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Tartaric Acid Recovery from Distilled Lees and Use of the Residual Solid as an Economic Nutrient for *Lactobacillus*

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The recovery of tartaric acid (TA) from distilled vinification lees coming from the white and red winemaking technology was optimized using response surface methodology and Statistica 5.0 software. The sequential treatment of dissolving TA and further calcium tartrate (CaT) precipitation could be used to recover up to 92.4% of the initial TA when distilled white lees were used. The residual lees were employed as economic nutrients for lactic acid production by *Lactobacillus pentosus* CECT-4023 using hemicellulosic vine shoot hydrolysates as carbon source. Distilled lees after TA extraction used as nutrients provided values of lactic acid (18.4−18.9 g/L), global volumetric productivities (0.82−0.84 g/L·h), and product yields (0.69−0.70 g/g) similar to those achieved when using the general medium for *Lactobacilli* (18.6 g/L, 1.11 g/L·h, and 0.62 g/g, respectively) or lees without TA extraction (16.4−17.2 g/L, 0.96−1.21 g/L·h, and 0.61−0.66 g/g, respectively). This technology not only avoids pollutant disposal but also represents a commercial source of tartaric acid and economic nutrients for biotechnological processes.

KEYWORDS: Vinification lees; tartaric acid; lactic acid; *Lactobacillus pentosus*

INTRODUCTION

Vinification involves all of the steps carried out during wine elaboration from grapes. In spite of being a seasonal activity, it is one of the most important agricultural activities in Spain, representing 10% of the total agricultural production. The large volumes of wastes produced ($\sim 18 \times 10^6$ m^3/year, which is 6 times higher than the wine wastewater produced in France or Italy, mainly due to the low cost of the disposal) are characterized by their high content of organic biodegradable compounds and suspended solids including vegetal remains proceeding from the destemmed, mires obtained during the clarification, and bagasse of press and lees obtained from different decanting steps (*1*). Consequently, turning vinification wastes into valuable products is becoming an essential part of good winemaking practices, further reducing concerns of waste disposal and cutting costs for partly imported wine additives, for example, tartaric acid (*2*). After the must fermentation, a decanting process takes place when the supernatant wine is separated from the lees (mainly dead yeasts and grape pulp, skin, and seeds). These lees, usually 5% (v/v), are led to alcohol production, and after distillation, distilled vinification lees are obtained as a residue (*3*).

In the beginning, it was thought that the lees might be utilized as a supplement in animal nutrition, but the yeasts of distilled lees recovered from centrifugation after column distillation have an excessively poor nutritional value that does not make them suitable for this purpose (*4*), probably due to the high amount of polyphenols joined to the proteins, which make them unassimilable, or the presence of toxic elements from residues of treatments, which are accumulated in the lipids of the yeasts. In previous works (*5*−*8*) we have proposed the utilization of lees as a nutritional medium for several *Lactobacillus* species as an inexpensive source of essential microbial nutrients achieving interesting results. These lees were used directly without tartaric acid recovery. Among lactic bacteria, *Lactobacillus* is the most interesting genus. It is generally cultivated at laboratory scale in a complex medium proposed by Mercier et al. (*9*). The principal disadvantage of this medium is the amount of nutrients necessary to reach high lactic acid yields, as well as the high cost of some of these nutrients (including yeast extract and peptone), which can represent 30% of the final value (*10*). Therefore, sometimes the difficulty in developing industrial process prevents the use of applicable research for commercial proposes.

Besides the nutritive value of these distilled lees as nutrient for *Lactobacillus*, one of the most important byproducts in these wastes is tartaric acid (TA). The TA special characteristic is its relative microbial stability. Spoilage bacteria and yeasts can hardly metabolize and degrade tartaric acid. Therefore, products such as candy, bakery goods, for example, cookies, and beverages including sodas become more stable with less need for chemical or thermal preservation. As a purely natural acid, tartaric acid could be a popular alternative to the widely used

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citric or phosphoric acid in the food and beverage industries and also finds many applications in the pharmaceutical industries (*11*). In addition, it is used for a variety of other purposes, from textile coloring to galvanizing and mirror production (*2*). TA is scarcely soluble (0.53 g/L at 20 °C and 1.16 g/L at 60 °C), if compared with potassium hydrogentartrate (5.7 g/L at 20 °C and 24 g/L at 60 °C) (*12*). Although recently the recovery of TA from lees and creams of tartar was proposed using techniques of electrodialysis (*13*), solvent extraction (*14*), and adsorption with active carbon (*15*), the traditional process involves its dissolution and further addition of calcium salts to precipitate the calcium tartrate (CaT) (*16*, *17*). Special attention must be paid during the tartaric acid purification process if the final product is to be used as an acidulant compound in soft drinks or as an additive in medicines, cosmetics, etc. (*13*).

In all of these effluents it is possible to recover an important amount of tartaric acid salts. Nevertheless, it is necessary to develop new technologies, taking into account that the solutions to remove tartrates are very pollutant, with COD of 50000 and 200000 mg/L, and a tartrate content equivalent in tartaric acid of 100−400 g/L (*12*). This work evaluates the recovery of TA from distilled vinification lees from the white and red wine-making technologies and further use of the residual lees as a unique nutrient for *Lactobacillus pentosus* CECT-4023 using trimming vine shoot hemicellulosic sugar hydrolysates as carbon source.

MATERIALS AND METHODS

Lees Sampling and Storage. Lees previously distilled (to recover ethanol and aromatic flavors used for the production of aromatic spirits liquors) were kindly supplied by Cooperativa Vitivinícola do Ribeiro (Ourense, Spain) and stored at 4 °C. Distilled lees from white and red wines are noted in the text as "distilled white lees" and "distilled red lees", respectively.

