fermentation

MDPI

Article

Torulaspora delbrueckii Improves Organoleptic Properties and Promotes Malolactic Fermentation in Carbonic Maceration Wines

Candela Ruiz-de-Villa [1], Jordi Gombau [2], Montse Poblet [1], Albert Bordons [3], Joan Miquel Canals [2], Fernando Zamora [2], Cristina Reguant [3] and Nicolas Rozès [1,*]

[1] Grup de Biotecnologia Microbiana dels Aliments, Departament de Bioquímica i Biotecnologia, Facultat d'Enologia, Universitat Rovira i Virgili, c/ Marcel·lí Domingo s/n, 43007 Tarragona, Catalonia, Spain; candela.ruiz@urv.cat (C.R.-d.-V.); montserrat.poblet@urv.cat (M.P.)

[2] Grup de Tecnologia Enològica, Departament de Bioquímica i Biotecnologia, Facultat d'Enologia, Universitat Rovira i Virgili, c/ Marcel·lí Domingo s/n, 43007 Tarragona, Catalonia, Spain; jordi.gombau@urv.cat (J.G.); jmcanals@urv.cat (J.M.C.); fernando.zamora@urv.cat (F.Z.)

[3] Grup de Biotecnologia Enològica, Departament de Bioquímica i Biotecnologia, Facultat d'Enologia, Universitat Rovira i Virgili, c/ Marcel·lí Domingo s/n, 43007 Tarragona, Catalonia, Spain; albert.bordons@urv.cat (A.B.); cristina.reguant@urv.cat (C.R.)

* Correspondence: nicolasrozes@urv.cat

Abstract: This study investigates the impact of inoculating *Torulaspora delbrueckii* (Td) strains during the initial phase of carbonic maceration (CM) vinification, aiming to enhance the fermentative process and unique characteristics of CM wines. CM is a winemaking technique where whole bunches (without destemming and crushing) are enclosed in tanks filled with carbon dioxide, inducing intracellular fermentation. This study compares the effects of two Td strains on the MLF performance and sensory characteristics of CM wines using both inoculated and spontaneous MLF strategies. Although general physicochemical parameters remained consistent across conditions, organoleptic attributes showed significant differences due to *T. delbrueckii* presence. *T. delbrueckii* introduction during CM resulted in wines with increased anthocyanin content and a particular volatile profile. Isoamyl acetate, a key aroma in CM wines, was notably elevated, especially in the TdP strain. Sensory evaluations also revealed distinctions, with TdV wines displaying more pronounced aromas of red fruit, banana, and grass. Regarding MLF, *T. delbrueckii* presence notably enhanced performance, particularly in spontaneous MLF cases, accelerating fermentation completion. Inoculating the *Oenococcus oeni* strain OoVP41 also shortened MLF duration. These findings highlight the potential of Td strains to improve MLF efficiency and sensory attributes in CM wines. Using *T. delbrueckii* strains strategically enables winemakers to optimize MLF and improve sensory profiles, offering an opportunity to produce higher-quality CM wines.

Keywords: *Oenococcus oeni*; carbonic maceration; anthocyanins

Citation: Ruiz-de-Villa, C.; Gombau, J.; Poblet, M.; Bordons, A.; Canals, J.M.; Zamora, F.; Reguant, C.; Rozès, N. *Torulaspora delbrueckii* Improves Organoleptic Properties and Promotes Malolactic Fermentation in Carbonic Maceration Wines. *Fermentation* **2023**, *9*, 1021. https://doi.org/10.3390/fermentation9121021

Academic Editor: Simona Guerrini

Received: 14 November 2023
Revised: 8 December 2023
Accepted: 11 December 2023
Published: 14 December 2023

1. Introduction

Carbonic maceration (CM) vinification is a specific winemaking technique that is characterized by not crushing or destemming the grapes. Instead, the whole bunches are placed in tanks, which are then filled with carbon dioxide (CO_2) to create an anaerobic environment [1]. Within the grapes, an anaerobic fermentative metabolism initiates, known as intracellular fermentation, causing the berries to undergo physicochemical changes. As a result, the grapes begin to break down, releasing some of the juice or must, which accumulates at the bottom of the tank and undergoes alcoholic fermentation (AF) through the action of wild yeasts present in the skin of the grapes [1]. Furthermore, this process also triggers other metabolic processes, including the extraction of phenolic compounds from the grape skins, the release of volatile compounds, and the partial degradation of L-malic acid [1]. These grape berries result in a wine with improved organoleptic characteristics

due to intra-berry metabolic pathways. Finally, the second part of the process takes place, where the free-run wine and the press-wine finish the AF, mixed or separated depending on the oenologist's decision [1,2].

The CM vinification process offers a wide range of possibilities as it can be varied at different points, for instance, using rotating tanks to homogenize the whole grains with the free-run juice or prolonging maceration after the AF [1]. During the first part of the process, there may be a biochemical decarboxylation of L-malic acid in the free-run fermenting must through malolactic fermentation (MLF), depending on the population of lactic acid bacteria (LAB) present in the grape must. Consequently, if MLF has already begun in the first step in the free-run fermenting must, it is advisable to vinify the two wine fractions separately. This separation is important because the higher sugar content in the grape berry can potentially lead to lactic spoilage if both fractions are combined. In this context, AF is usually fast, between 2 and 7 days, and then MLF takes place. However, sometimes, due to improper management, both fermentations may occur simultaneously [1].

