Original Article

WM&R

Waste Management & Research
2014, Vol. 32(12) 1235–1240

DOI: 10.1177/0734242X14555805
wmr.sagepub.com
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Vermicomposting grape marc yields high quality organic biofertiliser and bioactive polyphenols

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Abstract

Grape is the largest fruit crop in the world, and most (80%) of the harvested fruit is used to make wine. The main by-product of the wine industry is called grape marc, which consists of the stalks, skin, pulp and seeds that remain after pressing the grapes. The aim of this study was to evaluate whether grape marc could be processed by vermicomposting on an industrial scale to yield both a high-quality organic, polyphenol-free fertiliser and grape seeds (as a source of bioactive polyphenols). Vermicomposting reduced the biomass of grape marc substantially (by 58%), mainly as a result of the loss of volatile solids. After 2 weeks, the process yielded a nutrient-rich, microbiologically active and stabilised peat-like material that was easily separated from the seeds by sieving. Although the polyphenol content of the seeds was considerably reduced, this disadvantage was outweighed by the ease of separation of the seeds. Separation of the seeds also eliminated the polyphenol-associated phytotoxicity from the vermicompost. The seeds still contained useful amounts of polyphenols, which could be directly extracted for use in the pharmaceutical, cosmetic and food industries. The procedure described is effective, simple and economical, and could easily be scaled up for industrial application.

Keywords

Winery by-products, grape marc, polyphenols, vermireactor, earthworms, vermicompost

Introduction

Grape is the largest fruit crop in the world. The annual worldwide production amounts to almost 70 million tonnes (FAO, 2014), and most of this (80%) is used to make wine. The main by-product of wine-making is grape marc, which consists of the stalks, skin, pulp and seeds that remain after pressing the grapes. A small fraction of the grape marc generated in the wine industry is used to produce ethanol, to extract organic acids and to produce grape seed oil and other food ingredients (Álvarez-Casas et al., 2014; Fontana et al., 2013; Negro et al., 2003). Grape marc has also been used to feed animals, although its high lignin content makes it rather indigestible (Kammerer et al., 2005).

This waste material is potentially a valuable resource that could be used as a nutrient-rich organic soil amendment; however, overproduction has led to inappropriate disposal of the material on agricultural land. Application of the untreated raw material can damage crops owing to the release of excessive amounts of phytotoxic polyphenols to soils (Inderjit, 1996). However, the polyphenols can also be recovered and used as functional compounds in the pharmaceutical, cosmetic and food industries (El Gharras, 2009; Fontana et al., 2013; Quideau et al., 2011); a large proportion of the polyphenols (ca. 60%) in grape marc are contained in the seeds (Yilmaz and Toledo, 2004).

The agronomic problems associated with the application of the grape marc to soil can be minimised or eliminated by vermicomposting (Domínguez et al., 2010), as earthworms can at least partly digest polyphenols (Hättenschwiler and Vitousek, 2000). Vermicomposting is a bio-oxidative process in which detritivorous earthworms interact closely with micro-organisms, thus strongly affecting decomposition processes, accelerating the stabilisation of organic matter (OM) and greatly modifying the physical and biochemical properties of OM (Domínguez, 2004). Decomposition is also enhanced by the action of endosymbiotic microbes that reside in the earthworms' guts and produce extracellular enzymes that degrade cellulose and phenolic compounds (Domínguez et al., 2010). Vermicompost, the end product of vermicomposting, is a fine porous peat-like material with a high water-holding capacity; it also contains many nutrients in forms that are readily taken up by plants (Domínguez, 2004). At the end

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Table 1. Changes in the chemical properties and microbial activity of the grape marc during the vermicomposting process. Values are means ±SE ($n = 5$). Different letters indicate significant differences between the values, based on post hoc tests (Tukey HSD).