Trimming Wastes and Acid Hydrolysis. Trimming wastes locally collected were dried, milled to a particle size of <1 mm, homogenized in a single lot to avoid compositional differences, and stored until use. Aliquots from the homogenized lot were analyzed following the process described by Vázquez et al. (*18*). The composition of trimming wastes was as follows: extracts, 7.1%; cellulose, 34.1%; hemicelluloses, 19.0% (xylan, 12.8%; araban, 0.9%; and acetyl groups, 5.3%); lignin, 27.1%; and other, 12.7%. Hydrolysates of trimming wastes were obtained in an autoclave at 130 °C with 3% H_2SO_4 solutions during 15 min, using a liquid/solid ratio of 8 g/g (*7*). The hydrolysates contained 17.4 g of xylose/L, 11.1 g of glucose/L, 4.3 g of arabinose/L, 4.0 g of acetic acid/L, 0.7 g of furfural/L, and <0.1 g of hydroxymethylfurfural/L.

Experimental Design and Statistical Analysis. The TA was recovered from 100 mL of lees using a sequential process as is indicated in **Figure 1**. The first step involves its dissolution with HCl and the second one its precipitation as calcium tartrate after the addition of calcium salts. Both steps were studied using incomplete 3^3 factorial designs (*19*). The experimental data were analyzed by response surface methodology using Statistica 5.0 software. **Tables 1** and **2** provide information about the dimensionless, coded independent variables used in both designs. 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 regression using the least-squares method), and x denotes the independent variables.

The independent variables considered during the first design, for distilled lees from the white or red winemaking technology, and their variation ranges were temperature (T), 20−80 °C; HCl volume (37%), (vol HCl), 1−10 mL; and reaction time (t), 5−30 min. The standardized (coded) adimensional variables employed, having variation limits (−1, 1), were defined as x_1 (coded temperature), x_2 (coded HCl volume), and x_3 (coded time). The correspondence between coded and uncoded variables was established by linear equations deduced from their respective variation limits (see **Table 1**). The dependent variables considered in this study were y_1 (tartaric acid dissolved in white distilled lees) and y_2 (tartaric acid dissolved in red distilled lees).

The second factorial design was carried out from the optimum value of the previous design. The new independent variables considered and the variation ranges were concentration of $CaCl_2$, ($CaCl_2$ concn), 0−100 g/L; pH (pH), 0.5−9.5; and reaction time (t), 10−240 min. The standardized (coded) adimensional variables employed, having variations limits (−1, 1), were defined as x_4 (coded concentration of $CaCl_2$), x_5 (coded pH), and x_6 (coded reaction time). The correspondence between coded and uncoded variables was established by linear

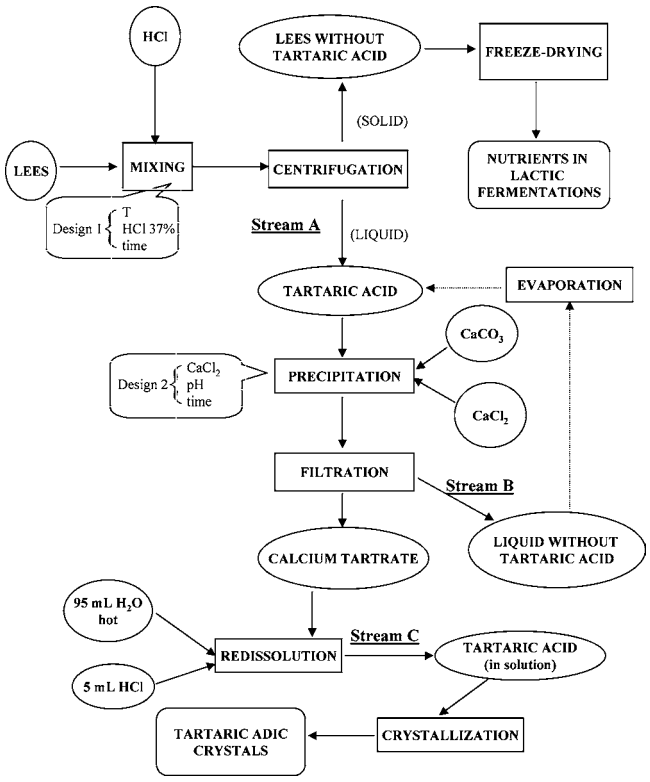

Figure 1. Flow diagram of the technology assayed.

Table 1. Dimensionless, Coded Independent Variables Used during the Tartaric Acid Dissolving

(**a**) Independent Variables

variable	nomenclature	units	variation range
temperature	T	°C	20–80
HCl volume (37%)	HCl vol	mL	1–10
reaction time	t	minutes	5–30

(**b**) Dimensionless, Coded Independent Variables

variable	nomenclature	definition	variation range
dimensionless temperature	x_1	(T - 50)/30	(–1, 1)
dimensionless HCl volume (37%)	x_2	(vol of HCl – 5.5)/4.5	(–1, 1)
dimensionless reaction time	x_3	(t – 17.5)/12.5	(–1, 1)

(**c**) Dependent Variables

variable	nomenclature
tartaric acid dissolved from white distilled lees, g/L	y_1
tartaric acid dissolved from red distilled lees, g/L	y_2

Table 2. Dimensionless, Coded Independent Variables Used during the Calcium Tartrate Precipitation

(**a**) Independent Variables

variable	nomenclature	units	variation range
$CaCl_2$ concentration	$CaCl_2$ concn	g/L	0–100
pH	pH		0.5–9.5
reaction time	*t*	minutes	10–240

(**b**) Dimensionless, Coded Independent Variables

variable	nomenclature	definition	variation range
dimensionless temperature	x_4	($CaCl_2$ concn − 50)/50	(−1, 1)
dimensionless HCl volume (37%)	x_5	(pH − 5)/4.5	(−1, 1)
dimensionless reaction time	x_6	(*t* − 125)/115	(−1, 1)

(**c**) Dependent Variables

variable	nomenclature
tartaric acid recovery, %	y_3

equations deduced from their respective variation limits (see **Table 2**). The dependent variable considered in this study was y_3 (percentage of tartaric acid recovery from stream A). To perform all of these assays, CaT was dissolved in hot water (100 mL) containing 5 mL of hydrochloric acid (37%) and analyzed by HPLC.