The distinctive organoleptic characteristics of CM wines, which have a lot of consumer appreciation, have contributed to the growing interest in studying this winemaking method [2–5]. These wines are known for having a higher content of esters, especially acetates, which increase their fruity aroma [3,5]. Specifically, the literature highlights the higher concentration of isoamyl acetate and ethyl cinnamate compared with wines produced using traditional fermentation methods [1,3]. Additionally, regarding color characteristics, CM wines have been found to have lower or higher levels of phenolic compounds and color intensity, which depends on the grape variety, the grape ripeness, the vintage, and the winemaking conditions [2,5,6].

Recent studies have focused on exploring the highly diverse autochthonous microbial population present in CM wines, with a large presence of non-*Saccharomyces* in the early stages [7,8]. The utilization of non-*Saccharomyces* commercial yeast presents an interesting prospect to maintain these special characteristics and have better microbiological control. For instance, the use of the species *Torulaspora delbrueckii*, commercialized as a starter culture, has been studied in sequential fermentation with *Saccharomyces cerevisiae* in white wines [9,10], red wines [11,12], rosé wines [13], and even in botrytized wines [14] owing to its impact on organoleptic properties. The utilization of *T. delbrueckii* in winemaking has been found to have significant effects on the composition of the volatile profile. The presence of this species leads to an increase in specific volatile compounds, such as fusel alcohols [10,15,16]. Additionally, the presence of *T. delbrueckii* during fermentative maceration in red wines has been associated with a greater release of phenolic compounds [11,12].

Furthermore, several research studies have focused on the potential of *T. delbrueckii* to enhance the performance of malolactic fermentation (MLF) by the main LAB in wine, *Oenococcus oeni* [17–19]. In the CM context, a recent study investigated how yeast species inoculation affects the bacterial population [20]. As described above, in the case of CM vinification, MLF may occur spontaneously, which can significantly impact the wine's flavor profile. In addition, it is important to note that a population of 10^6 CFU/mL and optimal conditions for LAB are necessary to carry out MLF. In cases where this does not occur, inoculation with LAB starter cultures and the use of *T. delbrueckii* could enhance the complete and successful L-malic acid degradation.

In summary, the objective of this research is to investigate the oenological implications of inoculating *T. delbrueckii* during the initial stage of CM vinification with the aim of improving the fermentative process and distinctive character of these wines. This study compares the effects of two different strains of this species on both the organoleptic characteristics and the performance of inoculated and spontaneous MLF.

2. Materials and Methods

2.1. Carbonic Maceration and Alcoholic Fermentation

This study evaluated three yeast species: one strain of *Saccharomyces cerevisiae*, CLOS YSEO (from now on referred to as Sc), obtained from Lallemand Inc. (Montreal, QC,

Canada), and two strains of *Torulaspora delbrueckii*: Viniflora Prelude (Td-P), obtained from Chr. Hansen Holding AS (Hoersholm, Denmark), and Viniferm NS Td (Td-V), obtained from Agrovin (Alcázar de San Juan, Spain). The yeast strains were inoculated from dry active yeast and rehydrated following the manufacturer's instructions. The *S. cerevisiae* strain was rehydrated at 37 °C for 30 min, while the *T. delbrueckii* strains were rehydrated at 30 °C for the same duration.

The fermentations were conducted using the Grenache grape variety (*Vitis vinifera* L.) supplied by the cellar Mas dels Frares of Rovira i Virgili University, which belongs to the AOC Tarragona. The grapes were harvested and processed in this experimental winery of the university. Semi-industrial scale fermentations were performed in a 15 L food-grade plastic container, maintaining a constant temperature of 22 °C. Initially, uncrushed grape berries, without stems, were placed in the tanks. The Control condition involved no inoculation during carbonic maceration (CM) and was left with the endogenous microbiota. The sequential conditions were inoculated with the two strains of *T. delbrueckii* at an initial concentration of 2×10^6 cells/mL. After yeast inoculation, CO_2 was pumped inside to create an anaerobic environment, and the tanks were hermetically closed. After five days of CM, the grapes were pressed, and the two must fractions (free-run must and press must) were mixed. The resulting fermenting must from each condition was transferred to another tank and *S. cerevisiae* was inoculated in all three conditions at a concentration of 2×10^6 cells/mL to finish AF. Thus, three different conditions were tested: Control, TdP, and TdV.

After CM (CM point) and at the final stage of AF (Final AF point), the samples were stored until analysis. In addition, the final wines were bottled to perform the sensory analysis.

Two media were used to determine inocula and population dynamics by plating a 1:10 serial dilution. For total yeast, YPD agar (10 g/L yeast extract, 20 g/L peptone, 20 g/L glucose, 17 g/L agar (Panreac Química SLU, Barcelona, Spain)) was used, and for non-*Saccharomyces* yeasts, the selective medium Lysine (Difco Laboratories, Detroit, MI, USA) was used.

An electronic densimeter (Densito 30PX Portable Density Meter (Mettler Toledo, Barcelona, Spain)) was used to monitor AF by measuring density each day. AF was considered finished when reductive sugars were under 2 g/L of the residual concentration. Residual glucose and fructose were determined using an enzymatic autoanalyzer Y15 Enzymatic Autoanalyzer (Biosystems S.A, Barcelona, Spain).

