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The composting potential of the by-product marc resulting from the white and red winemaking process

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Abstract. The production of an organic fertilizer was carried out, from waste generated in the winemaking process of white (Riesling Italian-RI) and red (Cabernet Sauvignon-CS) grapes obtained from the vineyard of Pietroasa (Romania). The potential of the biotransformation process of by-product marc in an open pile in the autumn-winter season was controlled and the thermophilic phase reached average values of 60 ºC at a neutral pH which gives it suitable characteristics to be used as a soil fertiliser. The values of the germination index (GI) of *Lepidium sativum* L. seeds, using water dilutions from the RI and CS compost marc, demonstrated that there was no degree of phytotoxicity (average GI 144.2% and 139.8%, respectively). The compost marc microflora was represented by bacterial and fungal isolates belonging to genera *Rhizopus* (10%), *Aspergillus* (80%), and *Penicillium* (17%), compared to by-product marc that only present fermentation yeasts. The structure of the microorganism populations in the compost marc dried by lyophilisation showed an almost double number of $CFUg^{-1}$ compared to the oven-dried compost. The presence of potential antagonistic microorganisms and the high number of $CFUg^{-1}$, demonstrated that this compost can have a suppressive effect on soil pathogens in addition to its quality as a fertilizer.

1 Introduction

The wine industry is associated with a great environmental impact, according to OIV data, it is estimated that for every 100 kg of processed grapes, around 25 kg of by-products are generated, an alarming value in the main wine-producing countries, such as Spain, France or Italy, where production can reach 1,200 tons per year [1]. The main solid residue during the winemaking process is the by-product marc, which determines the contamination of the soil with a phytotoxic effect on the roots [2] if it is incorporated directly without prior treatment. The polluting potential of grape marc depends on the high content of organic matter with an acidic pH, high content of polyphenols, tannin etc. [3]. Different authors have carried out and studied the composting process of grape marc with other organic residues and show that the compost obtained is an adequate fertilizer for the soil and crops ([4-7]). According to Nkoa (2014) [7] grape marc compost increases the biomass of microorganisms and their metabolic activity and provides organic carbon, nitrogen, phosphorus and potassium. Used as a substrate, grape marc compost determines a high rate of seed germination and hygienization in terms of pathogens for plants or human consumption [9].

In the composting process, microorganisms that represent between 2 and 20% of the total mass participate. Small temperature variations as a composting parameter (50-60 ºC optimal) affect microbial activity and compost biomass in comparison with pH, organic matter or C/N ratio [10]. The intense acid pH levels of grape marc affect microbial activity and the transition from the mesophilic to thermophilic stage. *Aspergillus*, *Penicillium* and *Rhyzopus* species are active at an acid pH (pH 3-4), but lignocellulosic residues with a buffer effect are normally used due to their complex composition ([11,12]).

Grape marc represents an important source of polyphenolic compounds. Yu and Ahmedna (2013) [13] estimate that the total polyphenol content of the dry matter is between 4.8% and 5.4%, being higher in red grapes than in white grapes. It has been shown that the lyophilization treatment of by-product marc has a positive influence, being a method for the conservation of active compounds (especially polyphenols) but it can also represent a method for the conservation of the active biomass of the compost [14].

The objectives of the work were to monitor some parameters of the open-pile composting process of Riesling Italian (RI) and Cabernet Sauvignon (CS) by-product marc and the reduction of phytotoxicity as well as the evolution of the biomass of microorganisms by different conservation methods.

2 Material and method

For this study, white grape marc (RI) and red grape marc (CS) from the Pietroasa Viticulture and Oenology Research and Development Station (SCDVV Pietroasa) were used (Fig. 1).

Figure 1. Varieties: Riesling Italian (RI) and Cabernet Sauvignon (CS) from Pietroasa vineyard.

The two compost piles were layered with white and red by-product marc (250 kg), respectively, with straw (3 kg) and compost activator (AGROKompostuse Zeolitem) (0.5 kg), directly on the ground and covered with plastic. In total, each compost pile was turned three times during the three months of composting (October to December), at which time hydration (10 L water/turn) of each compost mixture was also performed. The monitoring of the composting process parameters (temperature, humidity, pH and aeration) of the by-product marc from the RI and CS varieties was carried out (Fig. 2).

Figure 2. Monitoring the parameters of the composting process of by-product marc (RI and CS).

2.1 Compost phytotoxicity analysis

Seeds of *Lepidium sativum* L. (10 seeds/3 layers Whatman filter/Petri dish) were selected as phytotoxicity bioindicators for D_1 and D_2 dilutions (1:10 and 1:20, respectively) in distilled water (1 hour) of the composts obtained from white (RI) and red (CS) by-product marc. To observe the phytotoxicity effect, root elongation and germination percentage measurements were performed after 72 hours at 25ºC (FTC 90 E Velp Scientifica incubator), for the control using distilled water (Fig. 3). The humidity of the two composts was measured (Precise XM 60 thermal balance) and the pH (ph meter WTW inoLab pH 7110) of each compost extract. The germination index (GI) is calculated according to Zucconi et al. (1981) [15].