Microorganism. *L. pentosus* CECT-4023T (ATCC 8041), obtained from the Spanish Collection of Type Cultures (Valencia, Spain), was selected for its ability to ferment both pentoses and hexoses (*6*). The strain was grown on plates using the complete medium proposed by Mercier et al. (*9*), which contains 20 g of glucose/L, 5 g of yeast extract/L, 10 g of peptone/L, 5 g of sodium acetate/L, 2 g of sodium citrate/L, 2 g of K_2HPO_4/L, 0.58 g of $MgSO_4 \cdot 7H_2O$/L, 0.12 g of $MnSO_4 \cdot H_2O$/L, 0.05 g of $FeSO_4 \cdot 7H_2O$/L, and 20 g of agar/L at 31 °C for 12 h. Inocula were prepared by solubilization of cells from plates with 5 mL of sterile water. Biomass in inocula was measured by optical density at 600 nm and adjusted by dilution with water to reach a final concentration in the inocula of 4.0 g/L.

Lactic Acid Fermentation. Hydrolysates were neutralized with $CaCO_3$ to a final pH of 6.5, and the $CaSO_4$ precipitated was separated from the supernatant by filtration. The clarified liquors were supplemented with the complete medium proposed by Mercier et al. (*9*) as a positive control or 20 g of lees/L (with or without TA extraction), sterilized and used directly as fermentation medium. The concentration of 20 g of lees/L was selected according to previous works (*6*). Experiments were carried out in 250 mL Erlenmeyer flasks with a final volume of 100 mL. Thirty grams of calcium carbonate/L was added at the beginning of the fermentation to neutralize the lactic acid produced. Fermentations were carried out in orbital shakers at 150 rpm and 31.5 °C. Samples (2 mL) were taken at given fermentation times and centrifuged at 6000 rpm for 3 min. The supernatants were stored for analyses. Experimental data were carried out in triplicate, and means are reported. The volumetric productivities for lactic acid and acetic acid, $Q_{P,LA}$ and $Q_{P,AcH}$, respectively, were calculated for the fermentation times corresponding to the transition from high to low slope of the sigmoidal lactic acid profiles.

Analytical Methods. Organic compounds in the liquid fraction of lees (glucose, ethanol, malic acid, lactic acid, acetic acid, tartaric acid, and glycerol) as well as glucose consumed and lactic acid produced during fermentations were measured by high-performance liquid chromatography (Agilent, model 1100, Palo Alto, CA), with RI detection using a Transgenomic ION-300 column (Transgenomic Inc., San Jose, CA) eluted with 0.02 M H_2SO_4 at a flow rate of 0.4 mL/min.

The dry content in lees was oven-dried to constant weight at 102 °C. Ashes in lees were oven-dried to constant weight at 550 °C.

Nitrogen and carbon percentages in lees were analyzed using a Thermo Finnigan Flash Elemental Analyzer 1112 series, San Jose, CA.

Cu, Mg, Fe, Mn, Ca, Al, and Zn were analyzed in ashes using an Atomic Absorption Spectrometer 220 Fast Sequential, Varian, Palo Alto, CA. Previously, 0.15 g of ashes was digested with 5 mL of HNO_3 65%, 1 mL of H_2O_2 30%, and 0.5 mL of HF 40% in a Microwave Labstation MLS 1200 Mega, Milestone, Bergamo (Italy).

Phenolics concentration was determined by spectrophotometric measurements at 279 nm, because the absorbance at this wavelength is indicative of the presence of soluble lignin (*6*).

Table 3. Percentage of Solids (Grams per 100 g of Wet Lees) and Ashes in Lees and Carbon and Nitrogen Contents (Grams per 100 g of Dried Lees)[a]

lees	solids	ashes	N	C
white lees after distillation	17.2	23.4	2.0	31.1
red lees after distillation	3.3	16.9	2.1	37.4

[a] Data indicate the mean values of four replications. The standard deviations were below 2.8% of the mean.

Table 4. Concentration of Organic Compounds in Wet Lees (Grams per Liter)[a]

	glucose	ethanol	malic acid	lactic acid	acetic acid	tartaric acid	glycerol
white lees after distillation	0.0	8.6	0.0	5.9	2.3	0.0	0.0
red lees after distillation	0.0	2.3	0.0	2.4	11.4	0.0	0.0

[a] Data indicate the mean values of four replications. The standard deviations were below 2.2% of the mean.

RESULTS AND DISCUSSION

Lees Characterization. Solid, ash, nitrogen, and carbon contents are noted in **Table 3**. Dry content is mainly constituted by dead yeasts and solids in suspension (other microorganisms, colloids, organic matter, etc.). Nitrogen and carbon were analyzed from the dry fraction of lees, whereas ashes were determined by incinerating those solids. It can be observed that both dry content and ash percentage are higher when using lees from the white winemaking technology.

Carbon and nitrogen contents are significantly lower than those reported by Rivas et al. (*20*) for spent yeasts from xylitol production (42.2−46.2 and 5.7−6.3%, respectively) and also used as cheap nutrients for lactic acid production. This fact can be explained because in lees, not only spent yeasts are present but also pips, earth, grape skins, etc., are present. Ziegler (*21*) found a similar nitrogen percentage in lees (3−6%).