	Fresh grape marc	Worm-worked material	
	Day 0	Day 56	Day 112
pH	4.36 ±0.04[a]	8.17 ±0.06[b]	7.1 ±0.003[c]
Electrical conductivity (mS cm^{-2})	1.34 ±0.15[a]	0.49 ±0.01[b]	0.27 ±0.009[c]
Organic matter (%)	91.21 ±0.30[a]	92.29 ±0.71[a]	74.98 ±0.34[b]
Total carbon (g kg^{-1} dw)	484.23 ±1.60[a]	535.03 ±2.48[b]	375.96 ±1.47[c]
Total nitrogen (g kg^{-1} dw)	20.19 ±0.62[a]	20.56 ±0.95[a]	29.63 ±0.13[b]
C/N ratio	24.02 ±0.72[a]	26.12 ±1.14[a]	12.68 ±0.07[b]
Total phosphorus (g kg^{-1} dw)	4.03 ±0.08[a]	2.80 ±0.1[b]	8.36 ±0.32[c]
Total potassium (g kg^{-1} dw)	30.46 ±0.56[a]	16.13 ±0.39[b]	11.40 ±0.65[c]
Basal respiration (mg O_2 kg OM^{-1} h^{-1})	312.39 ±40.57[a]	121.81 ±11.16[b]	68.40 ±27.11[c]
Lignin (g kg^{-1} dw)	516.32 ±9.56[a]	495.76 ±8.96[a]	323.54 ±2.36[b]
Cellulose (g kg^{-1} dw)	225.3 ±10.39[a]	204.53 ±6.77[a]	58.26 ±10.48[b]
Hemicellulose (g kg^{-1} dw)	100.6 ±1.39[a]	90.83 ±0.89[b]	30.56 ±0.54[c]

[a,b,c]significant differences between the values.

of the vermicomposting process, the vermicompost can be easily separated from the more recalcitrant fractions of the material.

The objective of the study was to evaluate whether grape marc could be processed by vermicomposting on an industrial scale to yield both a high quality, polyphenol-free organic vermicompost, which could be used as a fertiliser, and grape seeds, which could be used as a source of bioactive polyphenols.

Material and methods

Grape marc

White grape marc (*Vitis vinifera* v. Albariño) was obtained from the Mar de Frades winery in Pontevedra (Galicia, NW Spain) and was stored at 4 °C until use. The grape marc was turned and moistened with water during the 2 days prior to the trial to achieve an adequate level of moisture for the earthworms (85%). Some chemical characteristics of the grape marc are summarised in Table 1.

Vermireactor set-up and sampling method

The system used was a rectangular metal pilot-scale (4 × 1.5 × 1 m) vermireactor housed in a greenhouse with no temperature control. The reactor was watered daily with an automatic watering system, to prevent desiccation. At the beginning of the trial, the vermireactor contained a layer of vermicompost (12 cm height) as a bed for the earthworms. The initial earthworm (*Eisenia andrei)* density was 214 ±26 individuals m^{-2}. The grape marc (158 kg fw, 6 cm height layer) was placed on top of a plastic mesh (5 mm mesh size) in the vermireactor. Use of the plastic mesh prevents mixing of the grape marc and the vermicompost bedding and also facilitates the removal of grape marc after processing by the earthworms.

The density and biomass of the earthworm population (adults, juveniles and cocoons) were determined periodically by collecting 10 samples (five from above and five from below the plastic mesh) of the material in the vermireactor every 14 days during the trial (112 days). The samples were collected with a core sampler (7.5 cm diameter and 12 cm height).

For analysis of polyphenols and the biological and physicochemical properties, five samples comprising 10 g of grape marc plus seeds were collected every 7 days during the trial. The samples were stored in plastic bags at −20 °C until analysis.

Analyses

Samples of the material (grape marc plus seeds) were either dried at 105 °C for 24 h, for determination of the moisture content, or combusted at 550 °C for 4 h, for determination of the OM content. Electrical conductivity and pH were measured in aqueous extracts (1:10 w/v) by using a Crison conductivity meter CM35 and a Crison MicropH 2000 pH meter, respectively. Total C and N contents were analysed in oven-dried (60 °C) samples, in a Carlo Erba (EA 1108 CHNS-O) 1500 C/N analyser. Total P and K contents were analysed in oven-dried (60 °C) samples by optical emission spectrometry with inductively coupled plasma (ICP-OES). Microbial activity was determined by measuring the oxygen consumption with the OxiTop® Control System (WTW, Weilheim, RFA), according to DIN ISO 16072. Cellulose, hemicellulose and lignin contents in the samples were determined by detergent fibre methods. Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) values were determined using the FibreBag System® (Gerhardt, Königswinter, Germany), as described by (Aira et al., 2006).

