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Evaluation of Vinification Lees as a General Medium for *Lactobacillus* Strains

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Lactobacillus species present high nutritional requirements, so it is necessary to find new low-cost nutrient components for fermentation media. This work compares the utilization of vinification lees (an important residue of wineries) from red and white winemaking technology, distilled or not. An amount of 20 g of lees/L was used as the unique nutrient to obtain lactic acid from glucose using *Lactobacillus* strains with different properties: *L. plantarum* CECT-221, *L. pentosus* CECT-4023, *L. casei* CECT-5275, and *L. coryniformis* subsp. *torquens* CECT-25600. Only *L. casei* using distilled lees showed values (P_{max} = 92.1 g/L and $Y_{P/S}$ = 1.05 g/g) similar to those obtained with the MRS broth. The UV spectra of white and red lees, distilled or not, allowed an interpretation of the different phenolic compounds present and their influence on the fermentation. Their detoxification by extraction with organic compounds and fermentation with *L. pentosus* was also considered. Time courses of glucose and lactic acid were modeled according to reported models to obtain more information about the process.

KEYWORDS: Vinification lees; lactic acid; *L. plantarum*; *L. pentosus*; *L. casei; L. coryniformis*

INTRODUCTION

Wine production is one of the most important agricultural activities in Spain, being 10% of the total agricultural production. Vinification involves all of the steps carried out during the elaboration of wine from grapes. This process generates different residues, as can be seen in the scheme of the process shown in **Figure 1** (*1*).

Vinification is a seasonal activity mainly performed during the fall, and 60−70% of the liquid streams generated are obtained 3 months after the vintage beginning (*2*). The main residues can be summarized as vegetal remains proceeding from the destemmed grapes, mires obtained during the clarification, bagasse of press, and lees obtained from different decanting steps. Some of these byproducts can be used for different applications. Thus, marc and seeds can be used to obtain anthocyanic colorants or oils and polymers of catechin, respectively (*3*, *4*). On the contrary, in the beginning, the utilization of lees (basically remains of dead yeasts) was considered for use as supplement in animal nutrition, but the yeasts of distilled lees (recovered for centrifugation at the exit of the column of distillation) have an exceedingly poor nourishing value that does not make them suitable for this purpose (*5*). This is probably due to the high amount of polyphenols joined to the proteins, which render them not assimilable, or the presence of toxic elements from residues of treatments, which are accumulated in the lipids of the yeasts. Many nitrogen-containing compounds are found in grapes and wines. These include inorganic forms, such as ammonia and nitrates, and many diverse organic forms including amines, amides, amino acids, pyrazines, nitrogen bases, pyrimidines, proteins, and nucleic acids. On the other hand, vitamin levels in wine are inadequate to be of major significance in human nutrition, but they usually are ample for microbial growth. For example, biotin and nicotinic acid contents are adequate for most yeast strains, covering the vitamin and growth factor levels required by lactic acid bacteria. During fermentation, and especially after, there is a slow release of nitrogen compounds into wine, probably owing to autolysis of dead yeast cells (*6*).

In a previous work (*7*) we have proposed the utilization of lees as nutritional media for *Lactobacillus rhamnosus* as an inexpensive source of essential microbial nutrients, achieving interesting results. 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. (*8*). 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 (*9*). Hujanen and Linko (*10*) demonstrated that the lactic acid production by *Lactobacillus casei* was markedly influenced by the type and initial concentration of the nitrogen source.

Yeast extract is an excellent nutrient for many microorganisms, but it is very expensive. Yeast extract can be obtained by

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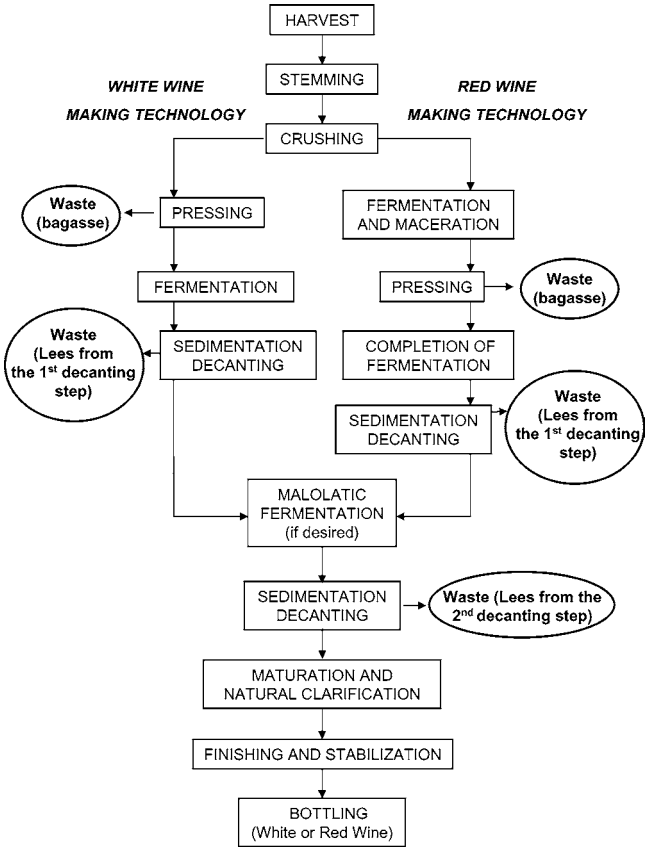