Organic Compounds. Glucose, ethanol, malic acid, lactic acid, acetic acid, tartaric acid, and glycerol concentrations are reported in **Table 4** for both kinds of lees. No glucose was detected in either case, indicating that sugars were consumed during the fermentation and transformed into ethanol, in spite of the concentration of ethanol being <9 g/L, because during distillation the alcohol is removed to obtain vinic alcohol.

The lack of malic acid was probably related to the development of a malolactic fermentation or other fermentation process during the storage of lees and also probably due to the grape liquid sugar that is produced by using must from grapes having a high degree of ripeness (*22*). Malolactic fermentation is carried out to decrease wine acidity and to give the wines better flavor and stability. During malolactic fermentations malic acid is decarboxylated into lactic acid with bacteria, mainly *Oenococcus oeni* (*23*). The rate of malolactic fermentation, and consequently the amount of final lactic acid, is related to the amounts of polyphenolic compounds such as gallic, caffeic, ferulic, and *p*-coumaric acids, catechin, and quercetin (*24*) due to their

Table 5. Concentration of Minerals in Lees (Expressed as Milligrams of Metal per Kilogram of Dried Ash)[a]

	Cu	Mg	Fe	Mn	Ca	Al	Zn
white lees after distillation	347.9	126.0	289.8	24.1	684.0	ND	18.6
red lees after distillation	39.3	ND	366.7	51.0	971.3	ND	19.5

[a] Data indicate the mean values of four replications. The standard deviations were below 3.2% of the mean. ND, not detected.

negative effect on fermentations. The lactic acid concentration oscillated between 2.4 g/L when using lees from the red winemaking technology and 5.9 g/L when using lees from the white winemaking technology.

The acetic acid concentration was 5-fold higher in lees obtained from the red winemaking technology due to acetic acid bacteria (*Gluconobacter oxydans*, *Acetobacter pasteurianus*, and *Acetobacter aceti*) that are present at all stages of winemaking, from the mature grape through vinification to conservation. Because red wine is made with must contacting grape skins, the possibility of finding these bacteria is higher, increasing consequently the amount of acetic acid in wine (*25*).

Tartaric acid was not detected in the liquid phase of lees because this acid is precipitated in the corresponding salts: potassium bitartrate and calcium tartrate, probably due to the long period of storage usually passing from the production of lees to their industrial treatment and conservation of lees into refrigerated chambers, which stimulates the precipitation. Glycerol was also not detected in the liquid phase of lees.

Minerals. Table 5 shows the concentration of minerals expressed as milligrams of Cu, Mg, Fe, Mn, Ca, Al, and Zn per kilogram of dried ashes in lees. The results shown were similar in both kinds of lees, in spite of the process technology difference that during the red winemaking technology must is in contact with grape skins, which have high metal concentrations. Nevertheless, taking into account that lees are usually mixed with bagasse (which also can have phytosanitary wastes containing metals) to be distilled together, the differences between red and white distilled lees were significantly reduced.

Tartaric Acid Recovery from Lees: Tartaric Acid Solubilization with HCl. The recovery of TA, which in lees is found precipitated as potassium bitartrate and calcium tartrate, usually requires two steps (see **Figure 1**). The first one involves the dissolution of the tartrate salts with HCl, whereas during the second step the TA is selectively precipitated to CaT, so that it can be isolated from the rest of the raw material compounds. To improve the process, both stages were optimized through incomplete factorial designs.

Because a systematic study of the effects caused by the operational variables on the susceptibility to recover TA from lees 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 use of several agroindustrial wastes (*8*, *26*−*31*).

Table 6 shows the set of experimental conditions assayed (expressed in terms of coded variables), as well as the experimental data obtained for variables y_1 and y_2. 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 13−15 are replications in the central point of the design measuring the experimental error. **Table 7** lists the regression coefficients and their statistical

Table 6. Operational Conditions Considered in This Study [Expressed in Terms of the Coded Independent Variables: Dimensionless Temperature, x_1, Dimensionless HCl Volume (37%), x_2, and Dimensionless Reaction Time, x_3] and Experimental Results Achieved for the Dependent Variables y_1 (Tartaric Acid Dissolved from White Distilled Lees, Grams per Liter) and y_2 (Tartaric Acid Dissolved from Red Distilled Lees, Grams per Liter)

	operational conditions			experimental results	
expt	x_1	x_2	x_3	y_1	y_2
1	–1	–1	0	10.971	12.814
2	1	–1	0	14.911	22.767
3	–1	1	0	82.092	39.611
4	1	1	0	58.509	42.843
5	–1	0	–1	53.408	40.832
6	1	0	–1	54.216	39.136
7	–1	0	1	47.274	37.9
8	1	0	1	66.52	50.355
9	0	–1	–1	10.533	13.629
10	0	1	–1	65.497	44.126
11	0	–1	1	15.624	13.584
12	0	1	1	59.049	45.824
13	0	0	0	61.79	42.801
14	0	0	0	63.471	42.719
15	0	0	0	58.721	43.101

Table 7. Regression Coefficients and Statistical Parameters.

(**a**) Regression Coefficients[a]

coefficient	y_1	y_2
b_0	–20.82***	3.612**
b_1	0.1796	0.03644
b_{11}	–0.001127	–0.0003330
b_2	19.5196*	10.63*
b_{22}	–0.9231*	–0.6452*
b_3	0.8264	–0.2989*
b_{33}	–0.03174***	–0.003316**
b_{12}	–0.05091**	–0.01245*
b_{13}	0.01229***	0.009434*
b_{23}	–0.05129	0.007748**

(**b**) Parameters Measuring the Correlation and Significance of Models

variable	R^2	R^2 corrected	F_{exptl}	P
y_1	0.96712	0. 90795	13.6376	0.930912
y_2	0.98371	0. 95438	309.59	0.996779

[a] ***, significant coefficient at the 99% significance level (*t* test); **, significant coefficient at the 95% significance level (*t* test); *, significant coefficient at the 90% significance level (*t* test).

significance (based on a *t* test). The same table includes statistical parameters (r^2 and F) measuring the correlation and the statistical significance of the models, respectively. It can be noted that both models showed good statistical parameters for correlation and significance and allowed a close reproduction of experimental data. Furthermore, panels **a** and **b** of **Figure 2** show the experimental and calculated data confirming the ability of the considered models for reproducing all of the experimental data obtained in this work.