To determine total and individual polyphenols, samples were extracted by means of pressurised solvent extraction (PSE) in a solvent extractor (ASE 150, Dionex, Co., Sunnyvale, CA, USA), as described by (Álvarez-Casas et al., 2014). The concentrations of total polyphenols (TPs) in grape marc extracts was determined

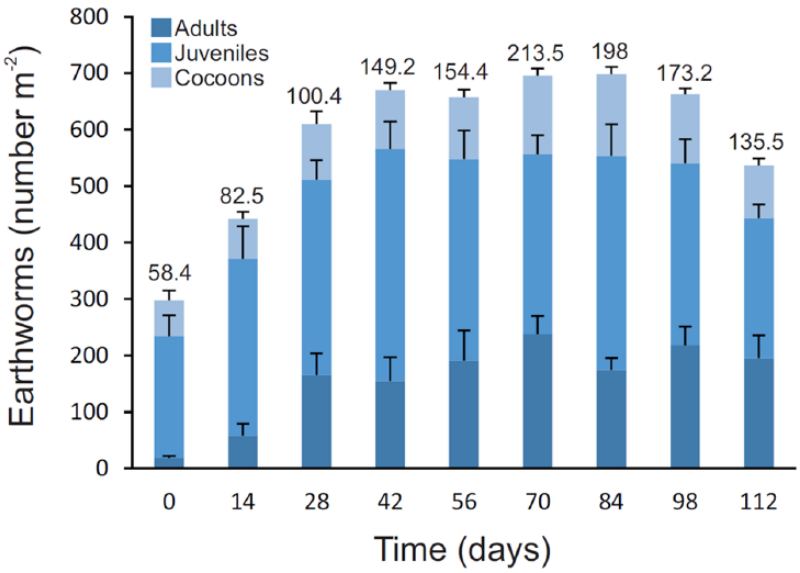

Figure 1. Earthworm density (number of adults, juveniles and cocoons per square metre) and earthworm biomass (g m^{-2} fw, numbers on top of the bars) during vermicomposting of grape marc. Values are means ±SE ($n = 5$).

according to the Folin-Ciocalteu colorimetric method (Singleton and Rossi 1965), and the absorbance values were measured at 760 nm (Spectrophotometer Shimazdu, UVmini-1240, Tokyo, Japan). TPs were quantified from a calibration curve prepared with gallic acid standard solutions and expressed as mg of gallic acid equivalents in the liquid extract (mg L^{-1} GAE). The concentrations of TPs were expressed as mg gallic acid per g of dry weight (mg gallic g^{-1} dw). A 5 mL aliquot of each PSE grape marc extract was concentrated to a final volume of 0.5 mL under an N_2 stream (VLM EC1 Sample Concentrator), while the extract was maintained at 40 °C. Finally, the concentrated extract was filtered through a 0.22 µm Polyvinylidene fluoride (PVDF) filter (Simplepure, USA) and analysed by high-performance liquid chromatography (Varian Prostar High Performance Liquid Chromatography (HPLC) system equipped with a diode array detector). The extracts were separated on a 3.9 mm × 150 mm, 4 µm, 60 Å, Waters Nova-Pak C18 column. The injection volume was 20 µL. The mobile phase solvents were (A) 1% formic acid/water and (B) 1% formic acid/methanol. The mobile phase gradient programme began with 5% B, increased to 20% B after 20 min, and then to 100% B after 25 min. The entire HPLC run time was 25 min, with a flowrate of 1.0 mL min^{-1} and column temperature, 50 °C. Polyphenols were detected at 280 nm and identified by comparison of their retention times and ultraviolet spectra to those of pure standards. The concentrations of the major polyphenols identified (gallic acid, catechin and epicatechin) were determined by examination of the respective calibration curves.

Statistical analysis

Data were analysed by repeated measures analysis of variance (rANOVA) with sampling time (0 to 16 weeks) as the within-subject factor. All variables fulfilled the assumption of sphericity (Mauchly's test), and significant differences in the main effects were further analysed by paired comparisons, with the Tukey HSD test. All statistical analyses were performed with SPSS v19 software.

Results and discussion

Population dynamics of earthworms

The initial population density of *E. andrei* in the vermireactor was 297 ±20 individuals m^{-2}, including 19 ±3 mature earthworms m^{-2}, 215 ±37 juveniles m^{-2} and 63 ±18 cocoons m^{-2}, of mean biomass 58.4 ±15 g live weight m^{-2} (Figure 1). The total number of earthworms and the number of mature earthworms, juveniles and cocoons increased significantly until Day 70, when the population reached its maximum density. No more grape marc was added to the vermireactor and after Day 84 the earthworm population density decreased thereafter until reaching a minimum on Day 112. The earthworm biomass followed a similar trend, reaching the maximum value on Day 70 and the minimum value on Day 112 (Figure 1).

