

Utilization of a Factorial Design To Study the Composting of Hydrolyzed Grape Marc and Vinification Lees

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Hydrolyzed grape marc (HGM) is the solid residue generated after the acid hydrolysis of grape marc to obtain hemicellulosic sugars for biotechnological purposes. In this work, HGM containing cellulose and lignin was composted together with vinification lees to obtain plant substrates on a laboratory scale. The effects of temperature (in the range of 20–50 °C), concentration of vinification lees (5–100 g/100 g of hydrolyzed grape marc), and concentration of CaCO₃ on the final properties of the composted HGM were studied by means of an experimental plan with factorial structure. The interrelationship between dependent and operational variables was established by models including linear, interaction, and quadratic terms. The most influential variable on the C/N ratio and P, K and Mg contents of composted substrates was the vinification lees concentration followed by the temperature, whereas on Na content and electrical conductivity the most influential variable was the temperature followed by the vinification lees concentration. The results of the incubation experiments indicated that optimal conditions for obtaining plant substrates can be achieved by composting 1:1 mixtures of hydrolyzed grape marc and vinification lees, in the presence of 5 g of CaCO₃/100 g of HGM. During composting the pH of the mixtures increased from 5.1–6.7 to 7.1–8.1, salinity and water-soluble carbon were reduced in most cases, and the initial phytotoxicity disappeared in all of the mixtures tested.

KEYWORDS: Grape marc; lignocellulosic fraction; vinification lees; compost; factorial design

INTRODUCTION

In Galicia (northwestern Spain), viticulture is one of the most important agricultural activities, producing a huge amount of residues consisting mainly of grape marc and vinification lees. Grape marc is mainly composed by cellulose, lignin, and hemicelluloses, and it is produced after pressing the crushing grapes, in white wine production technology, or after fermentation and maceration, in red wine production technology. After the must fermentation, a decanting process takes place when the supernatant wine is separated from the lees (mainly yeasts), and it is fed by pumps to empty tanks that are filled 100% for further stabilization. They are led to alcohol production and, after distillation, distilled vinification lees are obtained as a residue (1). These wastes are generally characterized by acidic pH values and high organic content, often >90%, with important concentrations of lignin, cellulose, and hemicelluloses (2, 3).

Nowadays, there is a growing interest in the exploitation of the residues generated by the wine industry. In particular, winery wastes could be an alternative source for obtaining lactic acid and biosurfactants (4). The treatment of grape marc with sulfuric acid allows the benefit of both hemicellulose and cellulose fractions contained in the feedstock. During this biotechnological process

an acid solid phase composed by cellulose and lignin is generated as a residue, which is called hydrolyzed grape marc (HGM). The hydrolysis of grape marc removes hemicellulosic sugars and produces structural changes in the cellulosic fraction that could facilitate the access of microorganisms to the carbon source during the composting of hydrolyzed grape marc (4), thus accelerating a process that is often long when applied to lignocellulosic materials. Some authors suggested that lignocellulosic residues could be saccharified to obtain solutions of monomeric sugars (glucose and celobiose), but their high lignin content would produce a poor susceptibility toward enzymatic hydrolysis (5). For this reason, from an industrial point of view, it would be interesting to look for other alternatives, different from enzymatic hydrolysis, to revalorize this acid cellulosic fraction from grape marc. In this way the composting of HGM could be an interesting alternative because no high investment is needed.

Although wine wastes can be potentially used as soil conditioner or for fertilizer production, the application of nondecomposed wastes or nonstabilized compost to soil may lead to immobilization of plant nutrients and be the cause of phytotoxicity (6). In fact, previous works have noted the phytotoxic effect of grape marc and vinification lees water extracts on the germination of cress (*Lepidium sativum* L.) (2, 7). Composting of organic matter is a simple and efficient manner of transforming agroindustrial wastes into products suitable for use as soil conditioner,

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Table 1. Independent Variables Used in This Study^a

variable	nomenclature	units	levels
temperature	<i>T</i>	°C	20–35–50
vinification lees concentration	VL	g of VL/100 g of HGM	5–55–100
CaCO ₃ concentration	CaCO ₃	g	5–10–15
dimensionless coded variable	nomenclature	definition	variation range
temperature	<i>x</i> ₁	(<i>T</i> – 35)/15	(–1,1)
vinification lees concentration	<i>x</i> ₂	(g of VL/100 g of HGM – 52.5)/47.5	(–1,1)
CaCO ₃ concentration	<i>x</i> ₃	(g of CaCO ₃ /100 g of HGM – 10)/5	(–1,1)

^aVL, vinification lees; HGM, hydrolyzed grape marc.

and extensive research has demonstrated that many biodegradable organic wastes, including winery wastes, can be composted in a convenient and economical way (2, 8–15).

In this work a novel feedstock, hydrolyzed grape marc, was composted at different temperatures in the presence of various concentrations of vinification lees and CaCO₃. An incomplete 3³ factorial design was used to study the effect of the initial composition of the mixtures and the experimental conditions on the final properties of the composts obtained.

MATERIALS AND METHODS

Materials. Grape marc, from white wine production technology, and vinification lees were supplied by the Cooperativa Vitivinícola do Ribeiro (Ourense, Spain). On the basis of previous work (4), grape marc was hydrolyzed by employing 3% H₂SO₄, at 130 °C for 30 min. After the hydrolysis of grape marc, the liquid phase can be employed for further lactic acid and biosurfactant production (4), whereas lignin and cellulose were recovered in the solid phase as a residue called hydrolyzed grape marc (HGM). Then this lignocellulosic phase was composted in the presence of vinification lees and CaCO₃, by applying the factorial design explained below.