With regard to the influence of independent variables on the variation of dependent variables considered, HCl volume, with a confidence level of 99% for both linear and quadratic terms, followed by reaction time, caused the strongest effects on TA solubilization in both white and red distilled lees, as can be seen from the absolute value of the corresponding coefficients listed in **Table 7**. Thus, panels **a** and **b** of **Figure 3** show the predicted dependence of the TA concentration of samples (y_1 and y_2) on the reaction time and HCl volume at 20 °C,

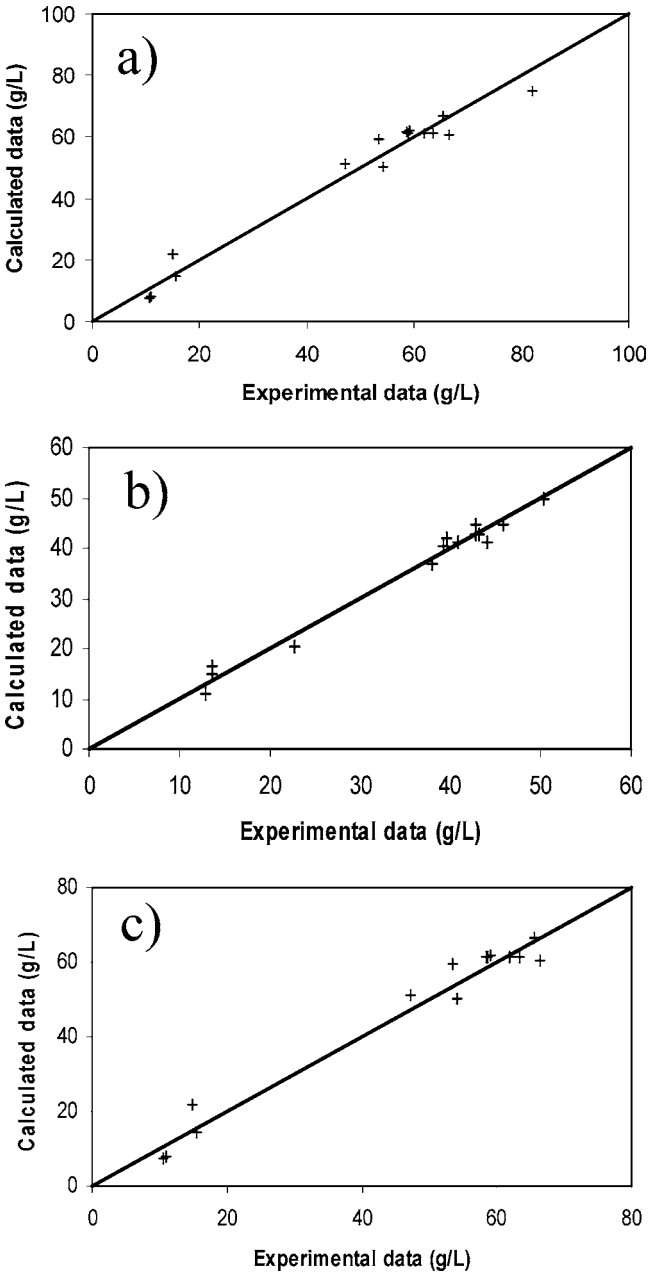

Figure 2. Comparison between experimental and calculated values of (**a**) variable y_1, (**b**) variable y_2, and (**c**) variable y_3.

Table 8. Composition of Streams A and C under Optimal Conditions Calculated for y_1 and y_3 and Percentage of Reduction of These Compounds

compound	stream A	stream C	reduction (%)
tartaric acid	77.5 g/L	71.62 g/L	7.59
lactic acid	2.84 g/L	1.24 g/L	56.34
acetic acid	1.24 g/L	0.50 g/L	59.68
ethanol	2.86 g/L	0.33 g/L	88.46
glycerol	0.25 g/L	0 g/L	100
phenolic compounds	58 abs[a]	1.93 abs[a]	96.67

[a] Determined as absorbance per dilution and expressed as units of absorbance.

