European Food Research and Technology (2022) 248:2497–2507
https://doi.org/10.1007/s00217-022-04064-1

ORIGINAL PAPER

Effect of acidification biotechnologies on the production of volatile compounds, lactic acid and colour in red wines after the use of pulsed light pretreatment in grapes

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Received: 24 March 2022 / Revised: 7 June 2022 / Accepted: 11 June 2022 / Published online: 14 July 2022

Abstract

The treatment of grapes with pulsed light is an emerging technique to reduce the populations of native microorganisms and thus implement fermentative biotechnologies in a more controlled manner with selected yeasts and bacteria. These biotechnologies can modify the profile of wines as the microbial populations thrive in different ways and produce different fermentative metabolites. The first objective of this research was to assess the use of pulsed light as a technology to reduce microbial population in grapes, and a second objective was to assess the viability of performing biological acidification in red wines with the use of *Lachancea thermotolerans* after the use of pulsed light. The treatment of grapes with pulsed light reduced the yeast and bacteria population 1.2 $\log_{10}$ CFU/mL and allowed the use of fermentative biotechnologies. Biological acidification with *Lachancea thermotolerans* produced more lactic acid than malolactic fermentation with *Oenococcus oeni*. Up to 4 g/L of lactic acid were reported in co-inoculations *of Lachancea thermotolerans* with *Saccharomyces cerevisiae*. On the other hand, the ester concentration, which provides floral and fruity aromas, was higher in wines that underwent malolactic fermentation. In this way, pulsed light treatment has produced wines with less aromatic volatiles and yet better perception of fruity and floral aromas as the lactic acid production was higher. Regarding colour, the wines were brighter and more intense due to the concentration of lactic acid although anthocyanins have decreased 9% and 18% in co-inoculation of *Lachancea thermotolerans* and *Saccharomyces cerevisiae* in wines with treated and untreated grapes, respectively. A deeper evaluation during large-scale winemaking is advised to assess pulsed light to reduce native microbiota before the implementation of acidification biotechnologies.

Keywords Non-thermal technology · Non-*Saccharomyces* · Red wine · Co-inoculation · Acidification · Freshness

Abbreviations

ADY	Active dry yeast
AF	Alcoholic fermentation
CI	Color intensity
FTIR	Fourier-transform infrared spectroscopy
HHP	High hydrostatic pressure
LAB	Lactic acid bacteria
LSD	Least significant difference
MLF	Malolactic fermentation
PCA	Principal component analysis
PEF	Pulsed electric fields
PL	Pulsed light
PMMA	Polymethyl methacrylate
TPI	Total polyphenol index
UHPH	Ultra-high-pressure homogenisation
UV	Ultraviolet
YEPD	Yeast extract, peptone, D(+)glucose

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Introduction

The fermentation biotechnology involved in winemaking is currently aiming towards producing wines with a more complex profile as they seek starter cultures to perform optimally in that specific way. Contrary to what happens in spontaneous fermentation, fermentative biotechnologies can control the quality and reliability of wine fermentations. These technological approaches may include starters for pure culture fermentation to stress the metabolic features of a single

strain, a practice that is widespread with the use of active dry yeast (ADY) of the species *Saccharomyces* spp. [1], and the increasing use of ADY of non-*Saccharomyces* yeasts. Another approach is the simultaneous fermentation or co-inoculation of two or more yeast strains that can thrive in synergy to improve the complexity of wines in parallel [2]. A third approach is the sequential fermentation which allows strains of first stages to express metabolically before another strain of higher fermentative power consumes residual sugars and carries on the fermentation [3].

One limitation on the successful implantation of these technologies during winemaking is the initial population of native microorganisms that avoid a correct growth of selected strains, or that prevents the expression of characteristic metabolic features. This is the case for some strains of the yeast species *Lachancea thermotolerans*. The production of lactic acid by this species, which helps improve the freshness in wines with a pleasant acidity [4], is inhibited in mixed fermentations with the presence of the apiculate species *Hanseniaspora vineae* [5]. On the other hand, *Hanseniaspora* spp. strains, as well as other non-*Saccharomyces* yeast species such as *Metschnikowia pulcherrima* and *Torulsapora delbrueckii*, are well known for their high enzymatic activity towards the production of aromatic esters [6]. This enzymatic activity, which contributes to increasing the complexity of the aromatic fraction, is greatly desired. Nonetheless, the competition of species during fermentation may endanger the performance of the selected culture yeasts. Therefore, and with the aim to reduce the initial microbial counts of native yeast and bacteria, the use of non-thermal emerging technologies is being tested to ensure the implantation of selected strains. Among the non-thermal technologies already tested for microbial reductions in grapes, the following are highlighted: high hydrostatic pressure (HHP) that allows the reduction of the use of SO_2 as an antiseptic additive for the preservation of wines [7]; ultra-high-pressure homogenisation (UHPH) able to eliminate viable cells of native microbiota [8] and also to prevent the proliferation of spoilage yeasts such as the species *Brettanomyces* spp. [9]; pulsed electric fields (PEF) which affect the cell wall structures and may increase the extraction of polyphenolic compounds on one hand [10], while also reducing the population of spoilage microorganisms on the other hand [11]. Another non-thermal technology rarely used in winemaking is the pulsed light (PL), which has been largely used in other food matrices, such as fresh fruits and vegetables, dairy products and meat [12–14] to improve the safety and shelf life of food products. The use of PL for winemaking purposes is proposed as a treatment for grapes after selection and before crushing [15].

This article summarises the findings after carrying out three fermentative biotechnologies for the biologic acidification of red wines produced with untreated grapes and grapes treated with PL.

