Chapter 8

Earthworms and Grape Marc: Simultaneous Production of a High-Quality Biofertilizer and Bioactive-Rich Seeds

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Additional information is available at the end of the chapter

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Abstract

Winemaking produces annually millions of tons of grape marc as a byproduct, which is a revaluable resource having many potential uses, including a nutrient-rich organic soil amendment. However, its application as untreated raw material can damage crops owing to the release of phytotoxic polyphenols. This agronomic problems can be minimized by vermicomposting, as earthworms can partly digest polyphenols. This chapter reports the results obtained in the processing of grape marc derived from white wine through vermicomposting on an industrial scale to yield both a high quality organic, polyphenol-free fertilizer and grape seeds as a source of bioactive compounds. Vermicomposting reduced substantially the residue biomass. In a very short-term, the process yielded a nutrient-rich, microbiologically active and stabilized peat-like material that can be easily separated from the seeds by sieving. The isolation of the seeds eliminates the polyphenol-associated phytotoxicity from the vermicompost and left those seeds prepared to be easily processed to get different bioactive compounds, mainly rich-polyphenols extracts but also rich-fatty acids seed oil. The procedure described is effective, simple, environmental-friendly and economical, and can easily be scaled up for industrial application yielding a variety of added-value products from the initial grape marc.

Keywords: wine and winemaking residues, earthworms, grape polyphenols, vermicomposting, vermicompost, earthworm humus

1. Introduction

The annual worldwide production of grapes in the world keeps increasing and accounts to nearly 78 million tonnes [1], and most of these grapes (up to 80%) are utilized to make wine. The main residue of winemaking is grape marc, also known as grape bagasse or grape pomace, which consists of the stalks, skin, pulp and seeds that remain after pressing the grapes. After the pressing process of the grapes to obtain the stum or grape juice, the grape marc is nearby 20% of the grapes weight. The overall composition of grape marc depends on the pre-treatment process in the winery and consists of 40% seeds and 60% skin and pulp grape, when stems are removed before pressing. When grapes are directly processed including their stalks, the composition of grape mars is 30% stems, 30% seeds and 40% skin and pulp grape.

Traditionally, grape marc has been used to produce pomace brandy spirits (orujo, grappa, zivania, törkölypálinka, …). Nowadays, a relative small fraction of the grape marc produced during the winemaking process in the wine industry is utilized for the production of ethanol, to extract organic acids and to produce grape seed oil and other food ingredients [2–4]. Due to its high acidity, it is easy to make silage and thus grape marc has also been used as fodder to feed livestock animals, although its high lignin content makes it rather indigestible [5].

This byproduct or subproduct is potentially a very valuable resource that could be used as a nutrient-rich organic soil amendment; however, overproduction in small geographic areas has led to inappropriate disposal of the material on agricultural land. Moreover, the application of the untreated raw material can damage crops owing to the release of excessive amounts of phytotoxic polyphenols to soils [6]. These phenolic compounds are responsible for the potential phytotoxic and anti-microbial activity of the grape marc, including potential negative effects on the physical, chemical and biological properties of the soil, potential phytotoxic effects on crops and potential groundwater pollution [7].

Since earthworms can digest polyphenols, at least partly [8], the agronomic problems associated with the application of the grape marc to soil can be minimized or eliminated by vermicomposting technologies [9].

From another point of view, polyphenols have well-known human health-promoting effects and other properties in different biological and food systems [3, 10]. Over the past 20 years, the level of scientific and public interest in grapevine polyphenols has increased greatly. Over this period, increasing numbers of potential human health applications for polyphenols have been suggested and experimental data supporting various uses have been accumulated, including anti-cancer and cardioprotective effects and also anti-inflammatory, anti-obesity, anti-ageing, anti-diabetic and neuroprotective properties. Polyphenols are potent antioxidants and can neutralize free radicals, thus halting lipid oxidation and other oxidative side effects [11, 12]. This characteristic makes polyphenols of interest for many different applications, such as the treatment of inflammation [13] and cancer [14, 15]. Moreover, these natural phenolic compounds have many industrial applications, for example, they may be used as natural colourants and have been reported to have excellent properties as food preservatives [11, 12], for anti-ageing purposes in cosmetics [16, 17] and for nutraceutical purposes [18].

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Polyphenols also have nootropic properties, i.e. they can enhance several brain functions, such as learning, memory, attention and motivation [19].

A large proportion of the polyphenols (ca. 60%) in grape marc is contained in the seeds [20]. Consequently, another interesting approach is to recover these polyphenols as functional compounds for the pharmaceutical, cosmetic and food industries [2, 3, 11, 21, 22].

A possible alternative to optimize the extraction of these polyphenols is to use the vermicomposting process as a pre-treatment technique of the grape marc. In this way, the potential agronomic problems associated with the application of grape marc to soil can be simultaneously minimized or eliminated [9, 23].

2. The process of vermicomposting

Vermicomposting is a bio-oxidative process in which detritivorous earthworms intensively interact with microorganisms, thus strongly affecting decomposition processes, accelerating the stabilization of organic matter and greatly modifying its physical and biochemical properties [24, 25]. Although microbiota produces the enzymes for the biochemical decomposition of organic wastes, earthworms are the crucial drivers of the vermicomposting process. Thus, they are responsible for the activation and acceleration of microbial activity through the processes of ingestion and breaking up of fresh organic matter, which result in a larger surface area accessible for microbial attack, altering significantly biological activity. Furthermore, the passage through the earthworm's gut and the associated process, as well as their interactions with other organisms in those detritivorous networks, changes the structure and function of the microbial communities [26, 27].