The initial population of earthworms in the vermireactor was low, and although it increased considerably as a consequence of the input of OM from the grape marc, it was far from the maximum capacity. As *E. andrei* lives in environments where the same material acts as the substrate and the food, the availability of this material improves the conditions for earthworm growth and reproduction, and leads to the presence of high numbers of earthworms. When large quantities of OM are available, the density of epigeic earthworms can be high, e.g. up to 8000 individuals m^{-2} in cow manure and 14,600 individuals m^{-2} in pig manure (Monroy et al., 2006).

Although micro-organisms are the main agents responsible for OM decomposition, earthworms affect the rates of decomposition directly by feeding and fragmenting activities and indirectly via interactions with micro-organisms (Domínguez, 2004; Domínguez

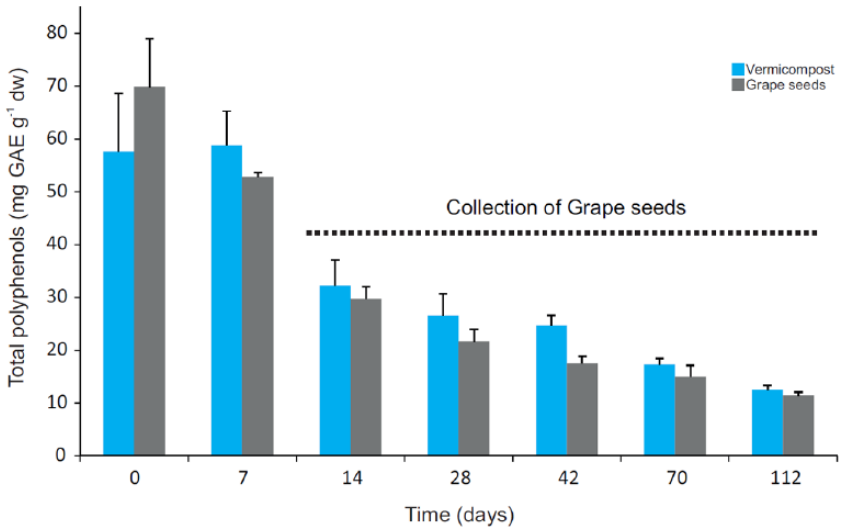

Figure 2. Changes in the TP index (mg GAE g^{-1} dw) during vermicomposting of grape marc. Values are means ±SE ($n = 5$).

et al., 2010). Thus, the decomposition rates are directly related to the earthworm population density (Aira et al., 2002, 2008).

Chemical and biological properties of grape marc during vermicomposting

The pH of the fresh grape marc was 4.36 ±0.04 and increased significantly until Day 56, reaching its maximum value and remaining neutral until the end of the study (Table 1). The electrical conductivity of the initial grape marc was 1.34 ±0.15 mS cm^{-2} and decreased significantly during vermicomposting, reaching the minimum value at the end of the study (Table 1). The OM content of the fresh grape marc was 91.21 ±0.30% and decreased over time until reaching a final value of 74.98 ±0.34% at the end of the trial.

The total carbon content of the initial grape marc was 484.23 ±1.60 g kg^{-1} dw. After Day 56 the total C was rapidly depleted and decreased significantly until reaching a final value of 375.96 ±1.47 g kg^{-1} dw at the end of the trial (Table 1). The total nitrogen content of the initial grape marc was 20.19 ±0.62 g kg^{-1} dw and this increased slightly until reaching a final value of 29.63 ±0.13 g kg^{-1} dw (Table 1). The C to N ratio decreased gradually from 24.02 ±0.72 to 12.68 ±0.07 after 112 days (Table 1). The total P content of the initial grape marc was 4.03 ±0.08 g kg^{-1} dw and increased significantly throughout the vermicomposting process until reaching a final value of 8.36 ±0.32 g kg^{-1} dw. The total K content of the initial grape marc was 30.46 ±0.56 g kg^{-1} dw and decreased significantly until reaching a final value of 11.4 ±0.65 g kg^{-1} dw (Table 1). The basal respiration of the initial grape marc was 312 ±41 mg O_2 kg OM^{-1} h^{-1} and decreased significantly over time until reaching a final value of 68 ±27 mg O_2 kg OM^{-1} h^{-1}.