Materials and methods

Yeast strains and growing media

The yeast strains used in this experimental setup were all isolated in the Food Technology Laboratory at the School of Agronomic, Food and Biosystems Engineering (Universidad Politécnica de Madrid). The species used were two non-*Saccharomyces* yeast strains *(Lachancea thermotolerans* (Lt) strain L3.1, and *Hanseniaspora opuntiae* (Op) strain A56, and the species *Saccharomyces cerevisiae* strain 7VA. The lactic acid bacteria (LAB) used for malolactic fermentation was a strain of the species *Oenoccocus oeni* Enoferm Alpha™ (Lallemand Bio, Madrid, Spain).

Since the initial microbial counts found on the grapes were below 1 $\log_{10}$ CFU/mL, yeasts were sprayed over the grapes to increase the initial population (Table 1). This pretreatment would simulate the yeast population typically found in the pruina of harvested grapes [16]. The treatment with pulsed light described in Sect. 2.2 was performed after this conditioning.

For the fermentation trails, strains from the species *Saccharomyces cerevisiae* 7VA and *Lachancea thermotolerans* L3.1 were used. These two yeast strains were grown for 24 h in liquid YEPD media at constant 24 °C to reach a population of 8.3 $\log_{10}$ CFU/mL and 8.4 $\log_{10}$ CFU/mL, respectively. The liquid medium was prepared by mixing 1% yeast extract (Laboratorios Conda; Madrid, Spain), 2% bacteriological peptone (Laboratorios Conda; Madrid, Spain), and 2% D(+)-glucose anhydrous (Panreac Química; Barcelona,

Table 1 Description of the addition of *H. opuntiae* and *S. cerevisiae* over the grapes to increase the initial population of yeasts before the PL treatment

Strain ID	Yeast species	Inoculum volume[a] (mL)	Inoculum population (CFU/mL)[b]	Population in must[2] (CFU/mL)
A56	*Hanseniaspora opuntiae*	10	8.4 $\log_{10}$	7 $\log_{10}$
7VA	*Saccharomyces cerevisiae*	5	8.2 $\log_{10}$	5.8 $\log_{10}$

[a]The volume used for the fermentation trials with the population described in the colum "Inoculum population"

[b]Measured in 1 mL of the inoculum; [2]Measured in 1 mL of must after the inoculation of A56 and 7VA

Spain). The growing medium was autoclaved for 15 min at 120 °C. The LAB were rehydrated in water free of chlorine at 20 °C for 15 min prior to inoculation, in accordance with the instructions for use given by the manufacturer. The population achieved for LAB was 9.9 $\log_{10}$ CFU/mL.

Pulsed light treatment

The PL treatment was applied to one set of non-immobilised grapes to reduce the blind spots created by shadows as a consequence of the geometry of the berries. It is also a way to emulate a continuous PL treatment for future investigations in this regard. The treatment consisted of a series of 120 pulses applied on destemmed berries placed on a two-layer arrangement in plastic trays. Every 40 pulses, the tray was removed from the chamber to reorganise the berries randomly without touching them directly. This step simulates the rotation that the berries are subjected to on a conveyor. The trays have the following dimensions: 13 cm × 20 cm × 5 cm. The PL apparatus (Claranor, Avignon, France) consists of a treatment chamber with a double xenon lamp with a flash width of 0.2–2 ms. The distance between the berries and the lamps was adjusted to 7 cm. The applied pulses reached 1 MW per flash, which translates to a maximum fluence of 30 J/cm^2. After the treatment, the trays were placed inside a laminar flow hood, Aeolus V (Telstar, Madrid, Spain), to carry on the microbial counts in an aseptic environment.

Must fermentation

Two sets of *Vitis vinifera* L. cv. Tempranillo grapes were crushed under sterile conditions inside a laminar flow hood. The first set corresponded to the untreated grapes, while the second corresponded to the PL-treated grapes. Both musts were left for 1 h with the skins, which were then removed from the juice. This maceration time intended to increase the concentration of phenolics and anthocyanins, but to limit the proliferation of microbial counts in the same time. The juice of each of the two musts was then divided into 9 flasks, 18 in total. The fermenters were 250 mL volume brown glass flasks filled with 220 mL to leave a headspace of 30 mL on top. The musts had a specific gravity of 1105 and pH values of 4.21 and 4.23 for untreated and treated grapes, respectively. The potential alcohol by volume expected in these musts was approximately 14.5% v/v. Microbiological analysis showed an initial population of 7.1 $\log_{10}$ CFU/mL and 5.8 $\log_{10}$ CFU/mL for inoculated *Saccharomyces* and non-*Saccharomyces* yeasts for the control must and the survival population after the PL treatment, respectively (see Table 1).

The experiment comprised three fermentative scenarios with treated grapes and the same three scenarios with untreated grapes. The fermentative scenarios were F1) alcoholic fermentation (AF) with *S. cerevisiae* and sequential malolactic fermentation (MLF) with *O. oeni*; F2) simultaneous AF and MLF with *S. cerevisiae* co-inoculated with *O. oeni*; and F3) co-inoculation of *S. cerevisiae* and *L. thermotolerans* with no MLF (see Fig. 1). The fermenters were closed with the use of Müller valves and they were placed at steady 20 °C right after the initial weight was recorded. Each fermentation scenario was carried out in triplicate. In scenario F1, the inoculum of *O. oeni* was added to the fermenters after the eighth day. A similar volume of sterile YEPD media was added to all the fermenters of scenarios F2 and F3 to keep the volume constant in all three fermentative scenarios. The evolution of the fermentations was followed-up until no changes in weight were observed. The fermentation lasted 24 days.

Oenological parameters

A Fourier-transform infrared spectroscopy (FTIR) equipment, OenoFoss™ (FOSS Iberia, Barcelona, Spain), was used to characterise glucose, fructose, gluconic acid, and nitrogen compounds in musts. One millilitre was needed for the analysis. The ethanol, glucose, fructose, and volatile acidity were determined in the finished wines. Malic acid and lactic acid were determined with an enzymatic analyser Y25 (Biosystems, Barcelona, Spain). Ethanol is expressed as % v/v, sugars and nitrogen compounds are expressed as g/L, and all organic acids are expressed as tartaric acid equivalents in g/L. The pH was determined in musts and finished wines using a GLP 21 Crison Instruments (Hach Lange Spain, S.L.U., Madrid, Spain). All samples were previously stirred with a vortex mixer to release the trapped CO_2 to avoid any errors in the measurements.