Experimental Design and Statistical Analysis. For the composting of hydrolyzed grape marc and vinification lees, an incomplete 3³ factorial design (16) was used to study the influence of temperature, addition of vinification lees, and addition of CaCO₃ on the composts' properties after 2 months of composting.

The experimental data were analyzed by the response surface method using the software Statistica 5.0. The interrelationship between dependent and operational variables was established by a model including linear, interaction, and quadratic terms

$$y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2$$

where *y* is the dependent variable, *b* denotes the regression coefficients (calculated from experimental data by multiple regressions using the least-squares method), and *x* denotes the independent variables.

The independent variables used in this study and their variation limits are shown in Table 1, and they were temperature (*T*, 20–50 °C), vinification lees concentration (VL (5–100 g of vinification lees/100 g of hydrolyzed grape marc), and CaCO₃ concentration (5–15 g of CaCO₃/100 g of hydrolyzed grape marc). The standardized (coded) adimensional variables employed, having variations limits (–1,1), were defined as *x*₁ (coded temperature), *x*₂ (coded vinification lees concentration), and *x*₃ (coded CaCO₃ concentration). The correspondence between coded and uncoded variables was established by linear equations deduced from their respective variation limits. Table 2 shows the set of experimental conditions assayed (expressed in terms of coded variables). These assays allowed the development of empirical models describing the interrelationship between operational and experimental variables by equations including linear, interaction, and quadratic terms. The sequence for the experimental work was randomly established to limit the influence of systematic errors on the interpretation of results. It can be noted that experiments 1–12 allowed the calculation of the regression coefficients, whereas

Table 2. Experimental Conditions of the Mixtures of Hydrolyzed Grape Marc (HGM) and Vinification Lees (VL)

mixture	<i>T</i> (°C)	VL (g/100 g of HGM)	CaCO ₃ (g/100 g of HGM)
1	0	–1	–1
2	0	1	–1
3	0	–1	1
4	0	1	1
5	–1	–1	0
6	–1	1	0
7	1	–1	0
8	1	1	0
9	–1	0	–1
10	–1	0	1
11	1	0	–1
12	1	0	1
13	0	0	0
14	0	0	0
15	0	0	0

experiments 13–15 were replications in the central point of the design to estimate the influence of the experimental error.

The dependent variables studied were those usually considered as relevant for the evaluation of compost quality and plant substrate suitability: pH, electrical conductivity (EC), water-soluble Na, organic matter (OM), total organic carbon (TOC), total nitrogen, water-soluble carbon (WSC), C/N ratio, water-soluble NO₃[–]-N, water-soluble NH₄⁺-N, water-soluble P, water-soluble K, water-soluble Mg, and germination index (GI).

Composting Process. Composting was carried out by employing different concentrations of vinification lees and CaCO₃ at various temperatures following the factorial design explained above. The composting process of the mixtures was carried out at laboratory scale under microaerobiosis conditions for 2 months. Reactors of 12 cm diameter and 7 cm height were filled with mixtures of 100 g of HGM and vinification lees and CaCO₃ at the doses required to fulfill the design conditions, with two replicates per treatment (the replicates were averaged prior to regression analysis). Aeration of the mixtures was achieved by turning the mixtures by hand. The conditions of constant temperature were obtained by maintaining the mixtures in thermostated chambers throughout the process.

Analytical Methods. For all analyses, fresh compost samples were sieved to <20 mm and homogenized. The samples were air-dried and milled prior to organic matter, carbon, and nitrogen analysis. Total organic matter content (OM) was determined by weight loss on ignition at 450 °C for 16 h. Total organic C (TOC) was calculated multiplying the OM concentration by a factor 0.58. Total N (TKN) was measured by Kjeldahl digestion and steam distillation. Aqueous extracts were prepared by agitating 10 g of fresh sample with 100 mL of distilled water during 1 h. Electrical conductivity (EC) and pH were determined in the filtered extracts using a glass electrode (Crison pH-meter Basic 20) and a conductivity meter (HANNA HI 9033), respectively. Water-soluble ammonium and nitrate were analyzed by steam distillation with MgO and Devarda alloy (17). Water-soluble carbon (WSC) was determined by the wet dichromate oxidation method. Water-soluble Mg was measured by flame atomic absorption spectroscopy, and K and Na were determined by flame atomic emission spectroscopy (Varian FS220), whereas P was

measured by colorimetric determination of the phosphomolybdc complex formed after reaction with ammonium molybdate, at 840 nm (Varian Cary 100). To test the presence of phytotoxic components, a germination–elongation test was run using seeds of *L. sativum* L. (cress), and a germination index (GI) calculated as described by Zucconi et al. (18)

$$GI = 100 \times \frac{G}{G_C} \times \frac{L}{L_C}$$

where G and L are the germination and radicle growth in the sample extract, respectively, and G_C and L_C are the germination and radicle growth of the control (distilled water), respectively. In the paper, the ratios $100 \times (G/G_C)$ and $100 \times (L/L_C)$ will be referred to as G (germination) and RG (root growth) for simplicity.

RESULTS AND DISCUSSION

Grape marc is rich in polymeric sugars that can be hydrolyzed to produce liquors containing monomers of xylose and glucose, which can be used to obtain lactic acid and biosurfactants by fermentation with *Lactobacillus pentosus* (4). After the hydrolysis, an acid solid phase remains as a residue named HGM, which is composed by lignin and cellulose. In this work, the possibility of reaching a simultaneous benefit of both liquid and solid phases from the prehydrolysis of grape marc was assessed, on the basis of the hypothesis that HGM can be composted together with vinification lees to obtain suitable plant substrates. Coupling lactic acid production from hemicellulosic sugars with HGM composting could be an interesting process for the benefit of both hemicellulose and lignocellulose fractions contained in grape marc.