Figure 3. Phytotoxicity analysis of by-product marc compost extracts (RI and CS).

2.2 Microbiological analysis of fresh and composted by-product marc

The microbiological analysis was carried out on the by-product marc obtained immediately after the white and red winemaking process and on the composted by-product marc obtained from the two varieties (RI and CS). Fresh and composted by-product marc fragments are placed in Petri dishes on PDA culture medium (Merk). Reading was done after 7 days of incubation at a temperature of 22 ºC. Fungal colonies were identified based on morphological characters. The results are expressed as incidence of different fungal isolates (%).

2.3 Microbiology of by-product marc compost oven-dried (RIcd and CScd) and lyophilized (RIcL and CScL)

Serial dilution method has been used for estimation of number of microorganisms of the by-product marc compost oven-dried (120 ºC for 48 hours) (RIcd and CScd) and lyophilized (minus 57.6 ºC and 10^{-2} mbar for 24 hours) (RIcL and CScL). Ten grams from each marc compost sample were put in 90 ml of distilled water. After homogenization for 30 minutes, serial dilutions (10^{-1}-10^{-6}) were prepared and aliquots (0.5 ml) were plated on PDA. Four repetitions were made for each dilution. All plates have been incubated at 22 ºC. The reading was carried out after 72 hours. The results are expressed in $CFUg^{-1}$ (colony-forming unit/g) of marc compost. Fungal colonies were identified based on morphological characters.

3 Results and Discussions

3.1 Control parameters of the by-product marc (RI and CS) composting process

The parameters followed such as temperature, humidity and aeration (turning) are related to the evolution of the by-product marc (RI and CS) composting process as well as the seasonal effect (October-December) (Figs. 4 and 5) ([16, 17]).

Both for the RI and CS by-product marc piles, it was necessary to turn three times to reactivate the composting process, due to the loss of humidity in the thermophilic phase (62.5 ºC for RI and 68 ºC for CS, respectively).

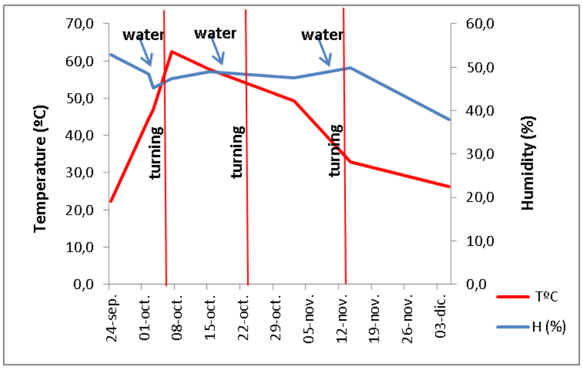

Figure 4. Control parameters of the composting process (T ºC and H%) of by-product marc (RI variety).

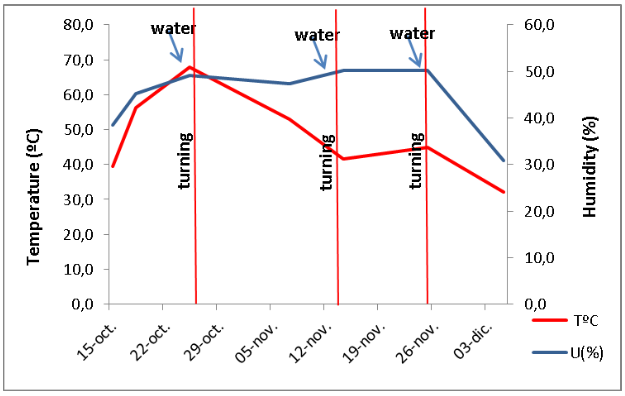

Figure 5. Control parameters of the composting process (T ºC and H%) of by-product marc (CS variety).

The mesophilic phase followed by the thermophilic phase of the composting process is performed sequentially ([16, 18]), the first phase was accompanied by alcoholic fermentation (pH average 4.5-5). Presence of *Basidiomycetes* and earthworms (biohumus) demonstrates the end of the composting process and followed by the maturation process, it also demonstrates the hygenization of the organic matter used [19] (Fig. 6).

Figure 6. *Drosophila melanogaster*, *Basidiomycetes* and earthworms in the composting process.

3.2 Compost phytotoxicity analysis

Both compost marc (RI and CS) used can be considered in the maturation process, the GI values exceed 60% (non-phytotoxic). In Table 1 we can see that the GI value was higher using D_2 for both composts (RI 144% and CS 139.8%, respectively).

The highest growth values of tigella and radicella were also demonstrated in the case of D_2 (CS: tigella 2.4 cm; RI: radicella 3.0 cm) compared to the control (tigella 1.8 cm, radicella 2.0 cm).

Table 1. Germination index (GI%) of *Lepidium sativum* L. seeds from the by-product marc (RI and CS) at two dilutions.