Major volatile compounds analysis

The volatile compounds were determined with the use of a gas chromatograph with a flame ionisation detector (GC-FID). The chromatograph is an Agilent Technologies™ 6850 (Palo Alto, CA, USA) with a column DB-624 (60 m × 250 µm × 1.4 µm). The injector's temperature was set at 250 °C and the detector's temperature was 300 °C. Finally, the temperature went from 40 °C for 5 min to 250 °C with a gradient of 10 °C /min and was maintained for 5 min. Hydrogen was used as the carrying gas with a flow of 2.2 L/min and the split ratio set at 1:10. The identification and the quantification of volatile organic compounds were performed with calibration curves for each of the following compounds (R^2): 2-phenylethyl acetate (0.99460), 2-phenylethanol (0.99933), ethyl acetate (0.99980), isobutyl acetate (0.99987), ethyl butyrate (0.99987), isoamyl acetate (PubChem CID: 31,276), acetaldehyde (0.99973), methanol (0.99917), 1-propanol (0.99938), diacetyl (0.99977),

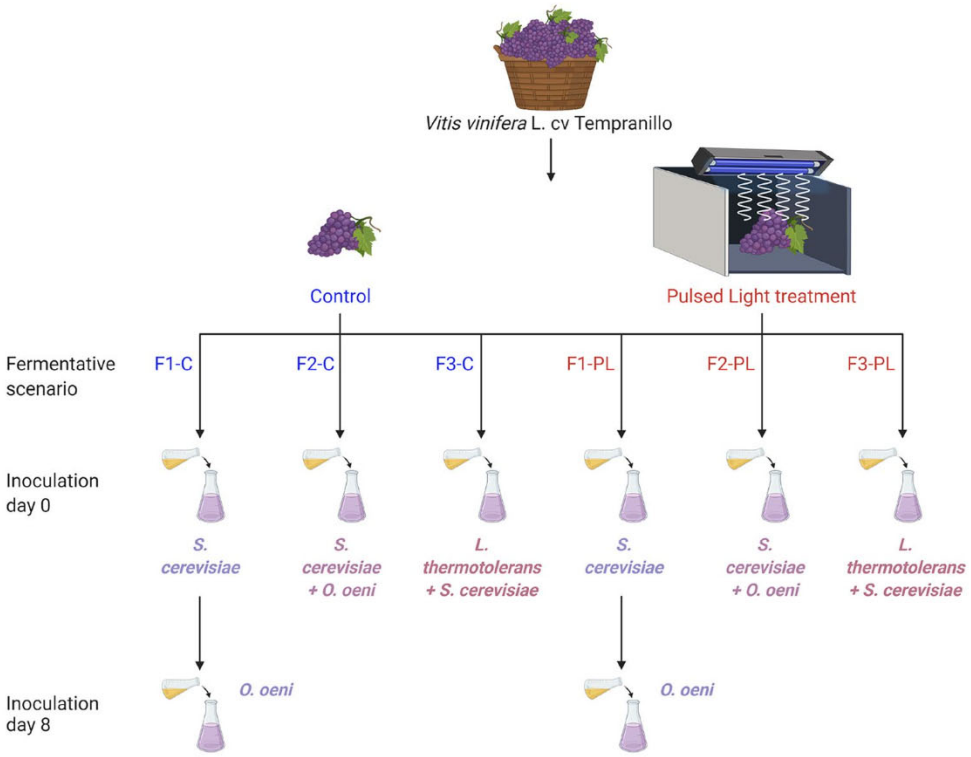

Fig. 1 Experimental setup for the fermentation of must from PL treatment on destemmed grapes

1-butanol (0.99932), 2-butanol (0.9954), isobutanol (0.99941), acetoin (0.99949), 2-methyl-1-butanol (0.99925), 3-methyl-1-butanol (0.99958), ethyl lactate (0.99916), 2,3-butanediol (0.99109), 1-hexanol (0.99935). Additionally, 100 µL of 4-methyl-2-pentanol (500 mg/L) were used as an internal standard in accordance with a procedure previously described [17].

Anthocyanins characterisation

The anthocyanins were determined with a liquid chromatograph Agilent Technologies™ 1100 (Palo Alto, CA, USA) with a column RP Kinetex C18 (100 × 4.6 mm; 2.6 µm) (Phenomenex, Torrance, CA, USA) and a diode array detector. This chromatographic technique was used to identify and characterise the following pigments: anthocyanins, both acylated and non-acylated; pyranoanthocyanins, including vitisins and vinylphenolic anthocyanins; and polymeric pigments. Two solvents were used: solvent A (water/formic acid 95:5 v/v) and solvent B (methanol/formic acid 95:5 v/v) with a gradient of 0–2 min, 85% A (working flow 0.8 mL/min); 2–10 min, 85–50% A linear; 10–12 min, 50% A; 12–13 min, 50–85% A linear; and 13–15 min until steady-state or re-equilibration. Malvidin-3-O-glucoside (Merck Life, Madrid, Spain) has been used as an external standard for the quantification of all pigments at a wavelength of 525 nm. The wavelength of maximum absorbance was used to identify the anthocyanins [18, 19]. The anthocyanins identified with this technique are: Delphinidin-3-O-glucoside (D3G), Cyanidin-3-O-glucoside (C3G), Petunidin-3-O-glucoside (Pn3G), Peonidin-3-O-glucoside (Pt3G), Malvidin-3-O-glucoside (M3G) grouped under the label non-acylated anthocyanins; Malvidin-3-O-glucoside-acetaldehyde adduct (vitisin B, Vit B), Malvidin-3-O-glucoside-acetic acid adduct (vitisin A, Vit A), Malvidin-3-O-glucoside-4-vinylphenol (M3GVPh) and Malvidin-3-O-glucoside-4-vinylguaiacol (M3GVg) grouped under the label pyranothanocyanins; Delphinidin-3-O-(6″-acetylglucoside) (D3GAc), Cyanidin-3-O-(6″-acetylglucoside) (C3GAc), Petunidin-3-O-(6″-acetylglucoside) (Pt3GAc), Malvidin-3-O-(6″-acetylglucoside) (M3GAc) and Malvidin-3-O-(6″-*p*-coumaroylglucoside) (M3GCm) grouped under the label acylated anthocyanins. The detection limit has been set to 0.1 mg/L [20].