The cellulose, hemicellulose and lignin contents of the initial grape marc were 225.3 ±10.39, 100.6 ±1.39 and 516.32 ±9.56 g kg^{-1} dw, respectively, and all of these decreased significantly until reaching final values of 58.26 ±10.48, 30.56 ±0.54 and 323.54 ±2.36 g kg^{-1} dw (Table 1).

The total mass balance was determined at the end of the trial (112 days) and the initial grape marc mass (158 kg fw, 31.07 kg dw) decreased significantly (58%), mainly as a result of the loss of volatile solids, until reaching a final mass of 66.65 kg fw (18.68 kg dw) at the end of the vermicomposting process.

Vermicomposting has been proven to be effective for the treatment of grape marc derived from red winemaking (Nogales et al., 2005; Romero et al., 2007) and white winemaking (Gómez-Brandón et al., 2010, 2011). In the present study, the changes in the earthworm population and the chemical and biological parameters indicated that the vermicomposting process was optimal and rendered good quality vermicompost. Vermicompost is a nutrient-rich, microbiologically active and stabilised fine peat-like material with a low C:N ratio and high water-holding capacity, and it constitutes a source of plant nutrients that are released gradually, through mineralisation, as the plant needs them (Domínguez, 2004). The quantity and quality of the nutrients in vermicompost can be explained by the accelerated mineralisation of OM, increased microbial activity, breakdown of polysaccharides and higher rates of humification achieved during vermicomposting (Domínguez and Gómez-Brandón, 2013). The biological properties of vermicomposts make these excellent organic fertilisers; when added to the soil or plant growing media, vermicompost increases germination, growth, flowering, fruit production and accelerates the development of plants. The enhanced plant growth may be attributed to various direct and indirect mechanisms, including biologically mediated mechanisms, such as the supply of plant-growth regulating substances and improved soil biological functions (Lazcano and Domínguez, 2011).

Changes in polyphenol contents of grape marc and seeds during vermicomposting

The polyphenol content of the grape marc was 58 ±10 mg GAE g^{-1} dw and decreased significantly throughout the vermicomposting process (Figure 2, $p < 0.0001$); the amount of polyphenols was reduced by almost one half in a period of only

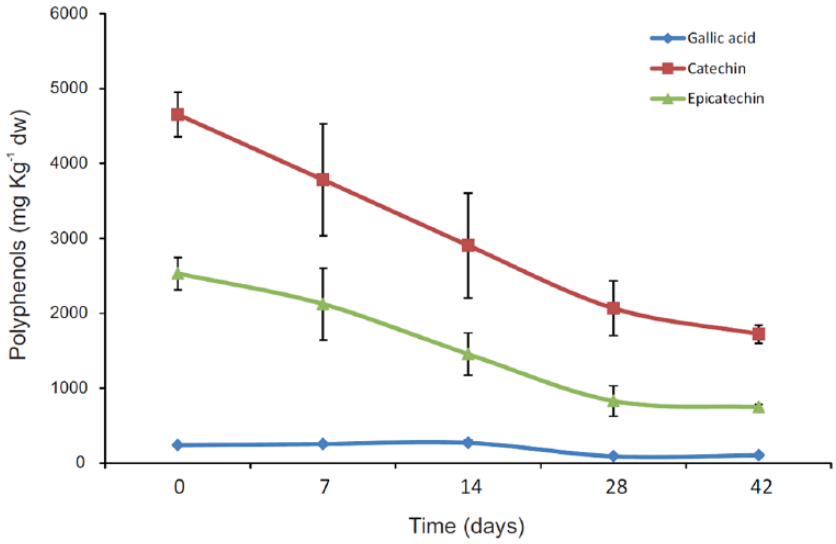

Figure 3. Changes in the concentration of gallic acid, catechin and epicatechin in wine grape seeds during the vermicomposting process. Values are means ±SE ($n = 5$).

14 days. At the end of the trial (112 days), the decrease was about 80% of the initial amount, with very low levels maintained during the last weeks, compared with the pre-vermicomposting levels, reaching a concentration of 12.5 ±0.7 mg GAE g^{-1} dw in the final product.