2.2. Malolactic Fermentation

After completing AF, replicates of each condition were mixed, and the resulting wines were divided into three conditions for MLF. The wines were stored at 4 °C for one week to stabilize them. The two strains of *Oenococcus oeni* used for inoculation were Lalvin VP41 (Oo-VP41) Lallemand Inc. and Viniflora CH11 (Oo-CH11) Chr. Hansen Holding AS. These strains were inoculated from commercial lyophilized products and rehydrated following the manufacturer's recommendations. *O. oeni* strains were rehydrated in wine at 20 °C for 15 min. Furthermore, a spontaneous MLF was performed (Sp).

As a result, the initial three AF conditions were expanded to a total of nine MLF conditions: Control, TdP, and TdV, each with their respective MLF condition of OoVP41, OoCH11, and Sp, with triplicate samples for each condition.

The MLF inoculations were performed at a population of 2×10^7 cells/mL. MLF was carried out in 1 L volumes at a temperature of 20 °C under anaerobic and static conditions. The progress of L-malic acid consumption was monitored daily until it reached a concentration below 0.1 g/L using a Y15 Enzymatic Autoanalyzer (Biosystems S.A, Barcelona, Spain).

The inoculum and populations of LAB were controlled by plating on modified MRS medium [21] (Difco Laboratories, Detroit, MI, USA). The medium was adjusted to pH 5 and supplemented with 4 g/L DL-malic acid (Sigma-Aldrich, Barcelona, Spain), 5 g/L

D-fructose (Panreac), 100 mL/L of tomato juice (Aliada, Madrid, Spain), 100 mg/L of nystatin (Panreac) to prevent yeast growth, and 25 mg/L of sodium azide (BioSciences, St. Louis, MO, USA) to prevent acetic acid bacteria growth. The plates were incubated at 27 °C in a 10% CO_2 atmosphere.

2.3. Area under the Curve (AUC)

The area under the curve (AUC) was calculated to assess significant differences in the AF performance. This was achieved by analyzing the decrease in density during AF and then integrating the values between two consecutive time points. The formula used for the calculation involved summing the areas of consecutive data points as follows: $\Sigma\,[((d_2 + d_1)/2) \times (t_2 - t_1) + \ldots + ((d_n + d_{n-1})/2) \times (t_n - t_{n-1})]$, where d_1, d_2, …, d_{n-1}, d_n represent the density at times 1, 2, n − 1, and n, respectively.

2.4. Physicochemical Analysis

2.4.1. General Oenological Parameters

The pH of the wines was determined using a Crison micro pH 2002 pH meter (Hach Lange Spain, L'Hospitalet, Spain). The content of citric acid, glycerol, and ethanol was determined following the procedure described in [22] using an Agilent 1100 HPLC system (Agilent Technologies, Waldbronn, Germany). Prior to injection, the wine samples were filtered using 0.22 µm pore filters (Merck, Barcelona, Spain). The HPLC system was equipped with a Hi-Plex H column (300 mm × 7.7 mm) inside a 1260 MCT (Infinity II Multicolumn Thermostat). Two detectors were coupled: a MWC detector (multi-wavelength detector, Agilent Technologies) for the determination of citric acid and a RID detector (1260 Infinity II refractive index detector, Agilent Technologies) for the determination of glycerol and ethanol.

The concentrations of acetic acid and L-malic acid were enzymatically determined using the Y15 Enzymatic Autoanalyzer (Biosystems S.A, Barcelona, Spain). Succinic acid was analyzed using a manual enzymatic method (Megazyme, Wicklow, Ireland) with the UV-Vis spectrometer POLARstar Omega (BMG LABTECH, Ortenberg, Germany).

To estimate the mannoprotein content, a precipitation with 95% ethanol followed by an acid hydrolysis at 90 °C was performed following the procedure described in [23]. The resulting mannoproteins were quantified in terms of mannose equivalents using a D-mannose and D-glucose enzymatic assay kit (Megazyme).

2.4.2. Volatile Composition

In the present study, the volatile composition of the wines was extracted using a liquid/liquid extraction method: 400 µL of dichloromethane in the presence of 2.5 g of ammonium sulfate $(NH_4)_2SO_4$ was used. Two internal standards (ISs), i.e., 4-methyl-2-pentanol (0.8 g/L) and heptanoic acid (0.7 g/L), were used. The extraction method was based on the protocol described by [24]. After the extraction, the organic phase was collected. Subsequently, 2 µL of the organic phase was injected into a gas chromatograph coupled to an FID detector (Flame Ionization Detector) (Agilent Technologies, Germany) using an FFAP column with the dimensions of 30 m × 0.25 mm × 0.25 µm. The injection was performed in split mode with a split ratio of 10:1 and a flow rate of 30 mL/min. The volatile compounds identified were the following: fusel alcohol acetates (isobutyl acetate, isoamyl acetate, 2-phenyletanol acetate), fusel alcohols (2-metil-propanol, 1-propanol, isoamyl alcohol, 1-pentanol, 1-hexanol, cis-3-hexen-1-ol, 2-phenylethanol), other alcohols (2-butanol, 1-butanol), ethyl esters of FA (ethyl butanoate, ethyl octanoate, ethyl decanoate, diethyl butanoate, ethyl dodecanoate, ethyl hexanoate), short-chain fatty acids (SCFAs) (propionic, isobutyric, butyric, butyric acids), and medium-chain fatty acids (MCFAs) (octanoic, decanoic and dodecanoic acids). All reagents were analytical grade from Sigma-Aldrich (Barcelona, Spain).