Colour assessment

The colour of the wines was determined with a UV–Visible spectrophotometer 8453 from Agilent Technologies™ (Palo Alto, CA, USA) and a DNA Phone Smart Analysis (Biosystems, Barcelona, Spain) for wine. The spectrophotometer has a photodiode array detector and uses a 1 mm path length quartz cuvette for the determination. A discrete wavelength mode was selected to acquire the absorbance at 420, 520, and 620 nm. The colour intensity is expressed as the sum of the absorbance of all three wavelengths multiplied by a

dilution factor of 10, and the hue is expressed as the quotient from dividing the absorbance at 420 nm by the absorbance at 520 nm [21]. The colour representation obtained with the DNA Phone is given in CIELab coordinates, which assess the luminosity or lightness (L), the green–red (a) and the blue-yellow (b) components, and CIELCh$_{uv}$ cylindrical coordinates that compare chroma (C) and hue (h). The samples were placed in a 1 cm path length polymethyl methacrylate (PMMA) cuvette. No sample preparation was needed to perform these two analyses.

Sensory evaluation

The analysis done for the sensory evaluation of the different wines obtained in this experiment was assessed with a panel of ten wine-tasting experts. The sensory evaluation was performed at the Chemistry and Food Technology Department of the School of Agricultural, Food and Biosystems Engineering (ETSIAAB) at Universidad Politécnica de Madrid (Spain). The panel was composed of five females and five males whose ages ranged from 25 to 55. The panel evaluated 15 basic wine descriptive attributes agreed upon by consensus earlier, two of which assessed the quality of the wines. The parameters were rated on a five-point scale from low perception (1) to high perception (5). The hue was rated on a separate scale from red (1) to orange (5). The descriptive attributes shown in the sensory evaluation form were evaluated in three distinctive sections: appearance (the intensity of the colour, the hue, and the transparency); aroma (intensity of the aroma, quality of the aroma, flowers, herbs, fruitiness, as well as reduction and oxidation notes); mouth (general acidity, astringency, body, bitterness); and a final general overall note. The pour size used for this tasting was 60 mL (approximately 2 oz). The results were treated with statistical analysis, and the average values were plotted in a radar chart.

Statistical analysis

Multivariate analysis was used to describe the differences observed in the wines produced, although the data is not extensive. All samples were compared to assess the production of lactic acid by the different fermentative media, and also to elucidate the profile of the wines produced by each fermentative condition. The means and the standard deviations were calculated, and the differences were examined using one-way ANOVA and the least significant difference (LSD) test. A principal component analysis (PCA) was obtained with the average concentration of oenological parameters, aromas, and pigments to illustrate the composition of wines produced in each fermentative condition. The calculations previously mentioned were made using PC Statgraphics v.XI software (Graphics Software Systems, Rockville, MD, USA). The significance has been set at $p < 0.05$.

Results and discussion

Microbial populations

The use of PL as a treatment for the reduction of native microbiota on grapes for winemaking is uncommon. The effect of the PL treatments on the reduction of initial populations found on the pruina of the grapes in this experiment was not powerful enough to eliminate the yeast strains in comparison to other non-thermal technologies such as ultra-high pressure homogenization (UHPH) [22]. As a matter of fact, the reduction observed on the sprayed yeast after the treatment was 1.2 $\log_{10}$ CFU/mL. Nonetheless, this result is comparable to what has been achieved by authors on other food matrices, such as spinach leaves and fresh-cut lettuce [14, 23], but the reduction was lower than what was observed on tomato fruit [24]. The PL treatments have greater results on the reduction of bacteria on foodstuff than reducing moulds and yeasts, as observed in avocado, watermelon and mushrooms [25]. One reason why this treatment is not as powerful may be that UV photons used in PL are less energetic than other photons [26] and they may also reduce their energy when the must is released from the grapes under treatment [27].

Regarding the energy dose used in this experiment, the density produced by the pulses was 0.8 J/cm^2, similar to the total fluence used in spinach leaves to reduce populations of *Listeria innocua* and *Escherichia coli* [23], but considerably lower than total fluences of 2.2 J/cm^2 and 4 J/cm^2 used for the reduction of *Saccharomyces cerevisiae* and moulds/yeasts in tomato and fresh-cut tomatoes, respectively [12, 24]. Besides the fluence produced in the PL chamber, the effectiveness of the pulses might also be limited by the amount of must released from the grapes during the treatment, as the grapes were destemmed, and shadows were created by the geometry of the berries, as geometry is important for the efficiency of PL treatments [28].

In terms of the effectiveness of the treatment on the fermentation of the Tempranillo must, there are no significant differences in the populations observed between treated and untreated grapes for the span of the experiment, except for the total counts found at day 0. At day 0, the populations were larger in all untreated musts due to the reduction of counts produced in the treated grapes. From this time on, all musts behaved similarly. In fermentative scenarios F1 and F2, the bacterial counts remained constant at ~ 7 $\log_{10}$ CFU/ml since day 0 in the first case, and from day 8 in the second. In these two scenarios, the population of *S. cerevisiae* decreased to ca. 4.4 $\log_{10}$ CFU/ml after the fourth

day. Fermentative scenario F3 did not show any significant difference in the population of *L. thermotolerans* at any time of the fermentation. All these results suggest that the microbial populations had similar development over the different stages, and the behaviour was similar for treated and untreated grapes for each fermentative scenario.