Properties of Wine Wastes. The main properties of the residues employed in this work are shown in **Table 3**. Both HGM and

Table 3. Main Properties of Hydrolyzed Grape Marc (HGM) and Vinification Lees (VL)^a

	HGM	VL
pH	1.0	3.5
EC (dS m ⁻¹)	14.8	2.5
OM (%)	97.9	88.1
TOC (%)	56.8	51.1
TKN (%)	1.2	4.1
C/N ratio	46	12.3

^a EC, electrical conductivity; OM, organic matter; TOC, total organic carbon; TKN, total Kjeldahl nitrogen.

vinification lees are characterized by their acidic pH, especially in the case of HGM. The acidity of grape marc is due to the H₂SO₄ employed for the hydrolysis of grape marc, whereas the acidic pH of the vinification lees is due to the metabolism of the lactic and acetic bacteria of wine, which transform carbohydrates into lactic and acetic acids. The pH of the lees is in the lowest range compatible with the microbial activity necessary for composting, which is considered to be approximately between 3 and 11 (19). The pH of the HGM is too acidic for this purpose, and therefore it must be neutralized. In both cases, the acidic pH could represent a problem if composting is carried out at an industrial level, as the transition from the mesophilic to the thermophilic phase could be negatively affected (20, 21). The electrical conductivity of these wastes can be also a problem for compost production due to their high values, and especially in the case of HGM, the value of which was near 15 dS m⁻¹, because EC values over 1.5 dS m⁻¹ are usually regarded as inadequate for the agricultural use of compost (22). To neutralize acidity and decrease HGM salinity, CaCO₃ was included in the composition of the tested mixtures. In this way, the sulfuric acid remaining in the HGM after the hydrolysis was removed by precipitation as calcium sulfate.

On the other hand, both wastes presented high contents of total organic matter and carbon, whereas the highest concentration of total nitrogen was that of the vinification lees, which as a consequence had the lower C to N ratio (**Table 3**). None of them presented values for this ratio in the range recommended for composting, between 25 and 35 (23). However, blending both materials could create adequate conditions for the beginning of composting, at least in this aspect.

Properties of the Mixtures during Composting. **Table 4** shows the pH and EC at the beginning and end of composting for all of the mixtures assayed. It can be seen that the three doses of carbonate tested were sufficient to neutralize pH and decrease EC to a level compatible with plant growth. It can be observed that the pH of the mixtures increased from 5.1–6.6 to 7.1–8.1 during the composting process, whereas EC slightly decreased after composting from 3.5–4.0 to 2.5–3.5 dS m⁻¹. As an exception, the mixtures composted at 50 °C with intermediate concentration of vinification lees (mixtures 8, 11, and 12) increased their EC to values between 4.9 and 5.9 dS m⁻¹. Arvanityannis et al. (24) reported pH values in the range of 6.5–8.5 and EC values in the range of 1.6–4.1 dS m⁻¹ for compost derived from winery wastes, whereas Diaz et al. (25) reported EC values of 5.2 and 8.9 dS m⁻¹ (1:5 w/v extracts) for compost obtained after composting grape

Table 4. pH, Electrical Conductivity, Na in the Aqueous Extract, and Organic Matter of the Mixtures of Hydrolyzed Grape Marc and Vinification Lees (Mean ± Standard Deviation)^a