2.4.3. Color and Anthocyanin Analysis

The following color parameters were determined: the CIELab coordinates, color intensity, and total polyphenolic index (TPI). The CIELab coordinates were determined as described by [25]. The colorimetric coordinates were calculated with MSCV software (https://www.unirioja.es/color/MSCV_7.exe, accessed on 10 February 2021), including the red-greenness (a*) and yellow-blueness (b*) and their derivate magnitudes: hue (H), lightness (L), and chroma (C). The color intensity (CI) was calculated from the sum of absorbances at 420, 520, and 620 nm, measured in a spectrophotometer, using the method described by [26]. The content of free anthocyanins, non-acylated and acylated anthocyanins, and pyranoanthocyanins, was determined at two points during AF: after MC and after AF. Samples from the final AF were only filtered with 0.22 µm pore filters (Merck) before injection. However, samples from the final CM were pre-treated using PVPP (Sigma-Aldrich) columns in order to eliminate sugars that could interfere with the HPLC measure. Briefly, 40 µL of the samples were injected into an Agilent 1200 series liquid chromatograph (HPLC-diode array detection) using an Agilent Zorbax Eclipse XDBC18, 4.6 × 250 mm, 5 µm column (Agilent Technologies). Chromatograms were recorded at 530 nm, and anthocyanin standard curves were made using Malvidin-3-O-glucoside chloride (Extrasynthèse, Genay, France). The compounds were identified by recording their UV spectra with the diode array detector and comparing these with the UV spectra reported in the literature. Anthocyanidin-3-monoglucosides, their respective acetylated and p-coumarylated anthocyanins, and pyranoanthocyanins of MC samples were quantified.

The total anthocyanin content was determined with spectrophotometry using the method described by [27].

2.5. Sensory Analysis

After AF and MLF, sensory analyses were conducted by a trained tasting panel consisting of 12 tasters. First, the panel compared three wines during a blind triangle test to identify any noticeable differences between them. Subsequently, a descriptive test was performed on wines that were found to be significantly different in the triangle test. In the descriptive test, the tasters evaluated the intensity of five attributes using a numerical scale (from 0 to 5): acidity, red fruit aroma, banana aroma, grass aroma, and global perception.

2.6. Statistical Analysis

All the fermentations were performed in triplicate to improve the consistency of the results. Statistical analysis of data was performed using ANOVA and the Tukey test with XLSTAT version 2022.5.1 software (Addinsoft, Paris, France). A *p*-value of less than 0.05 was considered statistically significant. A principal component analysis (PCA) was used to describe the volatile composition of the wines after MC and AF. Panel check V1.4.2.2012 was used for sensory data.

3. Results

3.1. Alcoholic Fermentation

As it has been described, CM offers several possibilities for conducting the vinification process. The following vinification process was carried out: after undergoing five days of CM under anaerobic conditions, the free-run must and press must were blended to observe and analyze the complete wine produced during CM, comparing samples with and without the presence of *T. delbrueckii*.

Subsequently, various parameters of the resulting wines were analyzed at two stages: after CM and AF. The initial parameters in the grape must were as follows: pH 3.3, titratable acidity 4.85 g/L, α-amino nitrogen 121 mg/L, and ammonium 87 mg/L. The initial density was 1085.3 g/L and as the yeast started the AF in the free-run must, the density gradually decreased. In the Control condition with spontaneous yeasts, the density only decreased to 1081.2 ± 0.4 g/L. However, in the CM condition with *T. delbrueckii*, the decrease was significantly more pronounced. Specifically, for TdP, the density decreased to 1063.6 ± 3.0 g/L,

and for TdV, it decreased to 1067.3 ± 2.8 g/L (Figure 1). This reduction in initial density facilitated a shorter duration of AF in wines containing *T. delbrueckii*. The TdP condition lasted for 13 days, while the TdV condition lasted for 14 days. In contrast, the Control condition with S. cerevisiae alone lasted for a duration of 17 days.