Wine composition

As shown in Table 2, the composition of the finished wines has no significant statistical differences in terms of volatile acidity, and although all wines are dry and have less than 2.1 g/L total sugars, the wines produced in scenario F3 had consumed the largest proportion of the sugars available in the must. The volume of ethanol reached (% v/v) is between 13 and 14.4%. This parameter is significantly higher in the fermentations with treated grapes, except for scenario F3, which obtained similar values to those with untreated grapes. This last result is in line with the fact during the early stages of fermentation, part of the sugars is consumed by non-*Saccharomyces* yeasts with lower fermentative power, whose populations are higher at the start of fermentation in untreated grapes. This slightly reduces the content of ethanol in finished wines [29]. Lastly, the main differences among fermentative treatments are visible in the pH values, the lactic acid produced, and the total acidity expressed as tartaric acid (g/L). As expected, the fermentations where strain 3.1 of the species *Lachancea thermotolerans* was used produced the highest concentration of lactic acid, up to 6 g/L, the lowest pH values (3.4), and the highest concentration of total acids with more than 8 g/L. The differences are significant between fermentative scenarios, but also between PL treatments. In this last matter, the samples that had PL treatment prior to the inoculation of Lt strain 3.1 stood out from those untreated trials. Fermentative wise, it has been observed that the strain L3.1 reduces the production of lactic acid when in the presence of other non-*Saccharomyces* yeast strains, especially with the apiculate genus *Hanseniaspora* spp. This phenomenon is stronger in the species *H. vineae* [5]. Therefore, the reduction of microbial counts with the PL treatment may lead to a less competitive fermentative environment for the *L. thermotolerans* to predominate, avoid inhibition, and increase the production of lactic acid.

Fermentative volatile compounds

Besides the differences observed in the oenological parameters, the wines produced also showed variations in the fermentative volatile compounds in accordance with the microbial populations thriving in the different fermentative scenarios. In this way, the untreated grapes had produced larger amounts of ethyl esters and acetate esters than their counterpart scenarios with treated grapes (see Fig. 1). This observation has been reported in either mixed or sequential inoculations of *H. vineae* and *S. cerevisiae* for the production of icewine with more floral and fruity aromas [30]. A larger initial population of apiculate *H. opuntiae* in untreated grapes may be responsible for this accumulation of aromatic metabolites. This metabolic characteristic is present in all species of the genus *Hanseniaspora* spp., and it is related to a high enzymatic activity that leads not only to the production of aroma compounds but also to a reduction of the concentration of higher alcohols [31]. This last feature was not noticeable in this experiment, since the concentration of higher alcohols is similar for the counterpart scenarios with treated and untreated grapes. Despite having a reduced amount of esters in the scenarios with treated grapes, the concentration achieved in these wines is above the threshold; therefore, it is expected for these wines to contribute to the aromatic complexity as well. For instance, the lowest concentration of ethyl acetate yielded in fermentative scenario F3-PL (12.1 mg/L—data not shown) can provide pear-like or banana-like aromas [32]. This ester, which odour threshold is 7.5 mg/L [33], is more abundant in wines that underwent MLF (scenarios F1 and F2) since *S. cerevisiae* strains have been shown to have alcohol acetyl transferase activity encoded by the *ATF1* gene [34]. Lastly, the concentration of carbonyl compounds, comprising acetaldehyde,

Table 2 Chemical composition of finished wines from treated and untreated grapes

ID	Ethanol (% v/v)	Glucose/Fructose (mg/L)	pH[1]	Total Acidity (mg/L)	Malic acid[2] (mg/L)	Lactic acid[2] (mg/L)	Volatile acidity[3] (mg/L)
F1-C	13.7 ± 0.1*b*	1.9 ± 0.3*a*	4.1 ± 0.0*a*	4.2 ± 0.3*c*	0.1 ± 0.1*b*	1.7 ± 0.2*bc*	0.2 ± 0.0*a*
F2-C	13.7 ± 0.1*b*	1.7 ± 0.2*ab*	4.1 ± 0.0*a*	4.1 ± 0.3*c*	0.1 ± 0.1*b*	1.4 ± 0.2*c*	0.2 ± 0.0*a*
F3-C	13.2 ± 0.3*c*	1.4 ± 0.3*c*	3.6 ± 0.1*b*	6.1 ± 0.6*b*	1.3 ± 0.4*a*	2.6 ± 1.3*b*	0.3 ± 0.0*a*
F1-PL	14.4 ± 0.1*a*	2.0 ± 0.1*a*	4.1 ± 0.0*a*	4.3 ± 0.1*c*	0.2 ± 0.1*b*	1.6 ± 0.0*bc*	0.2 ± 0.0*a*
F2-PL	14.2 ± 0.2*a*	1.9 ± 0.1*a*	4.2 ± 0.0*a*	4.1 ± 0.2*c*	0.1 ± 0.1*b*	1.3 ± 0.0*c*	0.2 ± 0.0*a*
F3-PL	13.7 ± 0.3*b*	1.3 ± 0.2*bc*	3.5 ± 0.1*b*	7.4 ± 0.8*a*	1.4 ± 0.1*a*	4.2 ± 0.3*a*	0.2 ± 0.1*a*

[1]pH electrode; [2]enzymatic analyser; [3]expressed as acetic acid

Average and standard deviation; $n = 3$. Different letters indicate statistical differences ($p < 0.05$) between treatments of each fermentative scenario

acetoin, diacetyl and mainly 2,3-butanediol, is more frequently produced in the wines with treated grapes than their fermentative counterparts. The main contribution to this group of compounds is given by the 2,3-butanediol, which is produced in large quantity by *S. cerevisiae* from the reduction of acetoin by yeast metabolism [35]. The concentration of 2,3-butanediol yielded in the wines from treated grapes ranged from 785 to 954 mg/L. Apiculate yeasts, mainly present in early fermentative yeasts and more abundant in untreated grapes, yield concentrations of 2,3-butanediol between 54 and 221 mg/L in pure fermentations [35]. In this experimental setup, the difference in the concentration of this volatile molecule found in wines with untreated grapes goes from −20 to −25% with the respective treated counterpart. Due to the contribution of this last volatile compound, whose wine-tasting descriptor can be perceived as creamy or oily on the palate, the concentration of total volatile compounds is also higher in the wines with treated grapes.