mixture	pH		EC (dS m ⁻¹)		Na (mg kg ⁻¹)		OM (%)		TOC (%)		TKN (%)		C/N	
	initial	final	initial	final	initial	final	initial	final	initial	final	initial	final	initial	final
1	6.1 ± 0.1	7.1 ± 0.1	3.7 ± 0.1	3.0 ± 0.05	136 ± 112	72 ± 0.1	79 ± 5	72 ± 0.7	46 ± 3	42 ± 0.4	1.2 ± 0.001	1.7 ± 0.02	38	25
2	5.1 ± 0.4	8.0 ± 0.1	3.7 ± 0.1	2.7 ± 0.2	70 ± 2	59 ± 1	81 ± 3	76 ± 3	47 ± 2	44 ± 2	2.9 ± 0.02	3.0 ± 0.01	16	15
3	6.7 ± 0.1	7.6 ± 0.4	3.5 ± 0	3.1 ± 0.01	108 ± 70	64 ± 1	66 ± 7	52 ± 5	38 ± 4	30 ± 3	1.0 ± 0.02	1.3 ± 0.02	38	23
4	5.6 ± 0.04	7.7 ± 0.5	3.4 ± 0.03	3.1 ± 0.02	61 ± 0.2	67 ± 4	69 ± 2	57 ± 2	40 ± 1	33 ± 1	2.3 ± 0.03	2.0 ± 0.03	17	17
5	6.6 ± 0.01	7.8 ± 0.4	3.6 ± 0.02	2.5 ± 0	101 ± 72	53 ± 2	71 ± 2	60 ± 3	41 ± 1	35 ± 2	1.1 ± 0.02	1.3 ± 0.02	37	27
6	5.7 ± 0.1	8.1 ± 0.1	3.6 ± 0.1	2.8 ± 0	72 ± 3	60 ± 1	74 ± 3	67 ± 1	43 ± 2	39 ± 0.6	2.2 ± 0	2.8 ± 0.05	20	14
7	6.6 ± 0.2	7.2 ± 0.1	3.6 ± 0.01	2.9 ± 0.2	100 ± 59	73 ± 2	74 ± 6	60 ± 0.02	43 ± 4	35 ± 0.01	1.2 ± 0.001	1.4 ± 0.02	36	25
8	5.8 ± 0.2	7.6 ± 0.03	3.6 ± 0.1	5.9 ± 0.2	66 ± 3	152 ± 2	72 ± 5	66 ± 0.3	42 ± 3	38 ± 0.2	2.7 ± 0.04	3.2 ± 0.03	16	12
9	5.2 ± 0.3	7.6 ± 0.01	4.0 ± 0.01	3.2 ± 0.1	68 ± 4	61 ± 0.1	79 ± 0.7	74 ± 0.7	46 ± 0.4	43 ± 0.4	1.7 ± 0	2.6 ± 0.01	27	17
10	6.1 ± 0.5	7.9 ± 0.1	3.7 ± 0.05	2.9 ± 0.1	125 ± 60	54 ± 1	67 ± 1	60 ± 4	39 ± 0.7	35 ± 3	1.6 ± 0	1.3 ± 0	24	27
11	5.5 ± 0.1	7.1 ± 0.4	3.8 ± 0.1	4.9 ± 0.1	176 ± 4	137 ± 2	79 ± 2	74 ± 1	46 ± 1	43 ± 0.6	1.7 ± 0.01	2.5 ± 0.03	27	17
12	6.0 ± 0	7.5 ± 0.03	3.6 ± 0.02	4.0 ± 0.05	112 ± 75	90 ± 1	59 ± 3	55 ± 0.4	34 ± 2	32 ± 0.2	1.8 ± 0.02	2.2 ± 0.02	19	15
13	5.7 ± 0.2	7.5 ± 0.04	3.7 ± 0.01	3.5 ± 0.2	62 ± 0	72 ± 3	69 ± 2	62 ± 0.8	40 ± 1	36 ± 0.5	2.2 ± 0.001	2.3 ± 0.03	18	16
14	5.4 ± 0.01	7.5 ± 0.1	3.7 ± 0.03	3.5 ± 0.2	59 ± 3	71 ± 3	67 ± 2	62 ± 0.3	39 ± 1	36 ± 0.2	2.0 ± 0.03	2.1 ± 0.001	20	17
15	5.7 ± 0.05	7.4 ± 0.02	3.6 ± 0.1	3.3 ± 0.1	60 ± 3	68 ± 0.1	72 ± 0.1	64 ± 2	42 ± 0.1	37 ± 1	2.3 ± 0.001	2.4 ± 0.01	18	15

^a EC, electrical conductivity; OM, total organic matter; TOC, total organic carbon; TKN, total Kjeldahl N.

Table 5. Water-Soluble C (WSC), N, and Nutrients in the Mixtures of Hydrolyzed Grape Marc and Vinification Lees (Mean \pm Standard Deviation)^a

mixture	WSC (g kg ⁻¹)		NH ₄ ⁺ -N (mg kg ⁻¹)		NO ₃ ⁻ -N (mg kg ⁻¹)		P (mg kg ⁻¹)		K (mg kg ⁻¹)		Mg (mg kg ⁻¹)	
	initial	final	initial	final	initial	final	initial	final	initial	final	initial	final
1	14.1 \pm 0.3	3.9 \pm 0.8	139 \pm 2	14 \pm 3	2 \pm 2	nd	225 \pm 3	96 \pm 0.2	2368 \pm 234	3906 \pm 164	120 \pm 0.1	123 \pm 1
2	11.3 \pm 0.3	11.0 \pm 2	67 \pm 0	478 \pm 0	nd	nd	190 \pm 6	105 \pm 11	5472 \pm 500	4929 \pm 1	80 \pm 3	13 \pm 0.3
3	12.6 \pm 0.4	3.5 \pm 0.8	126 \pm 0.1	39 \pm 6	nd	nd	197 \pm 4	105 \pm 1	2343 \pm 25	3372 \pm 80	108 \pm 5	92 \pm 2
4	10.2 \pm 1	8.0 \pm 0.7	68 \pm 1	401 \pm 24	nd	nd	173 \pm 7	34 \pm 1	4610 \pm 248	5766 \pm 122	77 \pm 2	35 \pm 1
5	13.2 \pm 0.2	7.2 \pm 2	131 \pm 1	9 \pm 3	nd	nd	207 \pm 3	106 \pm 1	2080 \pm 401	2810 \pm 35	107 \pm 4	79 \pm 1
6	10.9 \pm 0.5	8.9 \pm 1	66 \pm 4	176 \pm 2	2 \pm 3	nd	181 \pm 5	60 \pm 0.2	5493 \pm 507	4816 \pm 205	76 \pm 5	49 \pm 0
7	13.5 \pm 0.2	6.5 \pm 2	154 \pm 19	93 \pm 2	nd	nd	213 \pm 5	105 \pm 2	1977 \pm 140	4150 \pm 143	112 \pm 0.4	92 \pm 0.4
8	11.0 \pm 0.5	6.2 \pm 0.6	74 \pm 8	222 \pm 1	nd	nd	180 \pm 5	36 \pm 2	4287 \pm 663	11747 \pm 299	78 \pm 6	81 \pm 3
9	12.6 \pm 0.03	7.0 \pm 0.1	90 \pm 1	77 \pm 5	6 \pm 2	nd	210 \pm 3	70 \pm 3	4366 \pm 354	4408 \pm 157	90 \pm 4	79 \pm 4
10	11.2 \pm 0.4	7.2 \pm 3	75 \pm 1	47 \pm 2	9 \pm 1	nd	176 \pm 5	82 \pm 1	4512 \pm 260	3851 \pm 31	83 \pm 0.7	67 \pm 2
11	12.0 \pm 0.3	6.9 \pm 0.7	72 \pm 3	222 \pm 2	4 \pm 1	nd	189 \pm 1	61 \pm 0.4	4342 \pm 1068	8612 \pm 105	81 \pm 2	108 \pm 5
12	10.9 \pm 0.2	5.5 \pm 0.8	90 \pm 0.5	150 \pm 3	5.9 \pm 0.3	nd	182 \pm 3	46 \pm 1	3586 \pm 825	6728 \pm 25	86 \pm 0.2	85 \pm 0.1
13	11.7 \pm 0.1	6.6 \pm 1	83 \pm 1	100 \pm 1	6 \pm 2	nd	196 \pm 6	36 \pm 2	3520 \pm 631	4857 \pm 72	85 \pm 0.9	66 \pm 1
14	11.7 \pm 0.4	7.3 \pm 2	84 \pm 3	188 \pm 3	13 \pm 2	nd	185 \pm 0.2	47 \pm 1	2821 \pm 332	4741 \pm 26	89 \pm 2	70 \pm 3
15	11.6 \pm 0.3	4.5 \pm 2	77 \pm 6	150 \pm 1	3 \pm 5	nd	181 \pm 9	41 \pm 0.5	4063 \pm 1399	4832 \pm 34	89 \pm 2	66 \pm 0.2