Vermicomposting includes two different phases in relation to earthworm activity (**Figure 1**):

i. An active phase during which earthworms ingest, process and digest the dead organic matter; thereby modifying its physical and chemical properties and the structure and function of the microbial communities [27–29]; and

ii. A maturation phase characterized by the shift of the earthworms towards raw layers of unprocessed organic waste, during which microorganisms alone take over control of the decomposition of the earthworm's casts [9, 24].

The extent of the active phase is variable and depends on the species and density of earthworms (the main drivers of the process), and the rates at which they ingest and process the organic waste [30].

In the first instance, the effect of earthworms on the decomposition of organic waste during vermicomposting is due to gut-associated processes (GAPs). GAPs include all those modifications that the dead organic matter and the microorganisms undergo during the transit though the earthworm's gut. These alterations include the addition of carbohydrates, enzymes and other metabolites, change diversity and activity of the microbial and microfaunal populations and communities, physical homogenization of the ingested material and the inherent processes of digestion, assimilation and production of mucus and excretory substances such as urea and ammonia, which constitute an easily assimilable pool of nutrients for microorganisms (**Figure 1**). In addition, endosymbiotic microorganisms that live in the earthworms' guts and produce extracellular enzymes that degrade cellulose, polyphenols and other macromolecules [9] boost decomposition of the organic waste. The continuous burrowing activities of earthworms aerate and homogenize the substrate, producing important physical modifications of the substrate, accelerating microbial activity and further increasing the breakdown of organic wastes [24].

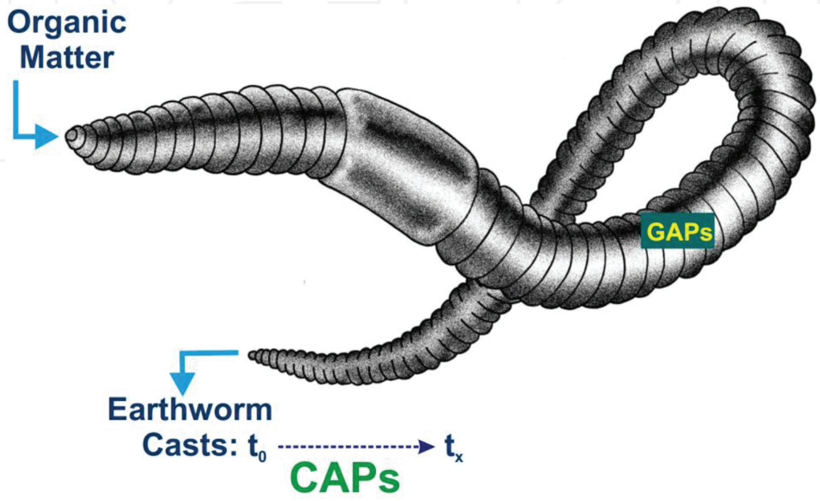

Figure 1. Earthworms influence the vermicomposting of organic matter primarily through gut-associated processes (GAPs, including ingestion, digestion and assimilation in the gut) and secondary through cast-associated processes (CAPs, ageing and microbial modifications of their excreta or casts).

Once GAPs are completed, the earthworm casts undertake cast-associated processes (CAPs), which are more related with ageing and maturation stages, with the physical and chemical changes of the egested casts and with the modifications that the microflora and microfauna present in the vermicompost exert over those cast materials [31]. During cast-associated processes, earthworms exert secondary transformations resulting from the GAPs (**Figure 1**). In vermicomposting systems, earthworm casts are mixed with other materials not ingested and/or digested by the earthworms, and depending on the heterogeneity of the organic wastes, the resulting vermicompost consists of a mixture of the two different portions. During this ageing process, vermicompost will reach an optimum stage in terms of its biological properties promoting plant-growth enhancement and suppression of plant diseases [32].

Vermicompost, the end product of vermicomposting, is a finely divided and porous peat-like material with a high water-holding capacity; it also contains many nutrients in forms that are readily taken up by plants [24]. At the end of the vermicomposting process, the vermicompost can be separated easily from the more recalcitrant fractions of the waste material.

3. Vermicomposting of grape marc

Grape marc derived from white grapes (*Vitis vinifera*, v. Albariño) was collected in a cellar of the Rías Baixas DO (Terras Gauda, O Rosal, Galicia, NW Spain). As a simple pre-treatment, the grape marc was moisturized and revolved before vermicomposting. The main chemical features of the grape marc are detailed in **Table 1**. The system used for the large-scale vermicomposting process was a pilot-scale vermireactor housed in a greenhouse. To prevent desiccation, the vermireactor was watered daily with an automatic system. At the beginning of the trial, the initial earthworm (*Eisenia andrei*) density was 214 ± 26 individuals m^2. The grape marc was then placed on top of a plastic mesh in the vermireactor to facilitate the removal of grape marc after processing by the earthworms [33].