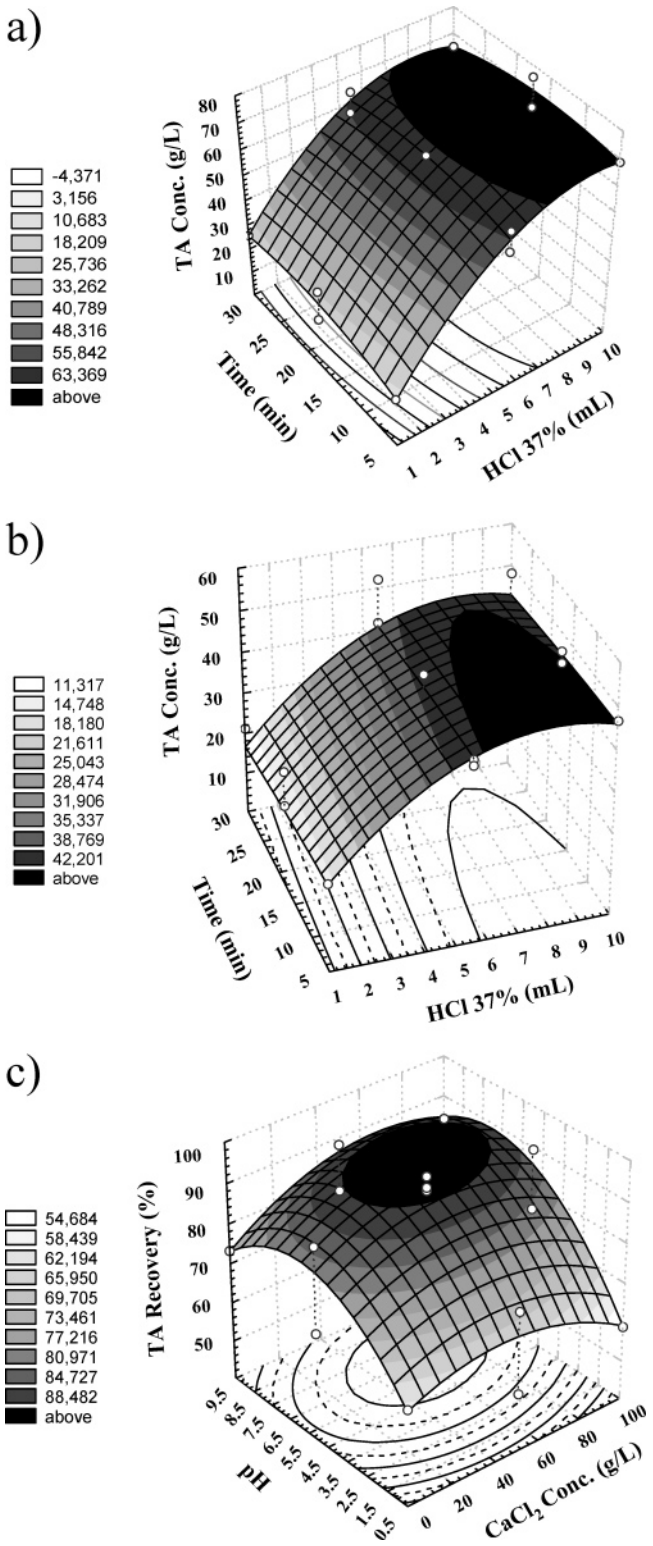

Figure 3. Dependence of the TA dissolved on volume of HCl (37%) and time predicted for samples carried out at room temperature ($x_1 = -1$) using (**a**) white distilled lees (variable y_1) or (**b**) red distilled lees (variable y_2) and (**c**) dependence of the percentage of TA recovered (variable y_3) on $CaCl_2$ concentration and pH for experiments carried out at intermediate times ($x_6 = 0$).

corresponding to room temperature (the most economical conditions). The surface response shows in both cases a slight continuous increase in y_1 and y_2, respectively, with the reaction time and a strong increment in the HCl volume to 8 mL followed by a slight decrease when the HCl was increased to 10 mL.

Using the "*solver*" application of Microsoft Excel, the maximum solubilization of tartaric acid from white distilled lees predicted from the model ($y_1 = 77.5$ g/L) was achieved when $x_1 = 20$, $x_2 = 9.77$, and $x_3 = 9.0$. Meanwhile, when using red

Table 9. Operational Conditions Considered in This Study (Expressed in Terms of the Independent Variables: Dimensionless $CaCl_2$ Concentration, x_4, Dimensionless pH, x_5, and Dimensionless Reaction Time, x_6) and Experimental Results Achieved for the Dependent Variable y_3 (Percentage of Tartaric Acid Recovery)

	operational conditions			initial TA (g)	TA (g) after filtration	TA (g) after redissolving	exptl
expt	x_4	x_5	x_6	(stream A)	(stream B)	(stream C)	results y_3
1	–1	–1	0	72.8	27.9	45.9	63.1
2	1	–1	0	72.8	28.5	49.8	68.4
3	–1	1	0	72.6	5.7	49.3	67.9
4	1	1	0	72.6	0.8	58.9	81.1
5	–1	0	–1	72.8	3.0	44.3	60.9
6	1	0	–1	72.9	1.0	63.7	87.4
7	–1	0	1	72.8	1.0	62.9	86.5
8	1	0	1	72.5	5.2	64.5	82.2
9	0	–1	–1	72.8	25.2	51.4	70.6
10	0	1	–1	72.6	0.6	52.4	72.2
11	0	–1	1	72.8	28.4	53.2	73.1
12	0	1	1	72.6	0.6	62.5	86.1
13	0	0	0	72.8	1.4	67.6	92.9
14	0	0	0	72.8	0.6	64.5	88.6
15	0	0	0	72.8	0.7	65.2	89.6

Table 10. Regression Coefficients and Statistical Parameters

(**a**) Regression Coefficients[a]

coefficient	y_3
b_0	46.78*
b_4	0.5405**
b_{44}	– 0.003732***
b_5	7.140*
b_{55}	– 0.8279*
b_6	0.09062
b_{66}	– 0.0003745
b_{45}	0.02200
b_{46}	– 0.001895**
b_{56}	0.01877**

(**b**) Parameters Measuring the Correlation and Significance of Models

variable	R^2	R^2 corrected	F_{exptl}	P
y_3	0.94026	0.83272	2.9997	0.259945

[a] ***, significant coefficient at the 99% significance level (*t* test); **, significant coefficient at the 95% significance level (*t* test); *, significant coefficient at the 90% significance level (*t* test).

distilled lees, the tartaric acid concentration (y_2) decreased to 45.6 g/L under similar conditions: $x_1 = 20$, $x_2 = 8.07$, and $x_3 = 5.0$.