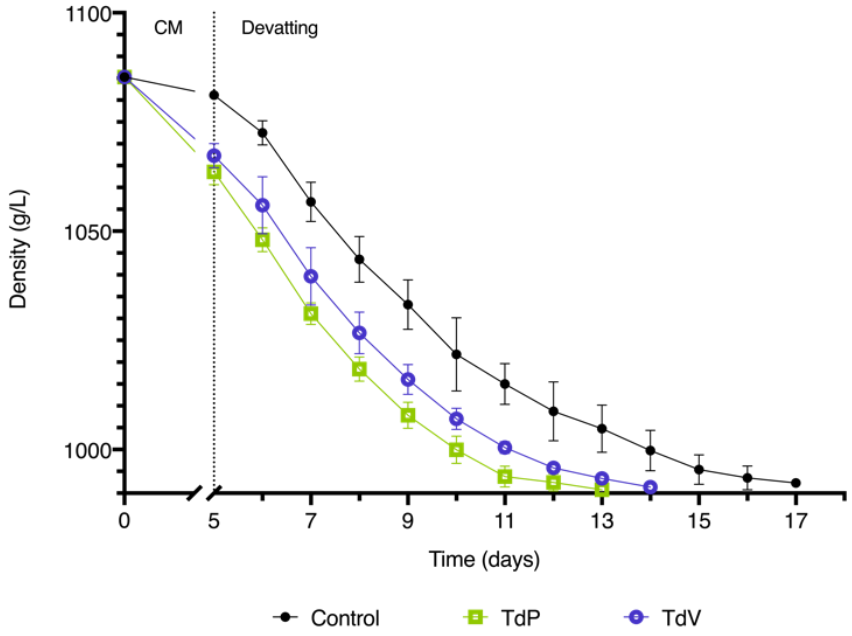

Figure 1. Kinetic of alcoholic fermentation after five days of carbonic maceration. Control corresponds to the pure fermentation with *S. cerevisiae* CLOS; TdP and TdV correspond to the fermentations with *S. cerevisiae* and CM with *T. delbrueckii* Prelude or *T. delbrueckii* Viniferm, respectively. All data are expressed as the arithmetic average of three biological replicates ± standard deviation (n = 3).

The AUC (area under the curve) values were calculated for wines sampled at the end of CM and sampled at the end of AF (Table 1). Significant differences were observed in the CM samples, where the presence of *T. delbrueckii* resulted in significantly lower AUC values compared with the CM Control, indicating a faster AF during CM. However, after inoculation with *S. cerevisiae* and the completion of AF, the Control wines exhibited higher AUC values than wines with *T. delbrueckii* (TdP and TdV). This indicates that despite *T. delbrueckii* consuming sugars during CM, *S. cerevisiae* alone leads to faster AF, which aligns with previous studies that reported *S. cerevisiae* to have faster AF compared with sequential or co-inoculated fermentations with non-*Saccharomyces* species due to the competition between species [12,28].

In terms of yeast populations, it was observed that the presence of non-*Saccharomyces* yeasts, detected in lysine medium, was higher in wines that were inoculated with *T. delbrueckii*, as expected (Figure S1). It was previously reported that when *T. delbrueckii* is used in sequential fermentation with *S. cerevisiae*, there is a high percentage of *T. delbrueckii* observed at the end of AF [19,28].

Few differences were observed in the general oenological parameters analyzed. A slight production of ethanol was observed during CM, between 1 and 3% (*v*/*v*), as stated in the literature [1]. However, in this sampling point, the ethanol content was higher in the musts inoculated with *T. delbrueckii* (TdP and TdV) than in the Control condition. This was predictable since AF in free-run must was less advanced in the Control condition due to the absence of inoculated yeast, producing only a 1.06% (*v*/*v*) of ethanol. In addition, ethanol production tends to increase after AF in TdP and TdV wines compared with Control wines. Many authors have stated that ethanol decreases in sequential AFs with the use of *T. delbrueckii* [16,29,30]. However, this decrease depends on vinification conditions [15]; thus, the reduction was not significant in these CM conditions. No significant differences

were observed in glycerol, pH, citric acid, or succinic acid among the samples. However, L-malic acid was consumed more in Td wines than in the Control wine after AF. This could be explained by the slight consumption of L-malic acid that has been observed in yeast for their metabolism [31]. It was reported that the consumption is higher in *T. delbrueckii* fermentations [32]. Variations were found in acetic acid levels, with a noticeable increasing trend in the Control samples at both the CM and AF stages. In sequential AF with *T. delbrueckii* and *S. cerevisiae*, the reduction in acetic acid was observed to a greater or lesser degree depending on the strain and winemaking conditions [12,14,17].

Table 1. The oenological parameters analyzed. End of CM corresponds to the sampling after carbonic maceration, before inoculating *S. cerevisiae*, and End of AF corresponds to the sampling after alcoholic fermentation. Control corresponds to the control fermentation; TdP and TdV correspond to the fermentations with *T. delbrueckii* Prelude or *T. delbrueckii* Viniferm, respectively. AUC, area under the curve.

General Oenological Parameters	End of CM			End of AF		
	CM Control	CM TdP	CM TdV	AF Control	AF TdP	AF TdV
AUCs	5416 ± 1^{a}	5372 ± 7^{b}	5381 ± 7^{b}	$11{,}287 \pm 49^{B}$	8119 ± 17^{A}	9165 ± 28^{A}
Glycerol (g/L)	2.04 ± 0.12^{a}	3.43 ± 0.79^{a}	3.38 ± 0.55^{a}	7.51 ± 0.76^{AB}	6.56 ± 0.59^{A}	7.55 ± 0.09^{B}
Ethanol (% *v*/*v*)	1.06 ± 0.04^{a}	2.99 ± 0.28^{b}	2.84 ± 0.16^{b}	11.06 ± 0.21^{A}	11.58 ± 0.16^{A}	11.70 ± 0.09^{A}
Ammonia (mg/L)	68 ± 9^{b}	40 ± 11^{a}	46 ± 6^{a}	n.d.	11 ± 4^{B}	7 ± 5^{AB}
α-amino nitrogen (mg/L)	109 ± 3^{b}	60 ± 7^{a}	97 ± 7^{b}	19 ± 7^{A}	27 ± 4^{A}	22 ± 3^{A}
Succinic acid (g/L)	0.42 ± 0.02^{b}	0.27 ± 0.07^{a}	0.31 ± 0.10^{ab}	0.34 ± 0.04^{A}	0.31 ± 0.05^{A}	0.29 ± 0.03^{A}
Citric acid (g/L)	0.26 ± 0.04^{a}	0.34 ± 0.10^{a}	0.28 ± 0.04^{a}	0.23 ± 0.02^{A}	0.25 ± 0.03^{A}	0.25 ± 0.05^{A}
Acetic acid (g/L)	0.34 ± 0.07^{b}	$0. \pm 0.02^{a}$	0.61 ± 0.06^{ab}	0.54 ± 0.10^{A}	0.47 ± 0.05^{A}	0.31 ± 0.06^{A}
L-malic acid (g/L)	n.a.	n.a.	n.a.	1.73 ± 0.04^{A}	1.64 ± 0.04^{B}	1.58 ± 0.04^{C}
pH	n.a.	n.a.	n.a.	3.21 ± 0.03^{A}	3.25 ± 0.03^{A}	3.27 ± 0.04^{A}
Eq. mannose (mg/L)	n.a.	n.a.	n.a.	114 ± 13^{A}	301 ± 7^{B}	311 ± 8^{B}