	Grape marc	Vermicompost
pH	4.36 ± 0.04^a	7.1 ± 0.003^b
Electrical conductivity (mS cm^{-2})	1.34 ± 0.15^a	0.27 ± 0.009^b
Organic matter (%)	91.21 ± 0.30^a	74.98 ± 0.34^b
Total carbon (g kg^{-1} dw)	484.23 ± 1.60^a	375.96 ± 1.47^b
Total nitrogen (g kg^{-1} dw)	20.19 ± 0.62^a	29.63 ±0.13^b
C/N ratio	24.02 ± 0.72^a	12.68 ± 0.07^b
Total phosphorus (g kg^{-1} dw)	4.03 ± 0.08^a	8.36 ± 0.32^b
Total potassium (g kg^{-1} dw)	30.46 ± 0.56^a	11.40 ± 0.65^b
Basal respiration (mg O_2 kg OM^{-1} h^{-1})	312.39 ± 40.57^a	68.40 ± 27.11^b
Lignin (g kg^{-1} dw)	516.32 ± 9.56^a	323.54 ± 2.36^b
Cellulose (g kg^{-1} dw)	225.3 ± 10.39^a	58.26 ± 10.48^b
Hemicellulose (g kg^{-1} dw)	100.6 ± 1.39^a	30.56 ± 0.54^b

Values are means ± SE (n = 5). Different letters indicate significant differences between the values, based on post hoc tests (Tukey HSD).

Table 1. Chemical properties and microbial activity of the fresh grape marc and the final vermicompost.

The density and biomass of the earthworm population were determined periodically by collecting 10 samples with a core sampler (five from above and five from below the plastic mesh) of the material in the vermireactor every 14 days during the whole trial (112 days). For the analysis of polyphenols and the biological and physicochemical properties, five samples (10 g) were collected every 7 days during the trial. The samples were stored in plastic bags at 20°C until analysis [33].

Samples of the material were dried at 105°C for 24 h, for the determination of the moisture content, and combusted at 550°C for 4 h, for the determination of the organic matter content. Electrical conductivity and pH were measured in aqueous extracts (1:10 w/v). The total C and N and total P and K contents were analysed in oven-dried (60°C) samples, using a C/N analyser and optical emission spectrometry with inductively coupled plasma (ICP-OES), respectively.

Microbial activity of the grape marc during vermicomposting was determined according to DIN ISO 16072, by measuring the oxygen consumption with the OxiTop® Control System. The total contents of cellulose, hemicellulose and lignin in the grape marc and vermicompost samples were determined by detergent fibre methods. Values of neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined, as described by Aira et al. [34], using the FibreBag System® [32].

To determine the total and individual polyphenols, samples were extracted by means of pressurized liquid extraction (PLE), as described by Alvarez-Casas et al. [2]. The concentration of total polyphenols (TP) in grape marc extracts was determined according to the Folin-Ciocalteu colorimetric method, and the absorbance values were measured at 760 nm. TP were quantified from a calibration curve prepared with gallic acid standard solutions and expressed as mg gallic acid per g of dry weight (mg gallic g^{-1} dw). A 5 mL aliquot of each PLE grape marc extract was concentrated to a final volume of 0.5 mL under an N_2 stream at 40°C. Finally, the concentrated extract was filtered through a 0.22 μm PVDF filter and analysed in a high-performance liquid chromatography (Varian Prostar HPLC system with a diode array detector). The determination chromatographic method is described in detail elsewhere [33].

Data were statistically analysed by repeated measures analysis of variance (rANOVA) with sampling time as the within-subject factor. Mauchly's test confirmed that all variables satisfied the assumption of sphericity, and significant differences in the main effects were further analysed by paired comparisons, with the Tukey HSD test.

4. Evolution of the earthworm population during vermicomposting of grape marc

Before adding the grape marc to the vermireactor, the population density of earthworms (in this study belonging to the species *Eisenia andrei*) in the vermireactor was around 300 individuals m^2, including 19 ± 3 adult and mature earthworms m^2, 215 ± 37 juveniles m^2 and 63 ± 18 cocoons m^2. The total earthworm biomass in the vermireactor was 58.4 ± 15 g live weight m^2 (**Figure 2**).

The total population density of earthworms and the population density of adult earthworms, juveniles and cocoons augmented considerably until day 70, when the population density reached its maximum. Then, since no more grape marc was added to the vermireactor, the earthworm population density started decreasing thereafter until reaching its minimum value at the end of the trial (day 112). Earthworm biomass increased in the same way, with maximum values after 70 days of vermicomposting, decreasing then until day 112 (**Figure 2**). The earthworm population density in the vermireactor before adding grape marc was quite small. As a consequence of the input of earthworm food from the grape marc, it increased rapidly and noticeably, but it reached values far from its maximum capacity. Detritivorous earthworms as *Eisenia andrei* live in pure organic matter environments where the availability of food increases earthworm growth, development and reproduction, leading to very large earthworm populations. Thus, when large amounts of food are available, the population density of this type of earthworms can reach very high values, as for example up to 8000 earthworms m^2 in cow manure and 14,600 individuals m^2 in pig manure [35].