Figure 1. Flow diagram of winemaking technology.

physical treatments (including mechanical breakdown of cell membranes, autolysis at 50−55 °C, plasmolysis under high NaCl concentrations, and permeabilization) (*11*), chemical methods with solvents and detergents, or enzymatic processes. Inexpensive food and feed processing byproducts, such as malt sprout, grass extract, or corn steep liquor have been proposed as nitrogen sources for *Lactobacillus* media, but clearly yeast extract exhibited the most significant increment on lactic acid production (*10*, *12*).

This work evaluates the utilization of vinification lees (distilled or not, from the white or red winemaking technology) as a unique nutrient for *Lactobacillus* strains with different characteristics (*L. plantarum* CECT-221, *L. pentosus* CECT-4023, *L. casei* CECT-5275, and *L. coryniformis* subsp. *torquens* CECT-25600), comparing the results with those obtained in a previous work with *L. rhamnosus* (*7*) and with the results achieved with the costly MRS broth. This work also takes into account the phenolic compounds present in these lees, observing the differences between the UV spectra of white and red lees, distilled or not, allowing us to give an interpretation of their influence on the fermentation of glucose to lactic acid. We also considered their detoxification by extraction with organic compounds and fermentation with *L. pentosus*. Time courses of glucose and lactic acid were modeled according to reported models, to obtain more information about the process.

MATERIALS AND METHODS

Lees Sampling and Storage. Lees from the campaign of 2002 were kindly supplied by Cooperativa Vitivinícola do Ribeiro (Ourense, Spain) and stored at 4 °C. In white winemaking technology the unfermented grape juice is removed from the tanks and ferments separated from the grains and grape skins (see **Figure 1**). On the contrary, red wines are obtained after the fermentation of the juice in contact with grapes, grains, and skins in the same tanks. After fermentation, the wine rests in tanks or barrels, decanting the particles in suspension to the bottom before being removed. The number of decanting steps depends on the kind of wine, the amount of lees decreasing in each step. Lees are usually distilled to recover ethanol and aromatic flavors used for the production of aromatic spirit liquors, giving lees no use to wineries. Lees studied in this work came from white and red wines after the second decanting step, distilled or not (noted in the text as "white lees" or "red lees").

Inocula Preparation. *L. plantarum* CECT-221, *L. pentosus* CECT-4023, *L. casei* CECT-5275, and *L. coryniformis* subsp. *torquens* CECT-25600 were obtained from the Spanish Collection of Type Cultures (Valencia, Spain). The strains were grown on plates using the complete medium proposed by Mercier et al. (*8*), 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 10 g of agar/L at the adequate temperature of each microorganism (31 °C for *L. pentosus* and 37 °C for the others) during 24 h. Biomass in inocula was measured by optical density at 600 nm and adjusted to the desired value (3.0 g of dry cells/L) by dilution with water. Five milliliters of this suspension was taken and added to 95 mL of medium to achieve an initial biomass concentration of 0.15 g of dry cells/L.

Lactic Acid Fermentation. Experiments were carried out in 250 mL Erlenmeyer flasks with a final volume of 100 mL using between 100 and 110 g of glucose/L. A positive control was used using the complete medium proposed by Mercier et al. (*8*) (noted in the test as "MRS broth"). Experiments were carried out with 20 g of lees/L according to a previous study (*7*). In all cases calcium carbonate (100 g/L) was added to neutralize the lactic acid produced. After inoculation (5 mL), fermentations were carried out in orbital shakers at 200 rpm. Samples (2 mL) were taken at given fermentation times and centrifuged at 6000 rpm for 3 min. The supernatants were saved for glucose and lactic acid analyses. Experimental data were measured in triplicate, and means are reported. Standard deviations were below 3.4% of the mean. The volumetric productivities Q_P were calculated for the fermentation times corresponding to the transition from high to low slope of the sigmoidal lactic acid profiles.