Different lowercase letters indicate the existence of a significant difference between samples at the end of CM (p-value < 0.05). Different uppercase letters indicate the existence of a significant difference between samples at the end of AF (p-value < 0.05). All data are expressed as the arithmetic average of three biological replicates ± standard deviation (n = 3). n.a., not analyzed; n.d., not detected.

Furthermore, in terms of nitrogen content at the end of CM, wines fermented with *T. delbrueckii* exhibited significantly higher consumption compared with the Control wine, which can be attributed to a larger yeast population. However, the Control wine after AF showed complete consumption of ammonia and slightly higher consumption of α-amino nitrogen compared with the TdP and TdV wines. It is worth noting that after CM, the TdP wine displayed a higher consumption of α-amino nitrogen compared with the TdV wine. Additionally, there was an increase in the presence of mannoproteins at the end of AF, consistent with previous reports [19,23,33], indicating higher nitrogen concentration availability in the TdP and TdV wines after AF.

3.2. Volatile Composition

In order to examine the impact of both *T. delbrueckii* strains on the volatile composition of wines, a principal component analysis (PCA) was conducted (Figure 2). The identified volatile compounds were categorized into the following families: ethyl esters, fusel alcohols acetates, fusel alcohols, MCFAs, SCFAs, and other alcohols (Table S2).

On the one hand, Figure 2 shows that PC1 separated the wines into two groups. The samples after CM and Control wines after AF (AF control) are separated into one group, while wines with *T. delbrueckii* after AF (AF TdP and AF TdV) are grouped in another group. On the other hand, PC2 separated the wines with T. delbrueckii regarding the strain used (AF TdP and AF TdV). The Control condition with spontaneous yeast during CM did not undergo changes during the completion of AF, with the exception of some compounds. Instead, it remained similar to the samples after CM: it was positively correlated with the other alcohols and MCFA variables and negatively correlated with the rest (Figure 2). Only few compounds showed a significant increase after the completion of AF in all conditions,

such as 1-propanol, 2-methyl propanol, ethyl dodecanoate, and 2-phenylethanol (Table S2). Previous studies reported that a wide range of aromatic compounds in carbonic maceration wines are formed in the initial stage of the process (CM), which occurs under a CO_2 atmosphere [5,34]. Some differences were observed in relation to the presence or not of *T. delbrueckii* after the CM phase. Ethyl butanoate, 2-methyl propanol, ethyl dodecanoate and 2-phenyletanol were increased significantly (Table S2) in the CM TdP and CM TdV wines, regardless of the strain.