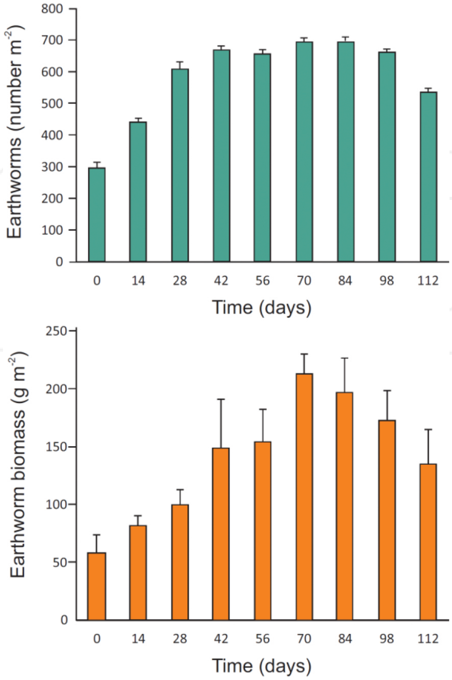

Figure 2. Earthworm density and earthworm biomass during vermicomposting of white grape marc.

Although microorganisms are the main agents responsible for organic matter decomposition, earthworms affect the rates of decomposition directly by feeding and fragmenting activities and indirectly through interactions with microorganisms [9, 24] (Domínguez 2004; Domínguez et al. 2010). Thus, the decomposition rates are directly related to the earthworm population density [31].

5. Vermicompost of grape marc

The pH of the fresh grape marc was quite acid and increased rapidly due to the action of the earthworms, reaching neutrality after seven weeks and remaining neutral in the final vermicompost (**Table 1**).

The rapid mineralization of the organic C of the grape marc leads to a significant reduction of the waste mass and volume. Thus, the mass of grape marc was reduced in 60% as a consequence of the vermicomposting process. On the other hand, this important reduction in mass implies increments in the concentration and availability of mineral nutrients.

Electrical conductivity (EC) of the grape marc was relatively high and decreased significantly during vermicomposting reaching quite low values in the final vermicompost (**Table 1**). The organic matter content of the fresh grape marc is very high and decreased rapidly over time reducing its values to almost 20%. During vermicomposting, the total carbon content of the grape marc depleted rapidly and reduced considerably in the vermicompost (**Table 1**). The nitrogen content of the grape marc was quite high (2%) and increased significantly during vermicomposting until reaching values of 3% in the vermicompost. The C to N ratio decreased gradually and quickly during the process until values around 12 (**Table 1**). While the total K content decreased significantly, the total P content increased significantly during vermicomposting. The microbial activity, measured as basal respiration, decreased very significantly over time reaching much lower values in the vermicompost (**Table 1**). Vermicomposting of grape marc drastically reduced the contents of cellulose, hemicellulose and lignin (**Table 1**).

Some other studies have demonstrated that vermicomposting can be an interesting and efficient alternative for the treatment of grape marc derived from the elaboration of red wine [36, 37] and white wine [38–40]. In the case study presented here, the positive and high dynamics of the earthworm population density together with the correct evolution of the chemical and biological properties indicate that vermicomposting was optimal and produced excellent quality vermicompost. Vermicompost is a mineral-rich, microbiologically active organic amendment that results from the interactions between earthworms and microorganisms during the breakdown of organic matter. It is a stabilized, finely divided peat-like material with a low C:N ratio, high porosity and high water-holding capacity, and it functions as a concentrated source of mineral nutrients that are released slowly and gradually, through mineralization, when plants require them [24]. The speeded breakdown and mineralization of organic wastes, the changes in the structure and function of the microbial communities and the high humification rates achieved during vermicomposting explain the quality and quantity of the nutrients in the vermicompost [40]. At the same time, the organic compounds, extracellular enzymes and other biological characteristics of vermicomposts make these outstanding biological fertilizers. Consequently, when added to the soil or to plant growing substrates, this complex mixture of earthworm faeces, humified organic matter and microorganisms also known as earthworm humus increases germination, growth, flowering and fruit production and accelerates the development of a wide range of plant species. The boosted plant growth may be indorsed to different direct and indirect effects, including biologically mediated mechanisms such as the supply of plant growth regulating substances and improvements in a vast array of soil biological functions [41].

6. Evolution of the polyphenol content of the grape marc and the grape seeds during vermicomposting

The polyphenol content of the initial white grape marc was 58 ± 10 mg GAE g^{-1} dw and decreased significantly throughout the vermicomposting process; the amount of polyphenols was reduced by almost one half in a period of only 14 days. At the end of the trial, the decrease was about 98% of the initial amount, with very low levels maintained during the past weeks, compared with the pre-vermicomposting levels (**Figure 3**), reaching a residual concentration in the final vermicompost.

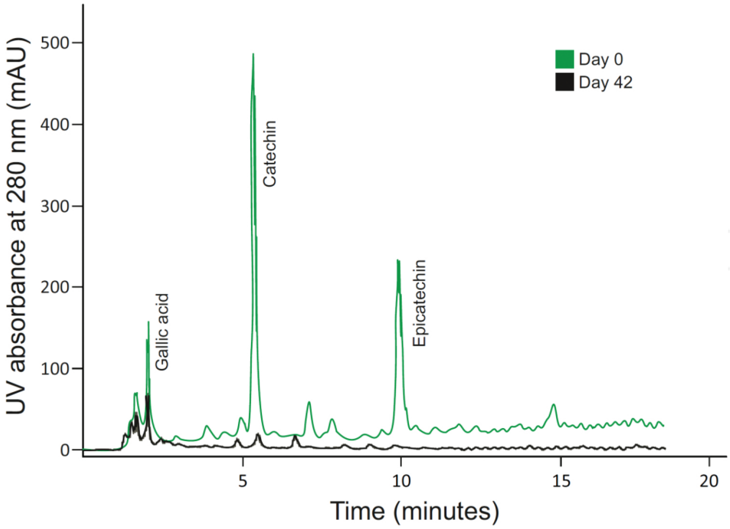

Figure 3. HPLC chromatograms of the fresh grape marc (green) and the vermicompost after 42 days (black), showing the dramatic reduction on the polyphenolic content during the vermicomposting process.