Solvent Extraction of Lees. Lees were extracted with ethyl acetate to remove phenolic compounds following the process described by Cruz et al. (*13*). The extraction was carried out in a single step using a lees/ethyl acetate volume ratio of 1:3 v/v. Ethyl acetate was recovered by vacuum evaporation and reutilized. These lees are noted in the text as "distilled lees extracted with ethyl acetate".

Analytical Methods. Glucose consumed and lactic acid produced during fermentations were measured by a high-performance liquid chromatograph (Agilent, model 1100, Palo Alto, CA), with RI detection using a Transgenomic ION-300 column (Transgenomic Inc., San Jose, CA) eluted with 0.01 N H_2SO_4 at a flow rate of 0.4 mL/min.

To compare the phenolic compounds, all lees were diluted with water to achieve a concentration of 2.5 g of lees/L, and UV spectra were recorded using a DAD spectrophotometer (Agilent, model 8453).

Fitting of Data. The commercial software Solver of Microsoft Excel 2002 was used to fit the experimental data to proposed models by nonlinear regression using the least-squares method. Lactic acid production was mathematically modeled following the equation proposed by Mercier et al. (*8*)

$$P = \frac{P_0 P_m e^{P_r t}}{P_m - P_0 + P_0 e^{P_r t}} \quad (1)$$

where t is time, P is lactic acid concentration, P_m is maximum concentration of lactic acid, and P_r is the ratio between the initial volumetric rate of product formation (r_p) and the initial product concentration P_0. From the series of experimental data lactic acid concentration/time, the model parameters P_0, P_m, and P_r can be calculated for each fermentation medium.

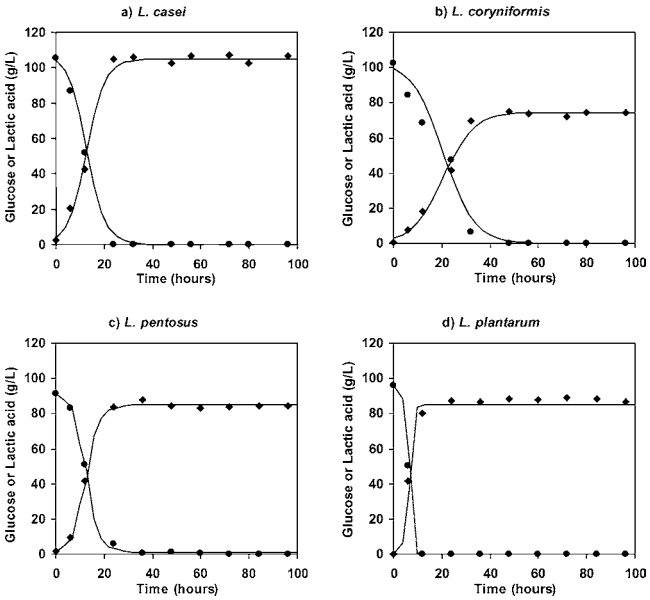

Figure 2. Experimental data and calculated time courses of lactic acid (◆) and glucose concentrations (●) during fermentations carried out with MRS broth using (**a**) *L. casei*, (**b**) *L. coryniformis*, (**c**) *L. pentosus*, and (**d**) *L. plantarum*. Results represent the average of three independent experiments. Standard deviations were below 3.1% of the mean.

Glucose consumption by *Lactobacillus* strains can be interpreted by the equation

$$S = S_0 - \frac{1}{Y_{P/S}}(P - P_0) \quad (2)$$

where $Y_{P/S}$ is the product yield, P_0 is the initial lactic acid concentration (g/L), P is the lactic acid concentration (g/L) for each time predicted for eq 1, S_0 is the initial glucose concentration (g/L), and S is the experimental glucose concentration (g/L) for each time. The model parameter $Y_{P/S}$ was calculated for each fermentation medium from the series of experimental data glucose concentration/time, P_0, and the P value for each time calculated from the regression parameters of eq 1.

RESULTS AND DISCUSSION

To evaluate vinification lees as nutritional media for *Lactobacillus*, we have chosen four different *Lactobacillus* strains: *L. casei*, which is homofermentative and produces mainly L-lactic acid (*14*, *15*); *L. coryniformis*, which is homofermentative and produces D-lactic acid (*14*, *16*); *L. plantarum*, which is also homofermentative but produces a racemic mixture of L- and D-lactic acid (*17*); and *L. pentosus*, which is heterofermentative, fermenting both hexoses and pentoses (*18*).