Anthocyanins characterisation

The PL may induce damage to the cellular structures located at the surface of the grape's skin. This damage is due to the photophysical effects, which produce the disruption of the vacuoles, among other structures, with the possible freeing of anthocyanins [36]. This phenomenon has not been reported as significant, although some minor changes in colour intensity have been described in previous experiments [15]. The reason why this technology does not increase pigment extraction considerably may be that PL has a low penetration depth in comparison to other technologies [37]. This characteristic can be reduced even further when liquid must is released from the grapes, since the propagation of the photons is less effective in liquid media [38]. The structural damage is then expected to be limited. Nonetheless, and despite this evidence, there is a tendency to have more pigments in wines from treated grapes when comparing treatments from the same fermentative scenarios (see Fig. 2). The effect has no statistical significance, and thus, it cannot be considered decisive.

Having said that, if the fermentative scenarios are considered, it is evident that scenario F3 has retained the least concentration of anthocyanins after fermentation, and the difference is significant when compared to scenarios F1 and F2. It is important to note that scenario F3 carried out a co-inoculation of *L. thermotolerans* with *S. cerevisiae* and did not undergo MLF. Different yeast strains behave differently towards anthocyanin reduction due to the interactions between the microbial wall and the molecules of anthocyanins [39]. In this way, the populations developed in fermentative scenario F3 interacted more with the anthocyanins in solution during fermentation, and thus, the concentration of pigments was reduced considerably. The effect was more intense in the wines produced with untreated grapes, where the initial inoculated populations increased the viable cells and the interactions between cell walls and anthocyanins. The reduction of anthocyanins in scenario F3-C reaches 18%, while the reduction is around 9% for scenario F3-PL. This interaction seemed less intense in scenario F1, followed by scenario F2, where LAB were used in co-inoculation and sequential fermentation, respectively. The use of PL reduces the chances of losing colouring matter as the initial yeast population decreases.

Colour assessment

The effect of the PL treatments on grapes is also evident in the colour intensity and the total polyphenol index values. Both parameters have consistently higher values on the wines elaborated with treated grapes (see Table 3) than their untreated counterparts. Analysing in more detail, it may be inferred that there was a higher polyphenol extraction with

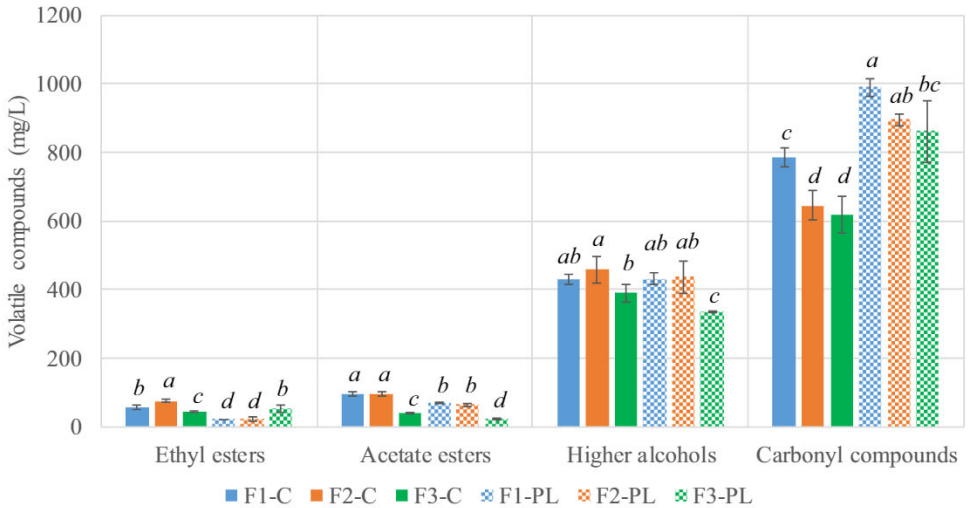

Fig. 2 The volatile fraction of major fermentative metabolites by GC-FID. Different letters indicate a significant difference between means ($p < 0.05$)

Table 3 Total polyphenol index (TPI), colour intensity (CI), tonality, CIE*Ch* and CIE*Lab* coordinates of all finished wines

ID	TPI	CI	Tonality	Chroma	Hue (°)	L	a	b
F1-C	34.8 ± 0.5*d*	7.1 ± 0.2*c*	0.9 ± 0.0*a*	63.0 ± 0.8*ab*	32.3 ± 0.7*b*	27.0 ± 1.2*a*	53.1 ± 0.3*ab*	33.8 ± 1.0*ab*
F2-C	35.1 ± 0.4 *cd*	7.2 ± 0.4*c*	0.9 ± 0.0*a*	61.6 ± 1.4*b*	32.9 ± 0.3*ab*	25.8 ± 0.2*a*	51.9 ± 0.9*b*	33.3 ± 1.1*bc*
F3-C	32.5 ± 0.8*e*	7.6 ± 0.2*c*	0.8 ± 0.0*b*	65.0 ± 2.2*a*	33.6 ± 0.9*a*	26.0 ± 0.5*a*	54.1 ± 1.3*a*	36.1 ± 2.0*a*
F1-PL	37.8 ± 0.6*b*	8.2 ± 0.3*b*	0.9 ± 0.0*a*	58.7 ± 1.5*c*	33.4 ± 0.3*a*	21.1 ± 0.5*b*	49.1 ± 1.5*c*	32.2 ± 0.6*bc*
F2-PL	39.3 ± 1.2*a*	8.7 ± 0.1*b*	0.9 ± 0.0*a*	54.9 ± 1.4*d*	32.1 ± 0.6*b*	18.6 ± 1.0*c*	46.5 ± 0.9*d*	29.1 ± 1.2*d*
F3-PL	36.2 ± 0.4*c*	9.5 ± 0.2*a*	0.7 ± 0.0*c*	57.9 ± 1.6 *cd*	32.1 ± 0.8*b*	18.9 ± 1.0*c*	49.1 ± 1.1*c*	30.7 ± 1.3 *cd*