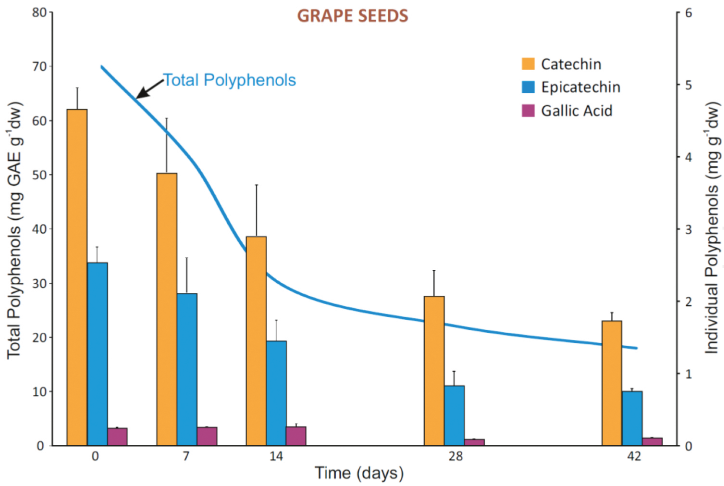

Figure 4. Evolution of the total polyphenol index (blue line) and of the concentration of gallic acid, catechin and epicatechin in white grape seeds during vermicomposting.

The initial concentration of polyphenols in the grape seeds was 70 ± 5 mg GAE g^{-1} dw. The polyphenol content also decreased gradually throughout the vermicomposting process (**Figure 4**). Grape seeds contain large quantities of polyphenolic compounds, being an interesting source for exploiting the biological properties of these natural substances on an industrial scale. After 2 weeks of vermicomposting, it starts to be possible the separation of the grape seeds and the earthworm by sieving. The optimal separation of the seeds is between weeks 4 and 6; latter, although the separation process is even easier the polyphenolic content of the seeds is lower. From the sixth week, the sieved biofertilizer does not contain polyphenols, due to the biodegradation of the 98% of the initial concentration, with residual values in the final vermihumus (**Figure 4**).

Gallic acid, catechin and epicatechin were the main polyphenols identified in the grape seeds (**Figure 4**). Their concentration was determined until the sixth week, corresponding to the end of the optimum time to collect the grape seeds, and because later the concentrations are much lower (**Figure 3**). The concentration of gallic acid and the flavanols, catechin and epicatechin, in the grape seeds decreased gradually and significantly during the first stages of vermicomposting (**Figure 4**).

The polyphenol content of the vermicomposted grape marc and seeds decreased gradually over time. The earthworm activity and the effects on decomposition are enhanced by the action of endosymbiotic microorganisms that produce extracellular enzymes that degrade phenolic compounds [32]. Seeds have greater resistance to the combined biodegradation action of earthworms and microorganisms than the remained vermicomposted grape marc, and this explains the higher concentration of polyphenols in the grape seeds during vermicomposting.

Earthworm activity during vermicomposting speeded the mechanical separation of vermihumus and grape seeds. They break down the grape marc acting as mixing machines, expanding the superficial area for microbial attack, and translocating materials and microbial-rich casts throughout the waste, thus homogenizing it. The most readily assimilable parts are rapidly decomposed to fine particles by the combined action of earthworms and microorganisms, whereas grape seeds stay almost entire. Polyphenols are mainly included in these more recalcitrant parts of the grape marc [20]. Grape seeds can easily be separated from the vermicompost after 2 weeks of vermicomposting. The total polyphenolic content of the seeds after these 2 weeks is lower than in the fresh grape marc, but the separation of seeds and vermihumus is easy, whereas this separation is much more difficult in the grape marc. The removal of the grape seeds also eliminates the phytotoxicity caused by the polyphenols in the vermihumus. Likewise, the lack of phytotoxic compounds is an indication of maturity in organic amendments [42]. This is important because the application of immature vermicompost can negatively affect crop development [43, 44].

Several studies have shown that grapes are a major source of polyphenolic compounds, especially benzoic acids, cinnamic acids, anthocyanins, flavonols, catechins and tannins, which are largely preserved in the grape marc [2]. The concentrations of gallic acid, catechin and epicatechin decreased in the same way as the total polyphenols, as they were degraded by earthworms and microorganisms. Nevertheless, the seeds obtained on day 14 still contain useful amounts of the three major polyphenols (**Figure 4**). These polyphenols have many

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beneficial properties mainly attributed to their anti-oxidant properties and anti-bacterial activities [3], making them interesting substances for use in the cosmetic and food industries. The three main polyphenols contained in the grape seeds act particularly well as hydrogen atom donors, the main mechanism by which these compounds express their anti-oxidant action [22]. Specifically, flavonols are used as natural anti-oxidants preventing degradation of lipids, as anti-microbial agents and functional supplements in foodstuffs to improve animal health and to preserve animal products, and as bioactive components in nutritional and dietary supplements [20].