Controls for the *Lactobacillus* Strains. Control fermentation runs were carried out using the fully supplemented medium MRS broth for all *Lactobacillus* strains. **Figure 2** shows the experimental data as well as the predicted values calculated by eqs 1 and 2 using the regression parameters listed in **Table 1**. All experiments show a kinetic pattern fairly described by the mathematical models with $r^2 > 0.976$ and 0.992 for glucose consumption and lactic acid production, respectively. It can be noted that *L. casei* presents the highest P_{max} (104.8 g/L) and $Y_{P/S}$ (0.97 g/g) followed by *L. pentosus*, *L. plantarum*, and *L. coryniformis*.

Evaluation of Lees as Nutritional Components for *Lactobacillus*. Taking into account the results obtained in a previous work (*7*) employing lees alone or in combination with corn steep liquor (CSL) as nutritional media for *L. rhamnosus*, 20 g of lees/L was proposed directly as the unique nutrient for other *Lactobacillus* strains. This study was carried out to determine if the results achieved with *L. rhamnosus* can be comparable with other *Lactobacillus* species, considering that each microorganism has a different hydrolytic activity (*19*). Lees came from the elaboration of white or red wines, after or without being distilled.

Lees from White Wine. Table 2 shows the kinetic parameters for lactic acid production and glucose consumption; meanwhile, **Figure 3** shows the kinetic pattern for lactic acid production using white lees, distilled or not, as well as the glucose consumed for all *Lactobacillus* strains. It can be emphasized from **Table 2** that in general P_{max} and $Y_{P/S}$ are higher when distilled lees were used. In these cases, the product yields calculated for distilled lees oscillate in the range of 1.05 g/g for *L. casei* and 0.74 g/g for *L. coryniformis*, which are similar to those values obtained with controls, 0.97−0.72 g/g (see **Table 1**). From a comparison of all the strains, the best results were achieved with *L. casei* (using distilled lees), reaching a maximum lactic acid concentration of 92.1 g/L (see **Table 2**), which is close to the 104 g/L achieved when the complete medium proposed by Mercier et al. (*8*) was used (see **Table 1**). *L. casei* produces lactic acid concentrations close to those obtained with *L. rhamnosus* in a previous work (*7*) (see **Table 2**). Curiously, both strains are homofermentatives, L-lactic acid producers, and show similar peptidase activities (*19*).

The use of lees as nutrients could be attributed to the fact that *Lactobacillus* strains possess high hydrolyzing activities toward substrates containing proline and alanylprolyl-*p*-nitroanilide (*19*), breaking the wall of the cells contained in lees. Additional experiments (data not shown) counting the number of yeast cells at the beginning and at the end of the fermentations showed that the hydrolytic activities were similar in all of the *Lactobacillus* strains studied. On the other hand, distilled white lees present adequate contents in nitrogen, vitamins, and growth factors, which are ample for the microbial growth. It seems that the amount of phenolic compounds found in white wines and, consequently, in the corresponding lees, consist in readily soluble non-flavonoids such as caftaric acid (caffeolyltartaric acid); related derivatives of *p*-coumaric acid and ferulic acid, catechins, and catechin-gallate polymers (*20*) are responsible for inhibiting the fermentations. Among the most typical inhibitory compounds, flavonols and other flavonoid phenols are solubilized slowly, being found in significant quantities only in juice macerated with the pomace, which happens during the red winemaking technology (as indicated in **Figure 1**). In this case, the soluble lignin of pipes and grains goes to the must, and from there to the lees during the fermentation step. These phenolic components could inhibit the growth of some *Lactobacillus* strains. **Figure 4** shows the UV spectra of distilled (**b**) and not distilled (**a**) white lees with a maximum of absorbance at 263 nm. It can be noted that distilled lees have modified their composition because of the different profile shown, reducing the percentage of phenolic compounds to 51.6%. This can explain the differences found during the fermentations carried out with distilled and not distilled lees, with the best results obtained when distilled lees were used. These results are interesting if we realize that lees are distilled in wineries to recover ethanol and aromatic flavors, the distilled lees being the real waste which must be used to obtain additional profits and to avoid contaminant problems (*7*).