^a nd, not detected.

marc with 10 and 20% of beet vinasses, respectively. It has to be noted that no works about the composting of HGM exist to date in the literature.

Similar to that observed for EC, the initial soluble Na concentrations were similar for all of the mixtures (59–136 mg kg⁻¹) and decreased to concentrations between 53 and 73 mg kg⁻¹ at the end of the experiment (Table 4), with the exception of samples 4, 8, and 13–15, which increased their concentrations to a range between 67 and 152 mg kg⁻¹. Although, in general, salinity tends to increase during composting as a consequence of the loss of weight of the pile, which produces a concentration of salts in the pile, the contrary was observed here in most samples. Given that no leaching was allowed during the experiments, the decrease in salinity must be attributed to changes in the Na fractions from soluble to nonsoluble forms, as well as to precipitation of salts after the treatment with carbonate.

As expected, the initial C concentrations of the mixtures decreased at the end of the experiment, whereas N concentrations increased (Table 4). OM decreased from initial values of 59–81 to 52–76% at the end of the experiment, corresponding to initial TOC values between 34 and 47%, and final C concentrations were between 30 and 44%. The C/N ratio also decreased from initial values in the range 17–38, to final values between 12 and 27. The evolution of these parameters can seem small in comparison with the composting of other wastes, but are in general agreement with data published by other authors on the composting of winery wastes (2, 8, 26).

Water-soluble carbon (WSC) is a direct measure of the water-soluble fraction of organic matter, which is the most accessible to microorganisms during composting, and it has been related to compost maturity by several authors (27, 28). In our experiments, WSC decreased in all mixtures from initial concentrations between 10.2 and 14.1 g kg⁻¹ to final values between 3.5 and 11.0 g kg⁻¹ (Table 5). As for the forms of soluble nitrogen, ammonium prevailed over nitrate in all of the mixtures, both at the beginning and the end of the experiment (Table 5), which could be an indicator of a slight oxygen deficiency during the process. During composting the soluble N forms are influenced by the equilibrium between mineralization of organic N, its loss as ammonia, and the immobilization in the microorganisms. This complex scheme for N availability can be one of the reasons why no consistent trend was observed for this element throughout the experiment.

The initial concentrations of water-soluble nutrients followed the relative sequence K \gg P > Mg (Table 5). At the initial

Table 6. Results of the Germination–Elongation Test with Cress of the Mixtures of Hydrolyzed Grape Marc and Vinification Lees (Mean \pm Standard Deviation)^a

mixture	initial			final		
	G	RG	GI	G	RG	GI
1	97 \pm 20	65 \pm 14	63 \pm 26	95 \pm 17	176 \pm 21	167 \pm 12
2	76 \pm 15	31 \pm 18	23 \pm 15	95 \pm 7	125 \pm 48	119 \pm 40
3	82 \pm 31	36 \pm 5	29 \pm 8	99 \pm 17	124 \pm 12	122 \pm 10
4	88 \pm 31	32 \pm 5	29 \pm 8	91 \pm 11	121 \pm 22	110 \pm 28
5	106 \pm 10	53 \pm 13	56 \pm 14	99 \pm 13	174 \pm 40	173 \pm 62
6	85 \pm 20	50 \pm 49	43 \pm 41	103 \pm 11	161 \pm 35	165 \pm 36
7	88 \pm 20	46 \pm 20	40 \pm 25	99 \pm 7	133 \pm 21	132 \pm 16
8	56 \pm 29	16 \pm 15	9 \pm 7	91 \pm 0	182 \pm 8	166 \pm 7
9	32 \pm 24	56 \pm 36	18 \pm 16	103 \pm 11	165 \pm 1	169 \pm 20
10	71 \pm 17	27 \pm 7	19 \pm 8	107 \pm 7	130 \pm 8	138 \pm 15
11	58 \pm 13	20 \pm 10	11 \pm 8	107 \pm 7	127 \pm 25	135 \pm 34
12	60 \pm 24	41 \pm 12	24 \pm 16	99 \pm 13	164 \pm 29	163 \pm 52
13	70 \pm 24	36 \pm 18	25 \pm 13	99 \pm 17	142 \pm 11	141 \pm 21
14	75 \pm 24	45 \pm 9	34 \pm 11	103 \pm 11	100 \pm 11	103 \pm 22
15	78 \pm 22	49 \pm 15	38 \pm 19	99 \pm 7	112 \pm 12	111 \pm 8

^a G, germination percentage; RG, root growth; GI, germination index.