7. Overview of the vermicomposting process of grape marc

Vermicomposting of grape marc has proven to be a very useful procedure that yields simultaneously an organic fertilizer and grape seeds. During the vermicomposting process, the activity of earthworms favours the mechanical separation of the different fractions of grape marc. The earthworms act as mechanical mixers, thus decomposing the organic material and increasing the surface area exposed to microorganisms; and moving the fragments and excreta rich in bacteria through the residue profile and thus, homogenizing the organic material.

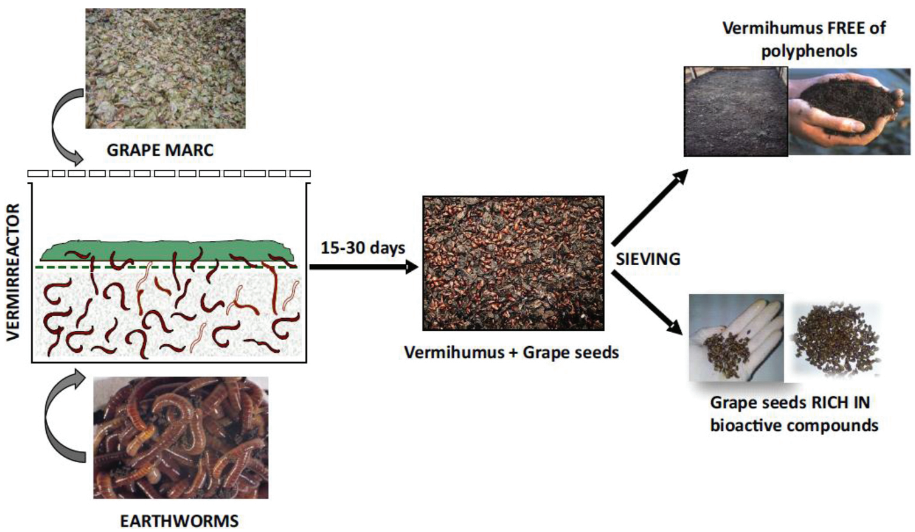

Figure 5. Schematic representation of the potential of earthworms during the vermicomposting process to obtain simultaneously a high-quality biofertilizer free of polyphenols and grape seeds rich in bioactive compounds.

Earthworms reduce the more digestible parts to a finer particle size, while seeds remain almost unaffected. These most recalcitrant parts of grape marc contain the highest amounts of polyphenols and, as mentioned previously, seeds can be easily separated from the vermicompost after 2 weeks of vermicomposting. Sieving the material at the earlier stages of the process led to the separation of the organic fertiliser (vermicompost) from the remaining residual material that mainly consists in grape seeds (**Figure 5**) (Patent no. ES2533501 [45]). The seeds maintain a high proportion of the initial polyphenol content, and separation of the material facilitates extraction of the polyphenols, which have several potential industrial applications.

The separation of the seeds also eliminates the phytotoxicity in the final vermicompost. The degradation of the phytotoxic compounds is a good indicator of the maturity of the vermicompost, an important fact because the immature earthworm humus can affect adversely the development of crops. Thus, a mature and stable product with great potential for use in agriculture is obtained.

Interestingly, the polyphenol content of a wine depends on how grapes have been processed in the winery. Consequently, the polyphenol content of the grape marc also depends on the winemaking process. During red wine vinification, skins and seeds remain for several days in contact with the fermentation broth, giving the red wine a high polyphenol concentration. However, in the white winemaking, the grape juice ferments without being in contact with the grape marc, which remains as a final residue of the process, retaining much of the initial polyphenolic load of the grapes.

8. Conclusions

In recent years, the wastes derived from the wine industry have been object of a growing interest due to several environmental and industrial issues. The excessive accumulation of this waste and the problems associated with its agricultural use led to the search for new techniques for its valorization. In the present study, the application of a vermicomposting process represents an interesting method for the treatment of grape marc, environmentally friendly and rendering a new resource with industrial and commercial interest.

The overall conclusion of this study is the patent viability of vermicomposting to:

– Transform rapidly the most labile parts of the grape marc into a high-quality, polyphenol-free organic fertilizer.

– Facilitate the mechanical separation of grape seeds with a high proportion of their initial polyphenol content.

– Increase promptly earthworm populations, susceptible to be used as fish bait, animal protein and source of bioactive compounds.

As well as yielding these beneficial added-value products, the process is inexpensive and environmentally friendly.

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References

[1] FAO. FAO STAT 2015 [Internet]. Available from: http://faostat.fao.org. [Accessed: 2016-04-01].

[2] Alvarez-Casas M, Garcia-Jares C, Llompart M, Lores M. Effect of experimental parameters in the pressurized solvent extraction of polyphenolic compounds from white grape marc. Food Chemistry.2014;157:524–532. DOI: 10.1007/s00217-015-2573-0

[3] Fontana AR, Antoniolli A, Bottini R. Grape pomace as a sustainable source of bioactive compounds: extraction, characterization, and biotechnological applications of phenolics. Journal of Agricultural and Food Chemistry.2013;61:8987–9003. DOI: 10.1021/jf402586f

[4] Negro C, Tommasi L, Miceli A. Phenolic compounds and antioxidant activity from red grape marc extracts. Bioresource Technology. 2003;87:41–44. DOI: 10.1016/S0960-8524(02)00202-X

[5] Kammerer D, Schieber A, Carle R. Characterization and recovery of phenolic compounds from grape pomace: a review. Journal of Applied Botany and Food Quality. 2005;79:189–196.

