mining wastes incubated with grape marc compost under laboratory conditions. *Waste Manag* 29:579–584. https://doi.org/10.1016/j.wasman.2008.06.019

Pellegrini A, Lanfranco J, Vacisek A, Gelati P (2014) Evolutionary Processes of Organic Waste for Vermicompost Production. 6 Credits. *Universidad Nacional de La Plata, Ed.; Segunda ed*

Pérez-Godínez EA, Lagunes-Zarate J, Corona-Hernández J, Barajas-Aceves M (2017) Growth and reproductive potential of Eisenia foetida (Sav) on various zoo animal dungs after two methods of pre-composting followed by vermicomposting. *Waste Manag* 64:67–78. https://doi.org/10.1016/j.wasman.2017.03.036

Saparrat MCN, Arambarri AM, Balatti PA (2007) Growth response and extracellular enzyme activity of Ulocladium botrytis LPSC 813 cultured on carboxymethylcellulose under a pH range. *Biol Fertil Soils* 44 (2): 383–386. https://doi.org/10.1007/s00374-007-0217-7

Saparrat MCN, Balatti PA, Arambarri AM, Martínez MJ (2014) Coriolopsis rigida, a potential model of white-rot fungi that produce extracellular laccases. *J Ind Microbiol Biotechnol* 41 (4): 607–617. https://doi.org/10.1007/s10295-014-1408-5

Saparrat MCN, Bucsinszky AMM, Tournier H Alfio, Cabello MN, Arambarri AM (2000) Extracellular ABTS-oxidizing activity of autochthonous fungal strains from Argentina in solid medium. *Rev Iberoam Micol* 17 (2): 64–68.

Saparrat MCN, Rocca M, Aulicino M, Arambarri AM, Balatti PA (2008) Celtis tala and Scutia buxifolia leaf litter decomposition by selected fungi in relation to their physical and chemical properties and lignocellulolytic enzyme activity. *Eur J Soil Biol* 44 (4): 400–407. https://doi.org/10.1016/j.ejsobi.2008.05.005

Schuldt M, Rumi A, Gutiérrez-Gregoric D (2005) Estimación de la capacidad de porte en lombricultivos de Eisenia fetida (Oligochaeta, Lumbricidae) con distintas materias orgánicas. *Producción Animal* 25 (1900): 101–109.

Singh UB, Malviya D, Khan W, Singh S, Karthikeyan N, Imran M, Rai JP, et al. (2018) Earthworm grazed-Trichoderma harzianum biofortified spent mushroom substrates modulate accumulation of natural antioxidants and bio-fortification of mineral nutrients in tomato. *Front Plant Sci* 9:1–15. https://doi.org/10.3389/fpls.2018.01017

Suthar S, Singh S (2008) Feasibility of vermicomposting in biostabilization of sludge from a distillery industry. *Sci Total Environ* 394 (2-3): 237–243. https://doi.org/10.1016/j.scitotenv.2008.02.005

Thalmann A (1968) Zur Methodik der bestimmung der dehydrogenaseaktivität im boden mittels triphenyltetrazoliumchlorid (TTC). *Landwirtsh. Forsch 21:* 21:249–258.

Tiquia SM, Tam NFY, Hodgkiss IJ (1996) Effects of composting on phytotoxicity of spent pig-manure sawdust litter. *Environ Pollut* 93 (3): 249–256. https://doi.org/10.1016/S0269-7491(96)00052-8

Troncozo MI, Figoli CB, Franco MEE, Mirífico MV, Bosch A, Rajchenberg M, Balatti PA, Saparrat MCN (2020) Biotransformation of grape pomace from Vitis labrusca by Peniophora albobadia LPSC # 285 (Basidiomycota). *Anais Acad Brasil Ci* 92 (1): 1–16. https://doi.org/10.1590/0001-3765202020181174

Troncozo MI, Lješević M, Beškoski VP, Anđelković B, Balatti PA, Saparrat MCN (2019) Fungal transformation and reduction of phytotoxicity of grape pomace waste. *Chemosphere* 237 https://doi.org/10.1016/j.chemosphere.2019.124458

Usmani Z, Kumar V, Rani R, Gupta P, Chandra A (2019) Changes in physico-chemical, microbiological and biochemical parameters during composting and vermicomposting of coal fly ash: A comparative study. *Int J Environ Sci Technol* 16:4647–4664. https://doi.org/10.1007/s13762-018-1893-6

Zucconi F, Monaco A, Forte M, Bertoldi M (1985) Phytotoxins during the stabilization of organic matter. *Composting of Agricultural and Other Wastes. London: Elsevier Applied Science Publishers,* 73–86. https://doi.org/www-personal.umd.umich.edu/~smtiquia/publications/BC1-2000-PDF.pdf