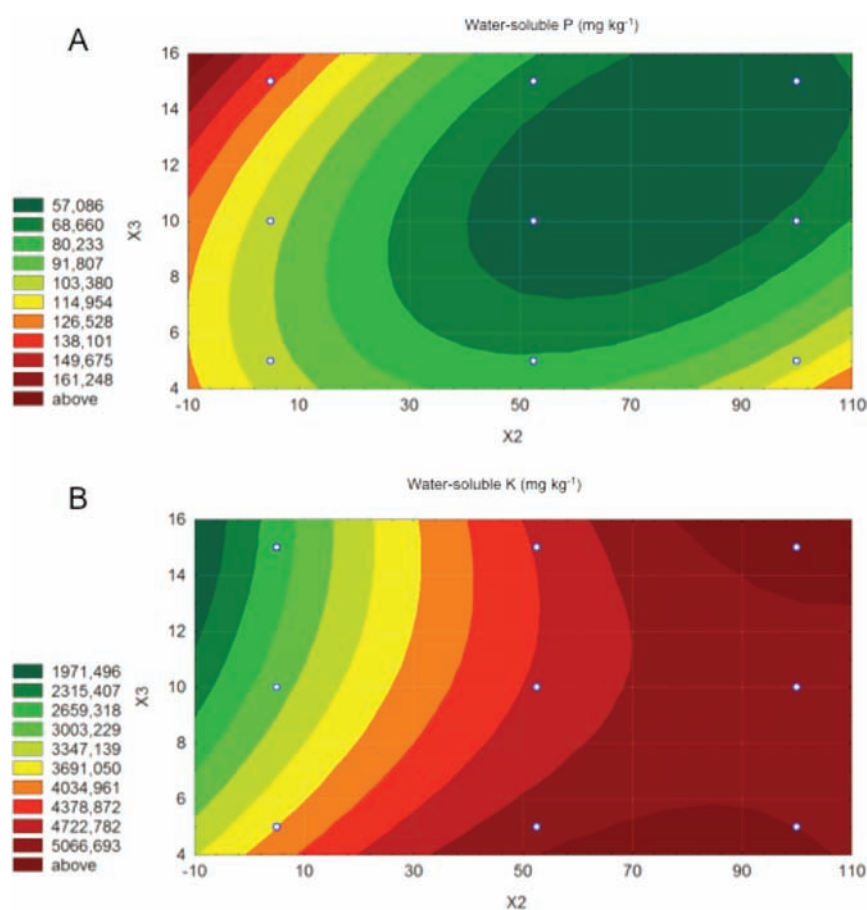
moment, K content ranged from 1977 to 5493 mg kg⁻¹, P from 180 to 225 mg kg⁻¹, and Mg from 76 to 120 mg kg⁻¹. The final concentrations of K (3372–11747 mg kg⁻¹) and Mg (13–123 mg kg⁻¹) showed no definite trend with respect to their initial ones, but the concentrations of P decreased after the experiment to values between 34 and 105 mg kg⁻¹, probably due to coprecipitation with carbonates. The K and Mg concentrations were higher than those found for other grape marc composts (29), due to the contribution of the lees, which have a high nutrient content (7). On the contrary, the P concentrations were lower, which we attribute to the reduction of availability provoked by the addition of carbonate.

Strong phytotoxicity has been highlighted as a problem for the agricultural use of winery wastes (7). According to Zucconi et al. (18), values for the GI of < 50% mean high phytotoxicity, values between 50 and 80% mean moderate phytotoxicity, and values > 80% indicate that the material presents no phytotoxicity. The values for GI can sometimes reach values > 100, thus indicating the presence of nutrients or germination promoters. In fact, all of the mixtures showed a strong initial phytotoxicity, with very low GI values in the cress test (Table 6). Initially, the GI values ranged from 9 to 63%, with two mixtures (1 and 5) classified as moderately phytotoxic and the rest as strongly

Table 7. Regression Coefficients, Significance Level (p), and R^2 and F Statistics of the Models To Predict Soluble P (y_1), Soluble K (y_2), Soluble Mg (y_3), C/N Ratio (y_4), Soluble Na (y_5), and Electrical Conductivity (y_6)

coefficient	y_1	p_{y1}	y_2	p_{y2}	y_3	p_{y3}	y_4	p_{y4}	y_5	p_{y5}	y_6	p_{y6}
b_0	41.3	0.000	4810	0.000	66.3	0.000	15.4	0.000	70.3	0.000	3.4	0.000
b_1	-8.8	0.001	294	0.005	11.5	0.000	-2.0	0.003	28.0	0.001	0.79	0.003
b_{11}	7.6	0.002	-386	0.007	14.0	0.000	1.5	0.010	17.1	0.004	0.43	0.019
b_2	-22.1	0.000	628	0.001	-26.0	0.000	-5.3	0.000	9.5	0.006	0.38	0.012
b_{22}	27.8	0.000	-543	0.003	-5.0	0.004	2.5	0.004	-2.9	0.115	-0.34	0.030
b_3	-8.1	0.001	358	0.004	-5.5	0.001	1.0	0.011	-6.8	0.012	-0.09	0.165
b_{33}	15.8	0.000	226	0.019	4.5	0.005	1.9	0.007	-1.9	0.219	-0.12	0.192
b_{12}	-5.8	0.003	-602	0.003	4.8	0.004	-0.02	0.883	18.0	0.003	0.68	0.007
b_{13}	-6.8	0.002	918	0.001	-2.8	0.011	-3.3	0.002	-10.0	0.011	-0.15	0.122
b_{23}	-20.0	0.000	343	0.008	13.3	0.000	0.9	0.030	4.0	0.062	0.07	0.324

variable	R^2	F_{exptl}	Pr > F (%)
y_1	0.93	7.0	99.4
y_2	0.89	4.6	97.6
y_3	0.80	2.3	86.4
y_4	0.93	7.3	99.5
y_5	0.87	3.7	95.7
y_6	0.78	2.0	81.9