[6] Inderjit. Plant phenolics in allelopathy. The Botanical Review. 1996;62:186–202.

[7] Barbera AC, Maucieri C, Cavallaro V, Ioppolo A, Spagna G. Effects of spreading olive mill wastewater on soil properties and crops, a review. Agricultural Water Management. 2013;19:43–53. DOI: 10.1016/j.agwat.2012.12.009

[8] Hättenschwiler S, Vitousek PM. The role of polyphenols in terrestrial ecosystem nutrient cycling. Trends in Ecology & Evolution. 2000;15:238–243. DOI: 10.1016/S0169-5347(00)01861-9

[9] Domínguez J, Aira M, Gómez-Brandón M. Vermicomposting: earthworms enhance the work of microbes. In Insam H, Franke-Whittle I, Goberna M, editors. Microbes at Work. Berlin: Springer; 2010. pp. 93–114. DOI: 10.1007/978-3-642-04043-6_5

[10] Aizpurua-Olaizola O, Ormazabal M, Vallejo A, Olivares M, Navarro P, Etxebarria N, Usobiaga A. Optimization of supercritical fluid consecutive extractions of fatty acids and polyphenols from *Vitis vinifera* grape wastes. Journal of Food Science. 2015;80:101–107. DOI: 10.1111/1750-3841.12715

[11] Makris DP, Boskou G, Andrikopoulos NK. Polyphenolic content and in vitro antioxidant characteristics of wine industry and other agri-food solid waste extracts. Journal of Food Composition and Analysis. 2007;20:125–132. DOI: 10.1016/j.jfca.2006.04.010

[12] Leopoldini M, Russo N, Toscano M. The molecular basis of working mechanism of natural polyphenolic antioxidants. Food Chemistry. 2011;125:288–306. DOI: 10.1016/j.foodchem.2010.08.012

[13] Kim J, Lee KW, Lee HJ. Polyphenols suppress and modulate inflammation: possible roles in health and disease. In: Watson RR, Preedy VR, Zibadi S, editors. Polyphenols in Human Health and Disease. MA, USA: Academic Press; 2014. pp 393–408.

[14] Hertog MGL. Flavonols and flavones in foods and their relation with cancer and coronary heart disease risk [thesis]. Wageningen, The Netherlands: Agricultural University; 1994.

[15] Cordova FM, Watson RR. Food and supplement polyphenol action in cancer recurrence. In: Watson RR, Preedy VR, Zibadi S, editors. Polyphenols in Human Health and Disease. MA, USA: Academic Press; 2014. pp. 191–195. DOI: 10.1016/B978-0-12-398456-2.00016-5

[16] Jean-Gilles D, Li I, Vaidyanathan VG, King R, Cho B, Worthen DR, Chichester CO, Seeram NP. Inhibitory effects of polyphenol punicalagin on type-II collagen degradation in vitro and inflammation in vivo. Chemico-Biological Interactions. 2013;205:90–99. DOI: 10.1016/j.cbi.2013.06.018. Accessed 2014 April 15

[17] Menaa F, Menaa A. Skin photoprotection by polyphenols in animal models and humans. In: Watson RR, Preedy VR, Zibadi S, editors. Polyphenols in Human Health and Disease. MA, USA: Academic Press; 2014. pp. 831–838.

[18] Gollücke AP, Peres RC, Odair A, Ribeiro DA. Polyphenols: a nutraceutical approach against diseases. Recent Patents on Food, Nutrition & Agriculture. 2013;5:214–219. DOI: 10.2174/2212798405666131129153239.

[19] Papandreou MA, Dimakopoulou A, Linardaki ZI, Cordopatis P, Klimis-Zacas D, Margarity M, and others. Effect of a polyphenol-rich wild blueberry extract on cognitive

performance of mice, brain antioxidant markers and acetylcholinesterase activity. Behavioural Brain Research. 2009;198:352–358. DOI: 10.1016/j.bbr.2008.11.013

[20] Yilmaz Y, Toledo RT. Major flavonoids in grape seeds and skins: antioxidant capacity of catechin, epicatechin, and gallic acid. Journal of Agricultural and Food Chemistry. 2004;52:255–260. DOI: 10.1021/jf030117h

[21] El Gharras H. Polyphenols: food sources, properties and applications—a review. International Journal of Food Science & Technology. 2009;44:2512–2518. DOI: 10.1111/j.1365-2621.2009.02077.x

[22] Quideau S, Deffieux D, Douat-Casassus C, Pouységu L. Plant Polyphenols: Chemical Properties, Biological Activities, and Synthesis. Angewandte Chemie International Edition. 2011;50:586–621. DOI: 10.1002/anie.201000044

[23] Gómez-Brandón M, Aira M, Lores M, Domínguez J. Changes in microbial community structure and function during vermicomposting of pig slurry. Bioresource Technology. 2011;102:4171–4178. DOI: 10.1016/j.biortech.2010.12.057

[24] Domínguez J. State of the art and new perspectives on vermicomposting research. In: Edwards CA, editor. Earthworm Ecology. Boca Raton, FL: CRC Press; 2004. pp. 401–424.