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 2, $p < 0.0001$). Grape seeds contain large amounts of polyphenols and are a potential source of these bioactive compounds on an industrial scale. From Day 14, the seeds and the organic fertiliser were able to be separated easily and efficiently by sieving. Optimal separation of seeds occurs at between approximately Days 28 and 42, before the concentration of polyphenols continues to decrease, although it can be done at any time up to the end of the process. From Day 42, the sieved organic fertiliser is virtually free of polyphenols, and the initial content decreases by 98% with a residual value of 1.37 ±0.4 mg GAE g^{-1} dw in the final vermicompost.

The main individual polyphenols identified in the seeds were gallic acid, catechin and epicatechin (Figure 3). The concentration of these compounds was determined until Day 42, coinciding with the end of the optimal collection period for grape seeds. The initial concentration of gallic acid in the seeds was 240 ±3 mg kg^{-1} dw. This concentration decreased until reaching a value of 104 ±9 mg kg^{-1} dw after 42 days. The concentration of epicatechin decreased from 2532 ±217 mg kg^{-1} dw (Day 0) to 748 ±38 mg kg^{-1} dw (Day 42). The initial concentration of catechin in the grape seeds was 4654 ±297 mg kg^{-1} dw, and it also decreased significantly to a concentration of 1723 ±121 mg g^{-1} dw on Day 42 (Figure 3).

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 micro-organisms that produce extracellular enzymes that degrade phenolic compounds (Domínguez et al., 2010). The higher concentration of polyphenols in the grape seeds than in the vermicomposted grape marc can be explained by the greater resistance of seeds to earthworm degradation and to attack by micro-organisms.

During the vermicomposting process, mechanical separation of the different fractions of the grape marc was enhanced by the earthworm activity. Earthworms act as mechanical blenders because they break down organic material, increase the surface area exposed to microbes, and move fragments and bacteria-rich excrement through the waste profile, thus homogenising the organic material. The most digestible parts are reduced to fine particles by the earthworms, whereas seeds remain almost unaltered. These more recalcitrant parts of the grape marc contain the highest amounts of polyphenols (Berg and McClaugherty, 2003; Yilmaz and Toledo, 2004). As already mentioned, the seeds were separated from the vermicompost after a 2-week period of vermicomposting. Although the polyphenol content of the seeds at this point is significantly lower than at the start of the trial, the seeds can be easily separated from the vermicompost by sieving, whereas in the first 2 weeks they can only be obtained by a tedious manual procedure. Separation of the seeds also removes the polyphenol-associated phytotoxicity from the vermicompost. Furthermore, the degradation of phytotoxic compounds is a good indicator of compost maturity (Wu et al., 2000). This is important because the application of immature vermicompost can negatively affect crop development (Hirai et al., 1983; He et al., 1995).

Several studies have shown that grapes are a major source of polyphenolic compounds, especially benzoic acids, cinnamic acids, anthocyanins, flavonols, catechins and tannins (García-Alonso et al., 2004), which are largely preserved in the grape marc (Álvarez-Casas et al., 2014). The concentrations of gallic acid, catechin and epicatechin decreased in the same way as the TSs, as they were degraded by earthworms and micro-organisms.

Nevertheless, the seeds obtained on Day 14 still contained useful amounts of the three major polyphenols (Figure 3). These polyphenols have many beneficial properties mainly attributed to their antioxidant properties and antibacterial activities (Fontana et al., 2013), making the extract of interest 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 antioxidant action (Quideau et al., 2011). In particular, catechins have been used as a natural antioxidant in oils and fats, as a supplement for animal feeds to improve animal health and to preserve animal products, as an antimicrobial agent in foodstuffs and as a functional ingredient in various food and dietary supplements (Yilmaz and Toledo, 2004).

The phenol content of wine depends on how the grapes are processed in the winery. The phenol content of the grape marc will, therefore, also depend on the winemaking process. During red winemaking, skins and seeds are in contact with the fermenting broth for several days, conferring red wine with a high concentration of polyphenols. However, in white winemaking, the grape juice ferments without the grape marc, which thus maintains its polyphenol content.

Conclusions

Vermicomposting of white grape marc has proven to be a useful procedure that yields an organic fertiliser and grape seeds. The process transforms the most labile parts of the grape marc into a high quality, polyphenol-free organic fertiliser. Sieving the material separates the fertiliser (vermicompost) from a residue that mainly contains grape seeds. 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. As well as yielding added value products, the process is inexpensive and environmentally friendly.

Declaration of conflicting interests

The authors declare that there is no conflict of interest.

Funding

This research was financially supported by projects CN 2012/305 and CN 2012/299 (Xunta de Galicia, Spain).

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