**Figure 1.** (A) Dependence of soluble P concentration of the composts (variable y_1) on vinification lees (x_2) and CaCO_3 (x_3) concentrations predicted for a fixed temperature of 25 °C. (B) Dependence of soluble K concentration of the composts (variable y_2) on vinification lees (x_2) and CaCO_3 (x_3) concentrations predicted for a fixed temperature of 25 °C.

phytotoxic. However, once the composting process finished, the GI values increased to >100% in all cases, so phytotoxicity disappeared in all mixtures. As usual in this test (30, 31), the germination percentage (G) was less sensitive than root growth (RG) to phytotoxicity.

Statistical Treatment of the Results. Because a systematic study of the effects caused by the operational variables (temperature,

VL concentration, and CaCO_3) on the composting of the mixtures of HGM and VL would require a great amount of experimental work, an incomplete factorial design of experiments was carried out. Several research groups have used phenomenological models based on experimental designs to study the chemical processing and/or bioconversion of lignocellulosic materials (4, 32–35) or to study the optimal conditions for

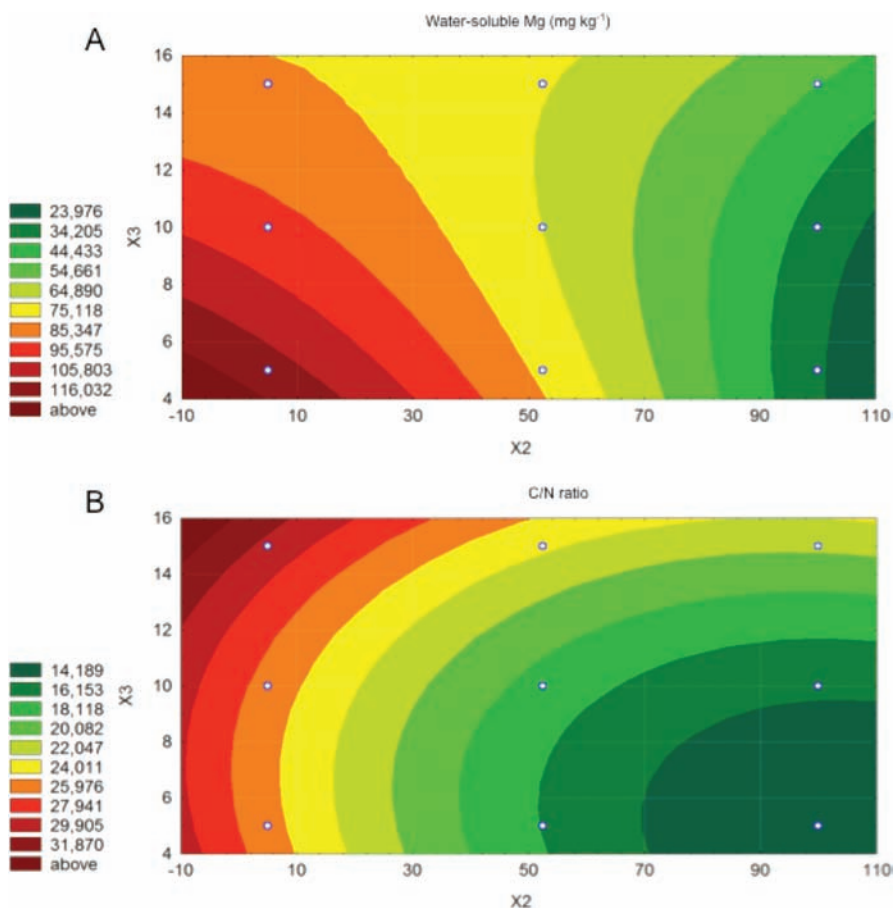


Figure 2. (A) Dependence of soluble Mg concentration of the composts (variable y_3) on vinification lees (x_2) and CaCO_3 (x_3) concentrations predicted for a fixed temperature of 25 °C. (B) Dependence of the C/N ratio of the composts (variable y_4) on vinification lees (x_2) and CaCO_3 (x_3) concentrations predicted for a fixed temperature of 25 °C.

extracting phytopigments from river bed sediments (36). In this work, an incomplete factorial design was employed to study the optimal conditions for the composting of the mixtures, in which three dependent variables were assayed at three levels. The experimental data allowed the development of empirical models describing the interrelationship between operational (independent) and experimental (dependent) variables by equations including, linear, interaction, and quadratic terms. The dependent variables modeled in this study with adequate correlation and statistical significance were water-soluble P, represented by the variable y_1 ; water-soluble K, variable y_2 ; water-soluble Mg, variable y_3 ; the C/N ratio, variable y_4 ; water-soluble Na, variable y_5 ; and EC, variable y_6 . Although the model for GI also showed good statistical parameters for correlation and significance, the corresponding data are not shown. Given that none of the mixtures was phytotoxic at the end of the experiment, the corresponding models would have no relevance. **Table 7** lists the regression coefficients and their statistical significance for the calculated models, as well as statistical parameters (R^2 and F_{exptl}) measuring their overall fit, testing the hypothesis that the differences found between the experimental values and the values calculated by the models were not significant. For the calculation of the F_{exptl} values, 9 degrees of freedom were considered. It can be noted that in all cases the models showed good statistical parameters for correlation and significance, allowing a close reproduction of the experimental data. In the range tested, in general, the addition of CaCO_3 caused only minor effects on the variables studied, as can be seen by the