$CI = \lambda_{420} + \lambda_{520} + \lambda_{620}$; $Tonality = \lambda_{420}/\lambda s_{520}$

Average and standard deviation; $n = 3$. Different letters indicate statistical differences ($p < 0.05$) between treatments of each fermentative scenario

the PL. The pigment concentration was higher in wines where *L. thermotolerans* was not used and slightly higher, with no significant difference, in the three fermentative scenarios using treated grapes. The extraction is probably due to modifications caused on cellular structures, such as the vacuoles containing anthocyanins, as photophysical changes occur as a consequence of the irradiation with PL [40]. This increment corresponds to the increase got in the absorbance measured at 520 nm, which indicates the presence of anthocyanins that absorb in the red range of the visible spectrum. The increment in CI also has an impact on the TPI, which is, as expected, also higher in the wines produced with treated grapes. Finally, the tonality expressed as the ratio between the absorbance of yellow (λ_{420}) divided by the absorbance of red (λ_{520}) has no statistical differences in the fermentative scenarios F1 and F2, where *O. oeni* was inoculated regardless of the use of treated or untreated grapes. Significant differences appeared when *L. thermotolerans* was used in fermentative scenario F3. Both assays, with treated and untreated grapes, had a lower hue; the wine with treated grapes (F3-LP) had the lowest value of all. This can be explained by the lactic acid accumulated in these wines decreasing the pH value to a higher extent, which has increased the quantity of anthocyanins in the ionised conformation or flavylium ions [41]. The higher the quantity of ionised anthocyanins, the more reddish a hue the wines would have. As per the CIE*Lab* and CIECh values, the vinifications produced with untreated grapes had higher values of chroma, *a* and *b* which relates to the colour and the saturation. On the other hand, treated grapes had lower luminosity values (L) which correlates with higher colour intensity (CI).

Wine sensory evaluation

To determine whether the differences observed through the analytical analyses can be perceived by consumers, the wines were submitted to a sensory evaluation. Figure 3 summarises the results obtained from the winetasting. Attributes such as aromatic intensity, herbaceous and floral notes, reduction, oxidation, and sweetness did not have any significant statistical differences. On the other hand, the parameters aromatic quality, fruity, bitterness, acidity, colour intensity, hue, and global perception yielded statistical differences. The wines produced in scenario F3 with *L. thermotolerans* were identified as wines with fruity aroma and better aroma quality, higher acidity, higher colour intensity, and better global perception. In particular, as previously seen in the colour assessment, wines produced in scenario F3 with treated grapes were perceived with a lower hue (reddish appearance), the highest acidity, and the best aroma quality, with fruiter scents. These two wines were perceived as fresher, as the attributes of acidity and fruitiness contribute

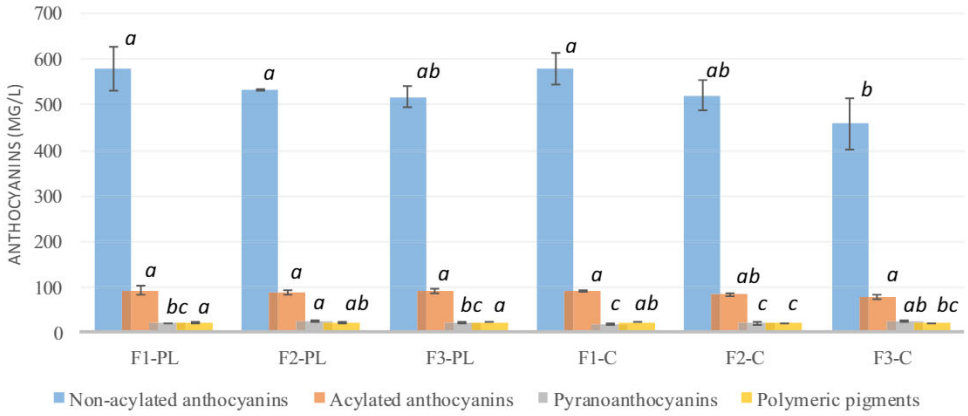

Fig. 3 Comparison of pigment content determined with HPLC–DAD and grouped by anthocyanin families. Different letters indicate a significant difference between means ($p < 0.05$)

to enhancing this characteristic, which is usually appreciated in white wines [42]. Freshness related to acidity is less common in red wines as it is in white wines, and it is difficult to achieve through MLF. At the same time, the wines elaborated with scenarios F1 and F2 produced wines with a flatter aroma, with more bitterness, and with a more yellowish tone as well. Nonetheless, and despite these results, the market's acceptance of this type of red wines, fresher than current commercially available wines, is yet to be assessed (Fig. 4).

Conclusion

The effect of PL on the reduction of native microbial populations found in grapes is not as noticeable as with the use of other non-thermal technologies, such as UHPH or PEF. Nonetheless, the pretreatment of musts with PL, even at low energy doses, can reduce the yeast populations enough to allow inoculated strains to thrive in such a way as to positively contribute to the overall perception of wines. The co-inoculation of *Lachancea thermotolerans* and *Saccharomyces cerevisiae* stands out from the rest of the fermentative biotechnologies in this matter. The reduction of native yeast populations allowed the production of higher concentrations of lactic acid. The concentration of lactic acid increased the freshness of wines which also increased the perception of floral and fruity aroma, although the concentration of volatile compounds responsible for floral and fruity aromas is slightly lower than the untreated counterpart wines. The fermentation of treated grapes also increased the amount of 2,3-butanediol, which enhanced the palate and balanced the lactic acid produced largely by *L. thermotolerans*. Lastly, biological acidification also improved the colour intensity in fermentations where the quantity of anthocyanins is reduced after their interaction with the lees cell walls. It is important to guarantee this effect in treatments with moving grapes in selection tables, as this may be the ideal time to treat the grapes during winemaking before the grapes are crushed and the tanks filled.