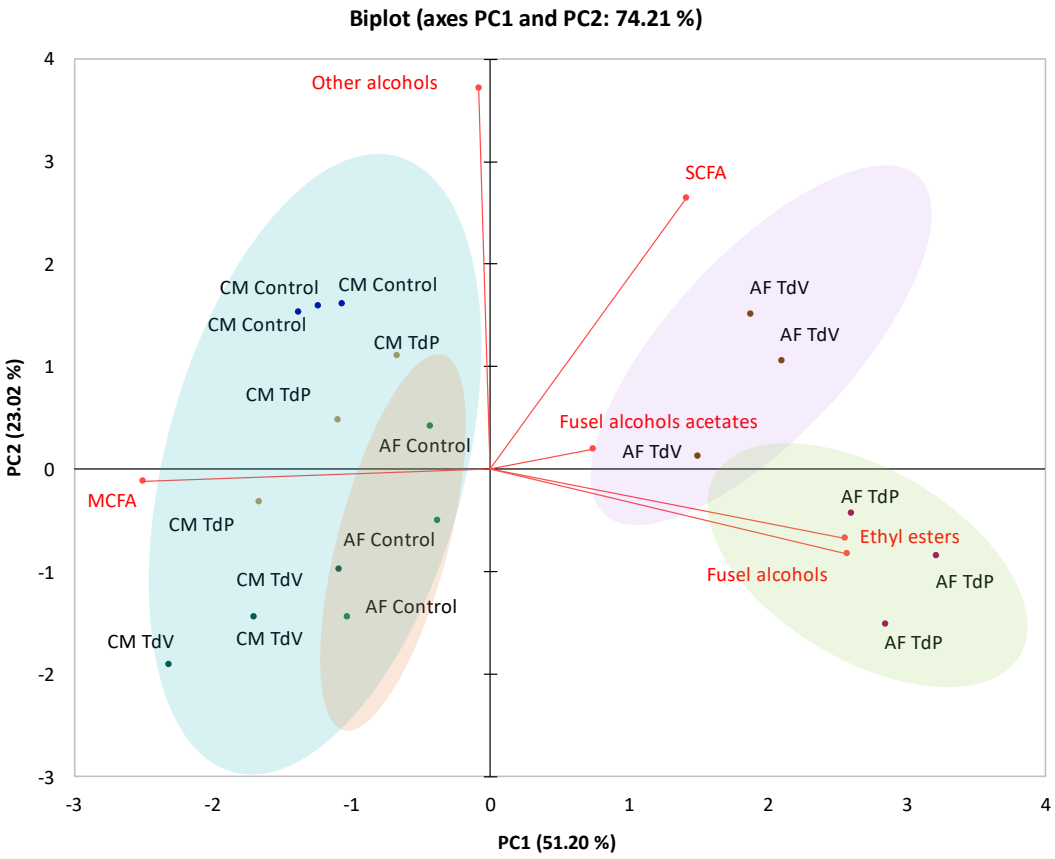

Figure 2. Principal component analysis biplot built from the following variables: ethyl esters, fusel alcohols acetates, fusel alcohols, MCFAs, SCFAs, and other alcohols. The samples are the following: CM Control: corresponds to the Control condition at the end of carbonic maceration; CM TdP and CM TdV: correspond to conditions inoculated with *T. delbrueckii* Prelude or *T. delbrueckii* Viniferm, respectively, at the end of carbonic maceration; Control: corresponds to the Control condition at the end of alcoholic fermentation; TdP and TdV: correspond to conditions inoculated with *T. delbrueckii* Prelude or *T. delbrueckii* Viniferm, respectively, at the end of alcoholic fermentation.

However, as shown in Figure 2, it is evident that several volatile compounds showed an increase only after AF in *T. delbrueckii* conditions (AF TdP and AF TdV) and were positively correlated with ethyl esters, fusel alcohols, SCFA, and fusel alcohol acetates. This implies that the final wines in the presence of *T. delbrueckii* after the entire process have higher concentrations of these volatile families. These compounds include 1-propanol, 2-methyl-propanol, isoamyl acetate, 2-phenylethanol acetate, ethyl decanoate, ethyl dodecanoate, and isopropanol, which are volatile compounds characterized by alcohol, wine, banana, roses, grape, and leave descriptors, respectively [35] (Table S2).

The modulation of aroma due to the presence of *T. delbrueckii* showed in this work confirmed previous results in red [16] and white wines [10] and even in rosé wine [13,36]. This effect has been also associated with an increase in esters and fusel alcohols, even though this impact depends on the strain and wine. Regarding the results of this study, the TdP strain increased significantly more than the TdV strain in the concentrations of

2-phenlyethanol, 2-methyl propanol, and isoamyl acetate (Table S2). Isoamyl acetate is a characteristic aromatic compound found in CM wines [1] and is characterized by its banana descriptor [35]. However, after CM, the concentration of isoamyl acetate in these wines only reached concentrations of 0.62 mg/L (Control), 0.63 mg/L (TdP), and 0.47 mg/L (TdV) (Table S2) without significant differences, maybe due to the grape cultivar. However, it is worth noting that the TdP and TdV wines showed a significant increase in the isoamyl concentration after AF, reaching a concentration of 3.37 mg/L and 1.32 mg/L, respectively, compared with the AF Control wine, which reached a concentration of 0.58 mg/L. Previous studies have reported that the CM vinification process leads to higher concentrations of this volatile compound compared with traditional vinification methods, with differences between cultivars and types of wines. For instance, French wines from a blend of cultivars [3] had concentrations of 3.20 mg/L in CM wines compared with 0.51 mg/L in traditional young red wines. However, [5] described concentrations of 2.78 mg/L in CM wines compared with 1.14 mg/L in traditional red wines.

In addition, it is worth highlighting that the negative correlation between AF TdP and MCFA and AF TdV and MCFA was due to a decreasing trend in this family (Figure 2), especially associated with decanoic acid. It has been reported that the presence of *T. delbrueckii* during AF leads to a reduction in MCFA. This reduction was associated with detoxification due to the presence of more mannoproteins in *T. delbrueckii* wines (Table 1). These findings indicate that the presence of *T. delbrueckii* during CM not only changes the volatile composition during the CM phase but also carries its influence throughout the AF process, especially with TdP.

3.3. Color Parameters and Anthocyanins Composition

Regarding the anthocyanins and color composition, interesting differences were found among the conditions. Table 2 presents the results of the anthocyanin composition and color parameters. It is evident that the concentration of anthocyanins is relatively low, which could be attributed to the grape cultivar [37] or maybe to the young age of the Grenache vines from which the grapes were harvested. It is noteworthy that the anthocyanin content and color characteristics in CM differ between different studies: some authors described these wines with high phenolic content and greater or similar color intensity than conventional wines [5]. However, traditionally, CM wines have been described as wines with less intense color and fewer total phenolic compounds and anthocyanins [2].

Table 2. The phenolic compounds and color parameters analyzed. End of CM corresponds to the sampling after carbonic maceration, before inoculating *S. cerevisiae*, and End of AF corresponds to the sampling after alcoholic fermentation. Control corresponds to the Control fermentation; TdP and TdV correspond to fermentations with *T. delbrueckii* Prelude or *T. delbrueckii* Viniferm, respectively.