Tartaric Acid Recovery from Lees: Tartaric Acid Precipitation as Calcium Tartrate. The solubilization of TA involves the dissolution of small amounts of other substances such as malic acid, glycerol, or phenolic compounds (see stream A of **Table 8**), which renders more difficult a further purification step. For that reason, it is desirable to recover the TA in higher concentration with a minimum amount of impurities. Besides, the exhausted wastes would represent an environmental problem if they are discarded (*13*). To avoid these problems, a selective precipitation of TA into CaT must be taken into account. The addition of calcium salts to precipitate the CaT in industrial wastes from vineries such as pomace of grape cultivars (*16*) or eluates, lees, and creams of tartar (*17*) is usual. This process was assayed using lees from the white winemaking technology due to the higher TA concentration reached in the previous step.

Calcium carbonate was added according to the stoichiometric reaction

$$C_4H_6O_6 + CO_3Ca \rightarrow CaC_4H_4O_6 + CO_2 + H_2O$$

Versari et al. (*17*) suggested that an excess of $CaCl_2$ improves the TA recovery. Thus, adding 7.6 g/L of $CaCl_2$ to eluates, which simulated the conditions of industrial precipitation, allowed a laboratory recovery of TA (62.4%) comparable to that obtained during the industrial process (58.3%). pH is also an important variable, although it must be considered that an excess of $CaCl_2$, as well as a pH value that is too high or too low, can render a lower yield of precipitation, because the enological wastes may contain colloids which flocculate at a high pH value corresponding to their isoelectric point. To consider all of these variables, a second factorial design was carried out from the optimum value of the previous design.

Table 9 shows the set of experimental conditions assayed (expressed in terms of coded variables), as well as the grams of TA in the starting optimal conditions, corresponding to the results achieved during the first experimental design and referred to 1 L (stream A in **Figure 1**), grams of residual TA after filtration (stream B), and grams of TA recovered at the end of the process, after the CaT was dissolved (stream C). Using this information, the yield of each experiment was calculated (variable y_3) and is reflected in **Table 9**.

Thereby, **Table 10** provides the regression coefficients and their statistical significance (based on a *t* test) as well as the statistical parameters (r^2 and F) measuring the correlation and the statistical significance of the model, respectively. These values indicate good statistical parameters for correlation and significance, allowing a close reproduction of experimental data. Furthermore, **Figure 2c** shows the experimental and calculated data confirming the ability of the model to reproduce all of the experimental data obtained. In the interval considered, pH (variable x_5) was the most relevant variable, with a confidence level of 99% for both linear and quadratic terms, followed by the $CaCl_2$ concentration (variable x_4), whereas the reaction time (variable x_6) hardly affected the precipitation treatment, as can be observed from the absolute value of the corresponding coefficients.

Table 11. Stoichiometric Parameters, Productivities, and Yields for Bioconversion Assays Carried out Using Different Nutrients during the Fermentation of Vine Trimming Waste Hemicellulosic Hydrolysates into Lactic Acid by *L. pentosus*[a]

medium	initial sugars (g/L)	LA_{max} (g/L)	AcH_{max} (g/L)	$Q_{P,LA}$ (g/Lh)	$Q_{P,AcH}$ (g/Lh)	$Y_{LA/S}$ (g/g)	$Y_{AcH/S}$ (g/g)
control	23.2	18.6	8.4	1.11	0.07	0.62	0.21
WDL after TA extraction	24.0	18.4	8.5	0.84	0.05	0.69	0.17
RDL after TA extraction	24.3	18.9	8.5	0.82	0.04	0.70	0.17
WDL before TA extraction	23.0	17.2	9.95	1.21	0.11	0.66	0.22
RDL before TA extraction	23.7	16.4	9.13	0.96	0.12	0.61	0.21

[a] WDL, distilled lees from the white winemaking technology; RDL, distilled lees from the red winemaking technology; TA, tartaric acid; initial sugars, glucose, xylose, and arabinose; LA_{max}, maximum concentration of lactic acid; AcH_{max}, maximum concentration of acetic acid; $Q_{P,LA}$, volumetric productivity of lactic acid; $Q_{P,AcH}$, volumetric productivity of acetic acid; $Y_{LA/S}$, lactic acid yield (g of lactic acid produced/(g of glucose, xylose, and arabionose consumed); $Y_{AcH/S}$, acetic acid yield (g of acetic acid produced/(g of glucose, xylose, and arabionose consumed).