Chemical compounds Delphinidin-3-O-glucoside (PubChem CID: 443650), Cyanidin-3-O-glucoside (PubChem CID: 441667), Petunidin-3-O-glucoside (PubChem CID: 443651), Peonidin-3-O-glucoside (PubChem CID: 443654), Malvidin-3-O-glucoside (PubChem CID: 443652), Malvidin-3-O-glucoside-acetaldehyde adduct (vitisin B, Vit B) (PubChem CID: 16138152), Delphinidin-3-O-(6″-acetylglucoside) (PubChem CID: 15385440), Cyanidin-3-O-(6″-acetylglucoside) (PubChem CID: 15714477), Petunidin-3-O-(6″-acetylglucoside) (PubChem CID: 44256961), Malvidin-3-O-(6″-acetylglucoside) (PubChem CID: 74977116), Malvidin-3-O-(6″-p-coumaroylglucoside)

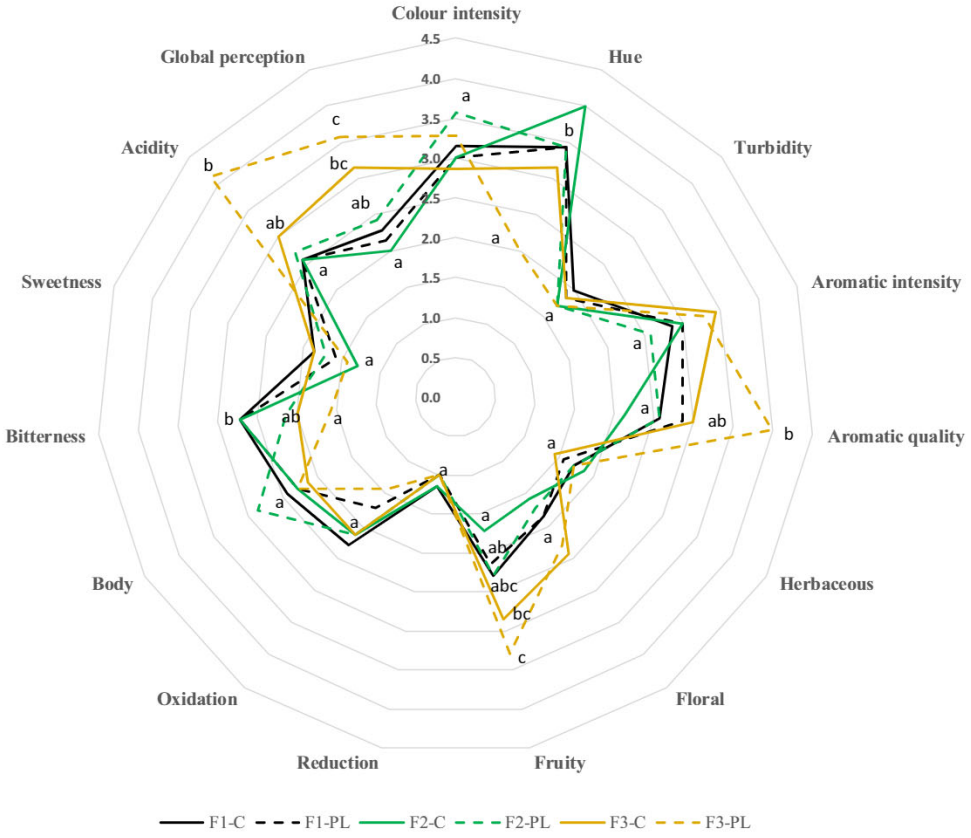

Fig. 4 Two-dimensional star plot of wine-tasting descriptors of a 60 mL pour size. Different letters indicate a significant difference between means ($p < 0.05$)

(PubChem CID: 71308234), Malvidin-3-O-glucoside-4-vinylphenol (PubChem CID: 44257035), Malvidin-3-O-glucoside-4-vinylguaiacol (PubChem CID: 44257037), 2-Phenylethyl acetate (PubChem CID: 7654), 2-Phenylethanol (PubChem CID: 6054), Ethyl acetate (PubChem CID: 8857), Isobutyl acetate (PubChem CID: 8038), Ethyl butyrate (PubChem CID: 7762), Isoamyl acetate (PubChem CID: 31276), Acetaldehyde (PubChem CID: 177), Methanol (PubChem CID: 887), 1-Propanol (PubChem CID: 1031), Diacetyl (PubChem CID: 650), 1-Butanol (PubChem CID: 263), 2-Butanol (PubChem CID: 6568), Isobutanol (PubChem CID: 6560), Acetoin (PubChem CID: 179), 2-Methyl-1-butanol (PubChem CID: 8723), 3-Methyl-1-butanol (PubChem CID: 31260), Ethyl lactate (PubChem CID: 7344), 2,3-Butanediol (PubChem CID: 262), 1-Hexanol (PubChem CID: 8103)

Author contributions Conceptualization: CE, CL. Methodology: IL. Formal analysis and investigation: CE, CV, IL. Writing—original draft preparation: CE. Writing—review and editing: CV, CL, IL. Funding acquisition: AM. Resources: AM. Supervision: CE, AM.

Funding This work has been supported by Ministerio de Ciencia, Innovación y Universidades (MICIU – Spain) [grant number RTI2018-096626-B-I00].

Declarations

Conflict of interest All authors declare not to have any conflict of interest.

Compliance with ethics requirements This article does not contain any studies with human participants or animals performed by any of the authors.

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