[25] Domínguez J, Gómez-Brandón M. Vermicomposting: composting with earthworms to recycle organic wastes. In: Kumar S, Bharti A, editors. Management of Organic Waste. Rijeka, Croacia: Intech Open Science; 2012. pp. 29–48. DOI: 10.5772/33874

[26] Domínguez J, Parmelee RW, Edwards CA. Interactions between *Eisenia andrei* (Oligochaeta) and nematode populations during vermicomposting. Pedobiologia. 2003;47:53–60. DOI: 10.1078/0031-4056-00169

[27] Lores M, Gómez-Brandón M, Pérez-Díaz D, Domínguez J. Using FAME profiles for the characterization of animal wastes and vermicomposts. Soil Biology and Biochemistry. 2006;38:2993–2996. DOI: 10.1016/j.soilbio.2006.05.001

[28] Aira M, Bybee S, Pérez-Losada M, Domínguez J. Feeding on microbiomes: effects of detritivory on the taxonomic and phylogenetic bacterial composition of animal manures. FEMS Microbiology Ecology. 2015;91:117. DOI: 10.1093/femsec/fiv117

[29] Aira M, Olcina J, Pérez-Losada M, Domínguez J. Characterization of the bacterial communities of casts from *Eisenia andrei* fed with different substrates. Applied Soil Ecology. 2016;98:103–111. DOI: 10.1016/j.apsoil.2015.10.002

[30] Aira M, Domínguez J. Optimizing vermicomposting of animal wastes: effects of dose of manure application on carbon loss and microbial stabilization. Journal of Environmental Management. 2008;88: 1525–1529. DOI: 10.1016/j.jenvman.2007.07.030

[31] Aira M, Sampedro L, Monroy F, Domínguez J. Detritivorous earthworms directly modify the structure, thus altering the functioning of a microdecomposer

food web. Soil Biology & Biochemistry. 2008;40:2511–2516. DOI: 10.1016/j.soilbio.2008.06.010

[32] Domínguez J, Edwards CA. Biology and ecology of earthworm species used for vermicomposting. In: Edwards CA, Arancon NQ, Sherman RL, editors. Vermiculture Technology: Earthworms, Organic Waste and Environmental Management. Boca Raton, FL: CRC Press; 2011. pp. 25–37.

[33] Domínguez J, Martínez-Cordeiro H, Álvarez Casas M, Lores M. Vermicomposting grape marc yields high quality organic biofertilizer and bioactive polyphenols. Waste Management & Research. 2014;32:1235–1240. DOI: 10.1177/0734242X14555805

[34] Aira M, Monroy F, Domínguez J. *Eiseniafetida* (Oligochaeta, Lumbricidae) activates fungal growth, triggering cellulose decomposition during vermicomposting. Microbial Ecology. 2006;52:738–747. DOI: 10.1007/s00248-006-9109-x

[35] Monroy F, Aira M, Domínguez J, Velando A. Seasonal population dynamics of *Eiseniafetida* (Savigny, 1826) (Oligochaeta, Lumbricidae) in the field. Comptes Rendus Biologies. 2006;329:912–915. DOI: 10.1016/j.crvi.2006.08.001

[36] Nogales R, Cifuentes C, Benítez E. Vermicomposting of winery wastes: a laboratory study. Journal of Environmental Science and Health, Part B. 2005;40:659–673. DOI: 10.1081/PFC-200061595

[37] Romero E, Plaza C, Senesi N, Nogales R, Polo A. Humic acid-like fractions in raw and vermicomposted winery and distillery wastes. Geoderma. 2007;139: 397–406. DOI: 10.1016/j.geoderma.2007.03.009

[38] Gómez-Brandón M, Lazcano C, Lores M, Domínguez J. Papel de las lombrices de tierra en la degradación del bagazo de uva: efectos sobre las características químicas y la microflora en las primeras etapas del proceso. Acta Zoologica Mexicana. 2010;26:397–408. DOI: 0065-1737

[39] Gómez-Brandón M, Lazcano C, Lores M, Domínguez J. Short-term stabilization of grape marc through earthworms. Journal of Hazardous Materials. 2011;187:291–295. DOI: 10.1016/j.jhazmat.2011.01.011

[40] Domínguez J, Gómez-Brandón M. The influence of earthworms on nutrient dynamics during the process of vermicomposting. Waste Management & Research. 2013;31:859–868. DOI: 10.1177/0734242X13497079

[41] Lazcano C., Domínguez J. The use of vermicompost in sustainable agriculture: impact on plant growth and soil fertility. In: Miransari M, editor. Soil Nutrients. New York: Nova Science Publishers; 2011. pp.230–254. DOI: 978-1-61324-785-3

[42] Wu L, Ma LQ, Martinez GA. Comparison of methods for evaluating stability and maturity of biosolids compost. Journal of Environmental Quality. 2000;29:424–429. DOI: 10.2134/jeq2000.00472425002900020008

[43] Hirai MF, Chanyasak V, Kubota M. A standard measurement for maturity. Biocycle. 1983;24:54–56.

[44] He XT, Logan TJ, Traina SJ. Physical and chemical characteristics of selected U.S. Municipal solid waste compost. Journal of Environmental Quality. 1995;24:543–552. DOI: 10.2134/jeq1995.00472425002400030022x

[45] Domínguez J, Lores M, Álvarez Casas M, Martínez-Cordeiro H. Procedimientopara la obtención y aislamiento de un fertilizanteorgánico y de semillas de uva a partir de residuos de uva. 2015. Patent number: ES2533501. 30/11/2015. Universidade de Vigo y Universidade de Santiago de Compostela. Spain.

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