absolute value of the corresponding regression coefficients. The most influential variable on the P, K, and Mg contents was the concentration of VL (as shown by the high values for the coefficients b_2 and b_{22}), followed by temperature and CaCO_3 . Increasing proportions of VL and CaCO_3 would produce the effect of increasing K concentrations and decreasing P and Mg. An increment in the temperature would reduce the concentrations of P and increase those of K and Mg. The most influential variable on the C/N ratio was the VL concentration, as can be seen by the absolute value of the corresponding regressions coefficients (b_2 and b_{22}), followed by temperature. Increments in both parameters would lead to a reduction of the C/N ratio. The most influential variable on water-soluble Na and EC (y_5 and y_6 , respectively) was temperature (high values for the coefficients b_1 and b_{11}). Increasing the VL proportion or temperature would increase the Na concentration and EC, whereas CaCO_3 would have the inverse effect.

Figures 1–3 show the model predictions for the final properties of the composts depending on the initial composition of the mixtures, for a composting temperature of 25 °C. **Figures 1** and **2A** show the model prediction for the concentrations of water-soluble P (y_1), K (y_2), and Mg (y_3). As the added vinification lees increased, higher values of K contents would be observed, whereas a decrease in the P and Mg concentrations would happen. It can also be observed (**Figure 2B**) that the C/N ratio would decrease with increasing proportions of vinification lees. **Figure 3A** shows that Na concentrations would decrease either with higher proportions of vinification lees or with higher CaCO_3 concentrations. The variation

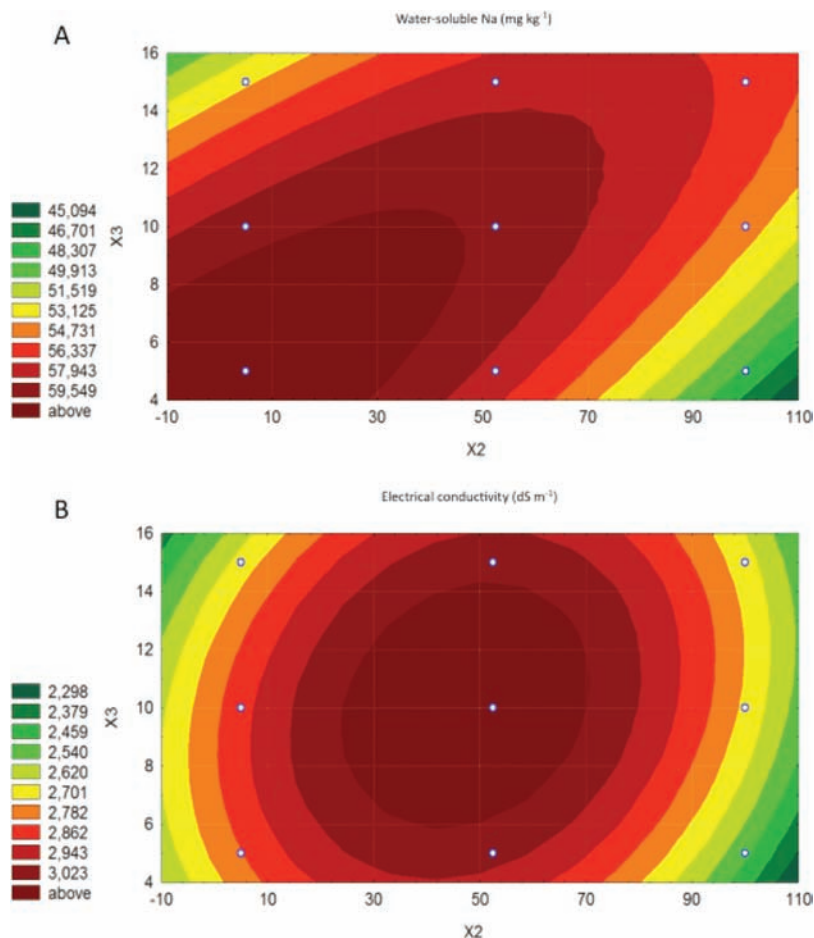


Figure 3. (A) Dependence of soluble Na concentration of the composts (variable y_5) on vinfication lees (x_2) and CaCO_3 (x_3) concentrations predicted for a fixed temperature of 25 °C. (B) Dependence of electrical conductivity of the composts (variable y_6) on vinfication lees (x_2) and CaCO_3 (x_3) concentrations predicted for a fixed temperature of 25 °C.

of EC (Figure 3B) presented a high complexity, once the temperature was fixed, due to the fact that temperature was the variable with the highest influence on this property.

Overall, blending hydrolyzed grape marc and vinfication lees could constitute a promising way to obtain suitable composts for agronomic applications. In the experiments reported here, the main changes taking place after the first 2 months of composting of the mixtures of these winery wastes can be summarized as follows: (1) an increment of the initial pH of the wastes; (2) a reduction of their initial salinity; (3) a reduction of the concentrations of WSC; (4) and the disappearance of the high initial phytotoxicity. The proportion of vinfication lees in the mixtures was the factor with the main influence in the final nutritive properties of the composts. However, relationships between the initial conditions of the experiments and other properties of the final composts (such as pH, total C and N, or water-soluble C) could not be established. To deduce the experimental conditions leading to composts with the best possible properties, the predictions of the models for soluble P concentrations, C/N ratio, and EC must be taken into consideration, and it can be concluded that a 1:1 mixture of hydrolyzed grape marc and vinfication lees, amended with no more than 5 g of CaCO_3 per 100 g of hydrolyzed grape marc, would produce the best results in terms of nutrient availability and salinity.

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