Compost dilutions	Tigella	Radicella	germinated sedes (%)	GI (%)
RI D_1	2.2±0.3	2.7±0.5	9.5	133.7
RI D_2	2.0±0.2	3.0±0.5	9.0	**144.2**
CS D_1	2.1±0.1	2.2±0.1	9.8	116.9
CS D_2	2.4±0.4	2.8±0.5	9.5	**139.8**
Control	1.8±0.1	2.0±0.1	9.5	105.4

D_1: 1:10 and D_2:1:20.

3.3 Microbiological analysis of fresh and composted by-product marc

Microbial activity is fundamental in the composting process for the decomposition of organic matter, so that the nutrients remain available for crops [20]. The microbiological composition of the fresh marc presented only fermentative yeasts (100% in RI and CS) demonstrating the hygiene of the winemaking process (Fig. 7).

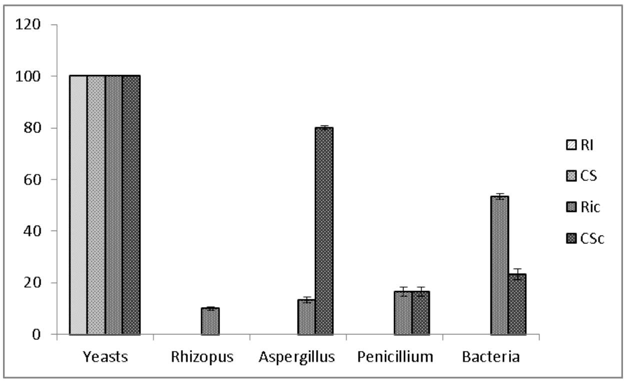

Figure 7. The microbiological composition (average %) of fresh (RI and CS) and composted by-product marc (RIc and CSc).

The by-product marc compost presented, apart from yeasts, microorganisms of the genera *Aspergillus* (13% in RIc and 80% in CSc), *Rhizopus* (10% in RIc and 0% CSc) and *Penicillium* (17% in Ric and 17% CSc), are microorganisms specific to this residue and known to have an antagonistic effect on pathogens ([20, 21]).

3.4 Microbiology of dry and lyophilized by-product marc compost

Table 2 shows the structure of the populations of microorganisms in the by-product marc compost and detected by the dilution method and expressed in $CFUg^{-1}$ (number/g of compost) and as incidence of genus/ species identified.

The results show that the structure of the microorganism populations in lyophilized marc compost

(RIcL and CScL) preserves the microflora an almost double number of $CFUg^{-1}$ compared to the oven-drying process of the same compost (RIcd and CScd) [22].

Table 2. The microbiological composition ($CFUg^{-1}$) of oven-dried (RIcd and CScd) and lyophilized (RIcL and CScL) by-product marc compost.

Microorganisms	$CFUg^{-1}$ marc compost			
	RIcd	RIcL	CScd	CScL
Bacteria	0.1×10^6	1.5×10^6	0.2×10^6	0.5×10^6
Yeasts	0.8×10^6	1.5×10^6	2.3×10^6	5.2×10^6
Aspergillus sp.	0.5×10^6	0	0	0
Penicillium sp.	0.9×10^6	2.8×10^6	0	1.3×10^6
Total CFU/g	2.4×10^6	5.8×10^6	2.5×10^6	7.0×10^6

The identified compost marc microflora was represented by bacteria as well by fungal isolates belonging to genera *Apergillus*, *Penicillium* and yeasts, which actively participate in the composting process. Differences were observed in the case of RI marc, a double total $CFUg^{-1}$ (5.8×10^6) being observed for RIcL compared to RIcd (2.4×10^6). For CS marc samples, the total $CFUg^{-1}$ recorded for CScL (7.0×10^6) was 2.8 times higher than CScd (2.5×10^6).

Grape marc is considered an ideal raw material that produces high-quality compost with a significant organic matter concentration (84.5%), compared to lignocellulosic residues resulting from vine pruning [23]. The presence of antagonistic microorganisms and the high number of $CFUg^{-1}$, demonstrated that this compost can have a suppressive effect on soil pathogens in addition to its quality as a fertilizer.

4 Conclusions

The solid-phase composting of the by-product marc, obtained by vilification of the RI and CS varieties, allowed obtaining an excellent fertilizer, compost mineralization being carried out under good conditions in an open pile system, in the autumn-winter season. The temperature in the thermophilic phase reached average values higher than 60ºC, and the composting took place over a period of three months, the presence of earthworms in the compost determining the formation of biohumus.

Following the analysis of the germination index (GI) of the *Lepidium sativum* L. seeds, using dilutions from the compost obtained from the fermentation of by-product marc (RI and CS), the percentage values of the GI exceed the value of 60% (non-phytotoxic).

The microbiological analysis of the by-product marc before and after composting demonstrated the fact that the fresh marc, resulting from the winemaking process, presents only fermentative yeasts on the culture medium, which demonstrates a total hygiene of the winemaking process. In the case of the composted by-product marc, the culture medium presented other yeasts and specific microflora known to have an antagonistic effect on pathogens (*Rhizopus*, *Aspergillus*, *Penicillium*).

Using the dilution method to determine the CFU/g of oven-dried and lyophilized marc compost, it demonstrates that lyophilization completely preserves the microflora specific to this by-product resulting from the composting process.

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