Finally, the remaining phenolic compounds present in distilled lees could be removed by extraction with organic solvents (*13*) (an economic process because the organic solvent can be

Table 1. Results Obtained by Regression of Lactic Acid and Glucose Concentration Data in Controls (MRS Broth)[a]

	lactic acid production					glucose consumption		
lees	P_0 (g/L)	P_{max} (g/L)	P_r (h^{-1})	r^2	*F* value	$Y_{P/S}$ (g/g)	r^2	*F* value
L. casei (**Figure 2a**)	3.5	104.8	0.261	0.993	531.41	0.97	0.994	875.2*
L. coryniformis (**Figure 2b**)	2.7	74.6	0.159	0.990	342.02***	0.72	0.976	260.9*
L. pentosus (**Figure 2c**)	1.3	84.7	0.342	0.999	2860.92***	0.92	0.998	3616.9**
L. plantarum (**Figure 2d**)	0.1	84.7	1.19	0.993	316.39***	0.88	0.999	6579590***
L. rhamnosus (*7*)	3.2	103.0	0.160	0.992	422.1**	0.97	0.997	2486.1*

[a] P_0 = initial lactic acid concentration (g/L); P_{max} = maximum concentration of lactic acid (g/L); P_r = ratio between initial volumetric rate of product formation (r_p) and initial product concentration P_0 (h^{-1}), $Y_{P/S}$ = product yield (g/g); r^2 = determination coefficient; *F* value = *F* test statistical parameter; *, significance level >95%; **, significance level >97.5%; ***, significance level >99%.

Table 2. Results Obtained by Regression of Lactic Acid and Glucose Concentration Data in Experiments Carried out with 20 g/L of Lees from the White Winemaking Technology[a]

	lactic acid production					glucose consumption		
lees	P_0 (g/L)	P_{max} (g/L)	P_r (h^{-1})	r^2	*F* value	$Y_{P/S}$ (g/g)	r^2	*F* value
L. casei with no distilled lees (**Figure 3a**)	7.2	65.8	0.085	0.985	221.52***	0.82	0.981	397.21*
L. casei with distilled lees (**Figure 3a**)	8.6	92.1	0.082	0.986	233.04***	1.05	0.989	735.22*
L. coryniformis with no distilled lees (**Figure 3b**)	2.4	25.6	0.085	0.992	198.96***	0.69	0.988	377.42*
L. coryniformis with distilled lees (**Figure 3b**)	3.5	29.3	0.055	0.968	102.05***	0.74	0.975	211.39*
L. pentosus with no distilled lees (**Figure 3c**)	6.4	50.1	0.089	0.978	146.86***	1.02	0.980	245.53*
L. pentosus with distilled lees (**Figure 3c**)	7.1	59.2	0.090	0.982	178.77***	0.89	0.971	270.99*
L. plantarum with no distilled lees (**Figure 3d**)	5.8	49.3	0.091	0.979	152.38***	0.86	0.992	761.53*
L. plantarum with distilled lees (**Figure 3d**)	8.3	67.4	0.089	0.986	231.31***	0.89	0.958	172.34*
L. rhamnosus with no distilled lees (*7*)	4.8	103.4	0.200	0.992	488.0**	0.91	0.985	349.1*
L. rhamnosus with distilled lees (*7*)	5.8	96.8	0.139	0.993	580.9**	0.93	0.989	583.8*

[a] P_0 = initial lactic acid concentration (g/L); P_{max} = maximum concentration of lactic acid (g/L); P_r = ratio between initial volumetric rate of product formation (r_p) and initial product concentration P_0 (h^{-1}), $Y_{P/S}$ = product yield (g/g); r^2 = determination coefficient; *F* value = *F* test statistical parameter; *, significance level >95%; **, significance level >97.5%; ***, significance level >99%.

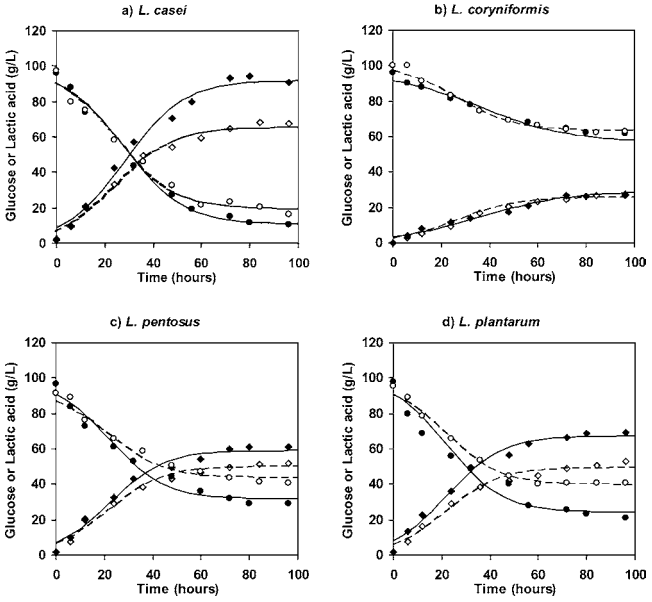

Figure 3. Experimental data and calculated time courses of lactic acid and glucose concentrations during fermentations carried out with 20 g/L of white lees (distilled or not) using (**a**) *L. casei*, (**b**) *L. coryniformis*, (**c**) *L. pentosus*, and (**d**) *L. plantarum*: glucose consumption using no distilled lees (○); glucose consumption using distilled lees (●); lactic acid production using no distilled lees (◇); lactic acid production using distilled lees (◆). Results represent the average of three independent experiments. Standard deviations were below 2.2% of the mean.