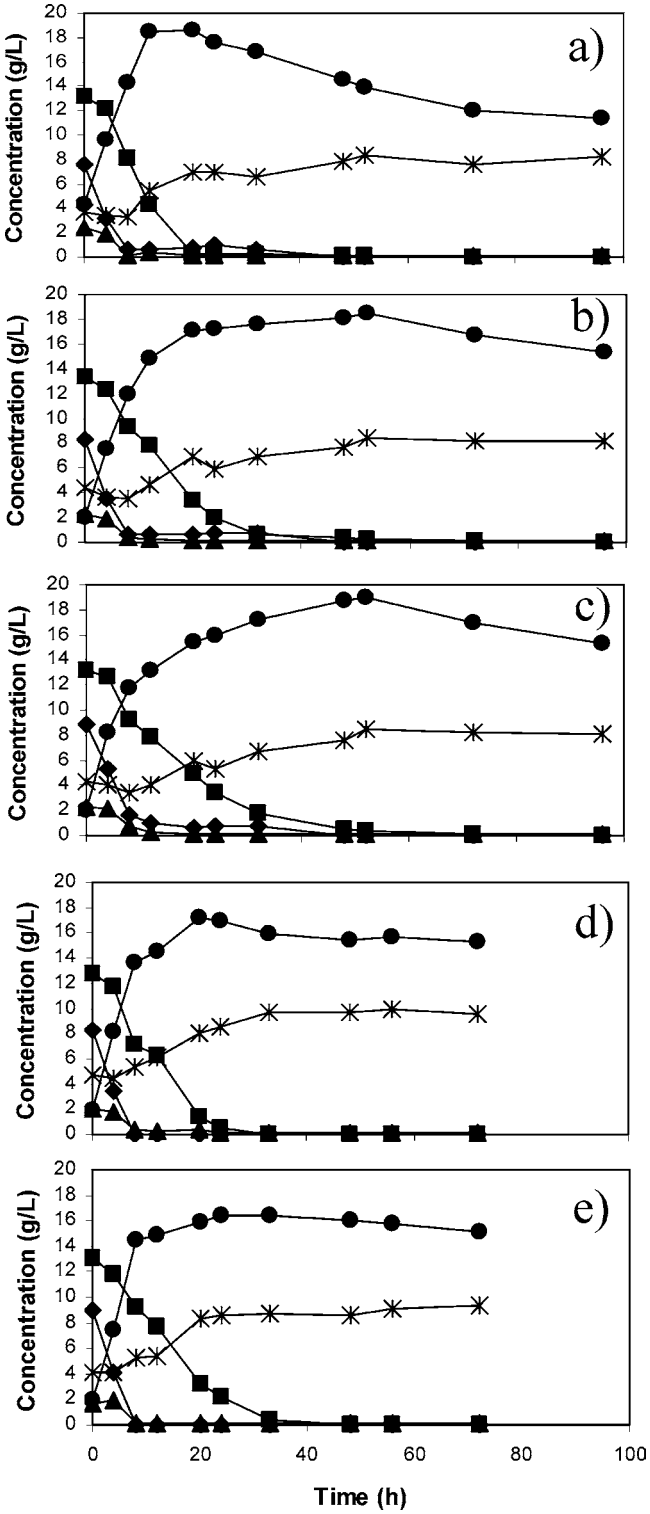

Figure 4. Course with time for the lactic acid production from hemicellulosic sugars hydrolysates by *L. pentosus* during fermentations carried out with (**a**) Mercier medium, (**b**) distilled white lees after TA extraction, (**c**) distilled red lees after TA extraction, (**d**) distilled white lees without TA extraction, and (**e**) distilled red lees without TA extraction: (◆) glucose; (■) xylose; (▲) arabinose; (●) lactic acid; (∗) acetic acid concentrations. Results represent the average of three independent experiments. Standard deviations were below 2.8% of the mean.

Figure 3c shows the percentage of TA recovered from distilled white lees (variable y_3) with respect to the most significant variables ($CaCl_2$ concentration and pH) in experiments conducted at an intermediate time (t = 125 min). For both variables, a maximum percentage was observed under intermediate parameters, which is in accordance with data from Versari et al. (*17*), who observed that an excess of $CaCl_2$, as well a pH too high or too low, decreases the precipitation.

Meanwhile, employing the *solver* application of Microsoft Excel, an optimum was calculated using x_4 = 51.3, x_5 = 6.84, and x_6 = 162.5. Under these conditions, the model predicts a maximum TA recovery of 92.4%. This value is considerably higher than that obtained at an industrial scale (58.3%) according to Versari et al. (*17*).

Taking into account the results of both streams A and C reflected in **Table 8**, it can be deduced that the CaT precipitation and further redissolving represent a significant reduction in secondary products, particularly glycerol, phenolic compounds, and ethanol with removal percentages of 100, 96.7, and 88.5%, respectively, making further crystallization steps useful. The residual TA and the removed substances remain in stream B. In our opinion, the ecological problems derived from the disposal of these wastewaters could be considered negligible compared with that of the initial untreated lees.

Lactic Acid Production from Vine Trimming Wastes Using Vinification Lees without Tartaric Acid as Nutrients. Lactic acid is a food additive widely used at an industrial scale (*32*). There are several works concerning the use of lignocellulosic materials as a carbon source for lactic acid production (*7*, *8*, *32*−*34*). Taking into account the previous results obtained by Bustos et al. (*5*, *6*), the biotechnological production of lactic acid, from the hemicellulosic sugars of vine trimming wastes, was assayed. In these fermentations the residual vinification lees that remain after the TA extraction were employed as nutrients. **Table 11** shows some fermentative parameters concerning the lactic acid and acetic acid production from these hemicellulosic sugars using *L. pentosus* and vinification lees. For comparative purposes a positive control was used using the complete medium proposed by Mercier et al. (*9*), the richest medium proposed in the literature. The main drawback of this medium is the high price, because it contains, among other, yeast extract and peptone, which reach prices as high as 7.3 and 10.3 $/kg, respectively (*35*). This table also includes fermentative parameters concerning the sugar consumption, where $Y_{LA/S}$ and $Y_{AcA/S}$ are the lactic acid and acetic acid yields, respectively. On the other hand, **Figure 4** shows the hemicellulosic sugar consumption as well as the product formation for all of the fermentations assayed.

From the results achieved it can be inferred that all of the fermentations assayed show tendencies similar to those obtained when using the control, although when lees from the red and white distilled lees, after TA extraction, were used, slightly higher concentrations of lactic acid were obtained compared with vinification lees without TA extraction. Moreover, yields in the control and using lees with or without TA extraction oscillated in the range of 0.61−0.70 g/g. Finally, the global volumetric productivities are slightly lower when TA is extracted from lees, probably because during the TA extraction some nutrients could be removed. In spite of that, this fact has to be balanced with the recovery of another valuable product, tartaric acid.

In conclusion, the sequential treatment of dissolving TA and further CaT precipitation can be used to recover up to 92.4% of the initial TA in lees. Moreover, the remaining lees can be employed as economic nutrients for lactic acid production from trimming vine shoot hydrolysates by *L. pentosus*, providing values of lactic acid, global volumetric productivities, and product yields similar to those achieved when using the general

medium for *Lactobacillus* or lees without TA extraction. This procedure has a triple profit because (a) a harmful environmental waste is removed, (b) tartaric acid, a valuable product, is recovered, and (c) the residual lees can be used as economic nutrients for biotechnological lactic acid fermentation in the presence of hemicellulosic sugars as carbon source.

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