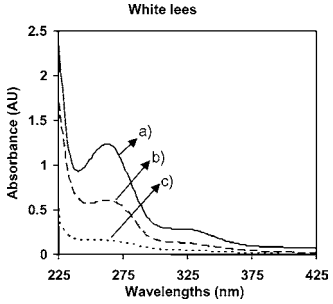

Figure 4. UV spectra of white lees: (**a**) no distilled; (**b**) distilled; (**c**) distilled and extracted with ethyl acetate.

recovered and reused). This step is especially interesting because of both the better susceptibility of detoxified lees toward the fermentation and the recovery of a phenolic fraction potentially valuable as a food additive owing to its antioxidant and antimicrobial activity, which is useful in the food industry to prevent rancidity and microbial spoilage. Besides, the lactic acid obtained is purer; consequently, it is not necessary to perform so many purification steps to recover it.

To evaluate the importance of removing the remaining phenolic compounds, we carried out a new fermentation with distilled lees extracted with ethyl acetate using *L. pentosus*. The selection of this microorganism was related with the poor results achieved (**Figure 3c**) and with its potential for its ability to ferment both glucose and xylose. **Figure 4c** shows the UV spectrum of distilled white lees extracted with ethyl acetate. From a comparison of the spectra of **Figure 4b**,**4c** it can be easily observed as the remaining phenolic compounds were almost completely removed (87.7% of absorbance reduction in relation with the not distilled lees). Consequently, the use of extracted lees as nutrients showed an enhanced susceptibility toward fermentation in relation with the use of unextracted lees, as can be seen in **Figure 5a**, where the kinetic pattern for lactic acid production and glucose consumed was similar to that obtained using the MRS broth (see **Figure 2c**). The same

Table 3. Results Obtained by Regression of Lactic Acid and Glucose Concentration Data in Experiments Carried out with 20 g/L of Distilled Lees from the Red and White Winemaking Technologies Extracted with Organic Solvents and Fermented with *L. pentosus*[a]

	lactic acid production					glucose consumption		
lees	P_0 (g/L)	P_{max} (g/L)	P_r (h^{-1})	r^2	*F* value	$Y_{P/S}$ (g/g)	r^2	*F* value
white lees (**Figure 5a**)	6.6	84.1	0.121	0.989	319.80***	0.87	0.989	750.51*
red lees (**Figure 5b**)	10.5	73.3	0.076	0.987	316.73***	0.88	0.983	561.25*

[a] P_0 = initial lactic acid concentration (g/L); P_{max} = maximum concentration of lactic acid (g/L); P_r = ratio between initial volumetric rate of product formation (r_p) and initial product concentration P_0 (h^{-1}); $Y_{P/S}$ = product yield (g/g); r^2 = determination coefficient; *F* value = *F* test statistical parameter; *, significance level >95%; **, significance level >97.5%; ***, significance level >99%.

Table 4. Results Obtained by Regression of Lactic Acid and Glucose Concentration Data in Experiments Carried out with 20 g/L of Lees from the Red Winemaking Technology[a]

	lactic acid production					glucose consumption		
lees	P_0 (g/L)	P_{max} (g/L)	P_r (h^{-1})	r^2	*F* value	$Y_{P/S}$ (g/g)	r^2	*F* value
L. casei with no distilled lees (**Figure 6a**)	9.0	66.2	0.064	0.978	156.61***	1.13	0.987	363.89*
L. casei with distilled lees (**Figure 6a**)	5.1	72.7	0.107	0.995	735.28***	0.92	0.987	610.66*
L. coryniformis with no distilled lees (**Figure 6b**)	1.6	8.9	0.049	0.99	354.07***	0.69	0.976	312.27*
L. coryniformis with distilled lees (**Figure 6b**)	1.4	11.6	0.051	0.988	274.89***	0.58	0.980	121.42*
L. pentosus with no distilled lees (**Figure 6c**)	5.1	30.6	0.072	0.987	252.46***	0.98	0.983	360.26*
L. pentosus with distilled lees (**Figure 6c**)	7.4	64.2	0.086	0.990	322.45***	0.85	0.954	147.45*
L. plantarum with no distilled lees **Figure 6d**)	5.0	30.2	0.074	0.988	273.42***	0.62	0.978	347.54*
L. plantarum with distilled lees (**Figure 6d**)	7.6	64.8	0.084	0.988	273.68***	0.87	0.977	334.37*
L. rhamnosus with no distilled lees (*7*)	9.2	90.2	0.074	0.988	351.2***	0.81	0.969	149.0*
L. rhamnosus with distilled lees (*7*)	9.6	75.2	0.065	0.971	118.7**	0.82	0.971	269.0*

[a] P_0 = initial lactic acid concentration (g/L); P_{max} = maximum concentration of lactic acid (g/L); P_r = ratio between initial volumetric rate of product formation (r_p) and initial product concentration P_0 (h^{-1}); $Y_{P/S}$ = product yield (g/g); r^2 = determination coefficient; *F* value = *F* test statistical parameter; *, significance level >95%; **, significance level >97.5%; ***, significance level >99%.

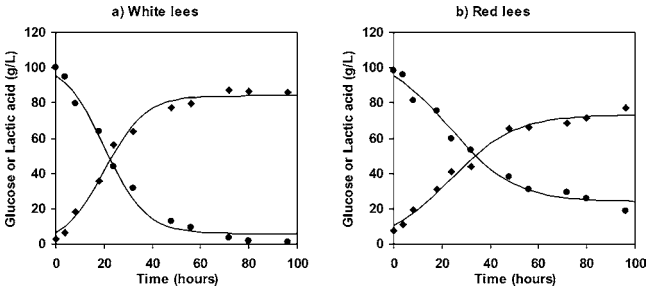

Figure 5. Experimental data and calculated time courses of lactic acid (◆) and glucose concentrations (●) during fermentations carried out with *L. pentosus* using (**a**) 20 g/L of white lees distilled and extracted with ethyl acetate and (**b**) 20 g/L of red lees distilled and extracted with ethyl acetate. Results represent the average of three independent experiments. Standard deviations were below 3.4% of the mean.

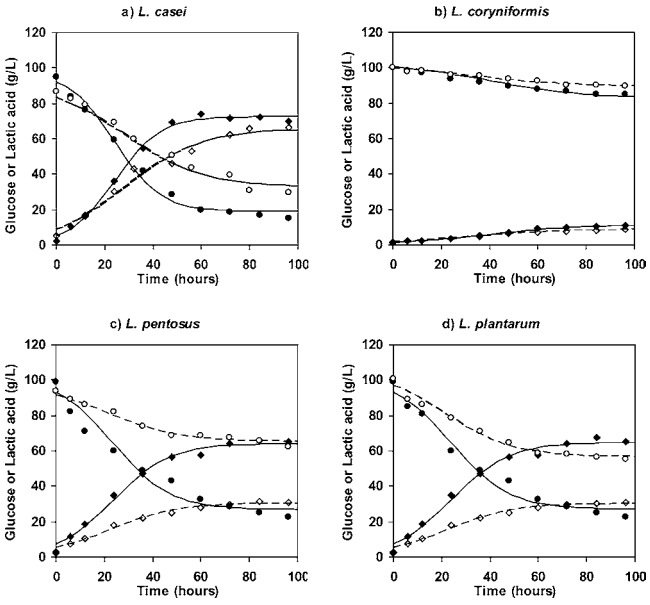

Figure 6. Experimental data and calculated time courses of lactic acid and glucose concentrations during fermentations carried out with 20 g/L of red lees (distilled or not) using (**a**) *L. casei*, (**b**) *L. coryniformis*, (**c**) *L. pentosus*, and (**d**) *L. plantarum*: glucose consumption using no distilled lees (○); glucose consumption using distilled lees (●); lactic acid production using no distilled lees (◇); lactic acid production using distilled lees (◆). Results represent the average of three independent experiments. Standard deviations were below 2.6% of the mean.

conclusion can be obtained by evaluating **Tables 1** and **3**, the maximum lactic acid concentrations being 84.7 g/L for the MRS broth and 84.1 g/L using the distilled white lees extracted with ethyl acetate.

Lees from Red Wine. Table 4 shows the kinetic parameters for lactic acid production and glucose consumption; meanwhile, **Figure 6** shows the kinetic pattern for lactic acid production using distilled and not distilled red lees, as well as the glucose consumed for all of the *Lactobacillus* strains considered. As above, it can be observed that the use of distilled lees showed a higher scope for lactic acid production. This can also be observed by comparing the P_r and P_{max} values listed in **Table 4**, which are always higher in fermentations carried out using distilled lees.

For red wine lees, the best results were achieved using distilled lees with *L. casei*, but in this case only 72.7 g of lactic acid/L was achieved (**Table 4**), in comparison with the 92.1 g/L obtained using white lees (see **Table 2**). These different behaviors of red and white lees could be due to the different compositions of the red and white wines. Whereas in white wines non-flavonoid phenolic components are basically present, the phenolic components of red wines are mainly flavonoid and anthocyanidin. In red wines, each anthocyanidin may be further complexed by acetic acid, coumaric acid, or caffeic acid,

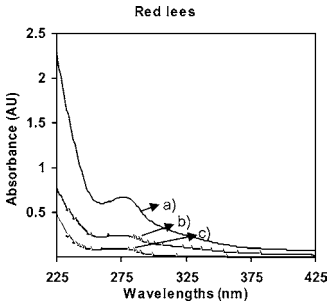

Figure 7. UV spectra of red lees: (**a**) no distilled; (**b**) distilled; (**c**) distilled and extracted with ethyl acetate.

bonding to the sugar component. Anthocyanin classification is based primarily on the position of the hydroxyl and methyl groups on the B ring of the anthocyanindin molecule. On this basis, grape anthocyanins are divided into five classes, namely, cyanins, delphinins, malvins, peonins, and petunins. The proportion and amount of each class vary widely among cultivars and growing conditions (*21*). Anthocyanins also are grouped on the number of sugar molecules per anthocyanidin. In most grape species, both mono- and diglucosidic anthocyanins are produced. In red grape varieties, anthocyanins tend to exist in loose complexes, either with themselves or with other compounds: flavonoids, phenols, hydroxycinnamoyl esters, and polyphenols (*22*). Various factors may lead to disruption of the anthocyanin complexes. For example, heating must destabilizes the structure (*6*). These phenolic components are in contact with lees, becoming part of their structure.

UV spectra of red wine lees are shown in **Figure 7**. Red lees showed a maximum absorbance at 279 nm, characteristic of soluble lignin. This peak of absorbance is not present in white lees. On the other hand, the amount of soluble lignin after distillation of lees decreased to 63.4% (**Figure 7a**,**b**) as happened with white lees. This can be due to the fact that during distillation, lees are submitted to high temperatures and the composition of the phenolic compounds varies owing to the disruption of the anthocyanin complexes, which produces changes in lees composition, that could be responsible for the improvement of the fermentation of *Lactobacillus* strains. The different behaviors observed in **Table 4** when distilled or not distilled lees were employed (particularly with *L. pentosus* and *L. plantarum*) could also be due to the differences detected by Bustos et al. (*7*) in the nitrogen percentage (distilled lees, 1.2%, and not distilled lees, 0.4%).

Finally, these lees were also extracted with ethyl acetate. The reduction in the relative size of absorbance at 279 nm observed in **Figure 7c** (85.8% with respect to not distilled lees) proves a selective removal of the phenolic compounds. The fermentation carried out using these detoxified lees with *L. pentosus* shows a slight increase in the fermentability (**Figure 5b** and **Table 3**), increasing the P_{max} from 64.2 to 73.3 g/L, although not enough to reach the 84.7 g/L obtained with the MRS broth. This fact, as well as the different maxima of absorbance detected in **Figures 4** and **7**, indicates the different natures of the phenolic compounds present in white or red lees, with more inhibitory capacity for the soluble lignin present in red lees.

Conclusions. When the costly MRS broth (which includes among other components yeast extract and peptone) was replaced with 20 g of vinification lees/L (a cheap nutrient obtained from wastes of wineries), the behaviors were different depending on the microorganism and the lees employed (distilled or not, coming from the red or white winemaking technology). In all cases distilled lees showed better performances due to some phenolic compounds changing their composition during the distillation, making the lees more appropriate for use as nutrients. In general, the results obtained using white lees were better than those obtained when red lees were used, probably because the soluble lignin released due to the skin fermentation with stem contact during the red wine production hindered slightly the fermentation, the inhibitory capacity being higher than that observed in the phenolic compounds present in the white lees.

The different *Lactobacillus* strains showed different behaviors, *L. casei* being the one with the better performance and similar to that achieved with *L. rhamnosus*. Both strains show similar peptidase activities (*19*). Using *L. pentosus* strains it was necessary to remove the remaining phenolic compounds by extraction with ethyl acetate to obtain high yields and high productivities of lactic acid. The UV spectra confirm the selective removal of these compounds, and the fermentation with *L. pentosus* can be carried out effectively, particularly with white lees. This is explained by taking into account the different phenolic compounds observed in the UV spectra of white and red lees, which is related to the different technologies used to produce red or white wines.

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