Phenolic Compounds and Color Parameters	**End of CM**			**End of AF**		
	CM Control	**CM TdP**	**CM TdV**	**AF Control**	**AF TdP**	**AF TdV**
Total anthocyanins (mg/L) quantified with spectrophotometry	61 ± 2^{a}	106 ± 4^{b}	118 ± 3^{b}	49 ± 3^{A}	114 ± 17^{B}	112 ± 7^{B}
Free anthocyanins (mg/L) quantified with HPLC	20 ± 7^{a}	85 ± 13^{bc}	97 ± 23^{c}	24 ± 2^{A}	69 ± 3^{BC}	66 ± 7^{C}
Pyranoanthocyanins (mg/L)	n.d.	2.43 ± 0.10^{d}	2.46 ± 0.12^{d}	1.76 ± 0.01^{B}	2.08 ± 0.06^{C}	2.06 ± 0.03^{C}
L*	53 ± 2^{bc}	45 ± 1^{a}	45 ± 3^{a}	59 ± 1^{C}	48 ± 2^{AB}	48 ± 4^{AB}
C*	48 ± 2^{b}	51 ± 1^{b}	53 ± 5.63^{b}	33 ± 3^{A}	53 ± 4^{B}	52 ± 3^{B}
H*	46.2 ± 4.8^{c}	22.5 ± 6.4^{b}	23.0 ± 1.9^{b}	17.9 ± 0.4^{AB}	12.6 ± 1.7^{A}	11.1 ± 1.6^{A}
a*	33 ± 3^{a}	47 ± 3^{b}	50 ± 4^{b}	33 ± 2^{A}	49 ± 7^{B}	51 ± 3^{B}
Bb*	34.6 ± 2.8^{c}	19.3 ± 4.9^{b}	21.2 ± 0.1^{b}	10.6 ± 0.4^{A}	10.8 ± 1.9^{A}	9.9 ± 0.8^{A}
CI	2.45 ± 0.18^{b}	2.79 ± 0.09^{b}	2.76 ± 0.13^{b}	1.48 ± 0.34^{a}	2.55 ± 0.26^{b}	2.52 ± 0.23^{b}

Different lowercase letters indicate the existence of a significant difference between samples at the end of CM (p-value < 0.05). Different uppercase letters indicate the existence of a significant difference between samples at the end of AF (p-value < 0.05). All data are expressed as the arithmetic average of three biological replicates. n.d., not detected.

In terms of microbiological treatments, the presence of *T. delbrueckii* during the CM phase significantly increased the concentration of anthocyanins in the wines, particularly in the TdV wines. This effect could be related to the oxidation of anthocyanins in the Control wines due to the fact that the AF took longer to start (Figure 1). Furthermore, the high anthocyanin content of wines fermented in the presence of *T. delbrueckii* was previously documented in traditional red wines with this specific TdV strain [12], as well as with other strains [11,32,38]. This phenomenon may be attributed to the elevated pectolytic activity observed in certain non-*Saccharomyces* yeast, such as *Metschnikowia pulcherrima* [39]; however, this has not been proven in *T. delbrueckii*.

After AF, the wines produced with TdP and TdV still maintained a higher proportion of free anthocyanins compared with the Control wine (Table 2). However, when comparing the concentration of anthocyanins after CM and after AF, it had a decreasing trend in the presence of *T. delbrueckii*, with a higher reduction observed in the TdV wine. This effect was not observed in the Control wine after AF. As it was previously reported, an AF with *T. delbrueckii* without the presence of grape skins can lead to a decrease in anthocyanin levels, as observed in rosé wines [36]. This reduction could be attributed to the formation of aglycones from anthocyanins, which are susceptible to oxidation [40], or it could be due to the high β-glucosidase activity of certain *T. delbrueckii* strains [41]. Additionally, it could be related to the absorption of pigments by the yeast walls, which has been shown to vary depending on the yeast species or strain [42,43].

Anthocyanidin-3-O-monoglucosides and other free anthocyanin concentrations, determined with HPLC-DAD, exhibited a similar trend to that reported for total anthocyanins measured using spectrophotometry. This correlation was anticipated, as spectrophotometric analysis includes the detection of other pigments, potentially leading to an overestimation of the total anthocyanin concentration. Conversely, HPLC-DAD methods solely detect free anthocyanins [44].

In terms of pyranoanthocyanins (Vitisin A and Vitisin B), significant differences were observed. In the Control samples at the end of CM, we did not detect any pyranoanthocyanins, while they were detected in the TdP and TdV samples after CM. However, after AF, pyranoanthocyanins were detected in all the wines, with a higher concentration in the TdP and TdV wines. It was also observed that in Td wines after AF, the concentration decreased in comparison with Td samples after CM. It is worth noting that in rosé wines, it was previously observed that a higher proportion of pyranoanthocyanins were present in wines fermented with *T. delbrueckii* compared with wines fermented with *S. cerevisiae* [38]. These derived pigments contribute to improved color stability in wines, as they are less affected by changes in pH and are less likely to experience discoloration due to the presence of sulfur dioxide [45].

At the CM stage, there were no significant differences in CI among the different conditions, although there was a slight decreasing trend observed in the Control CM sample. However, after the completion of AF, the Control wine exhibited a significant reduction in CI (Table 2). In this context, it was observed that the Control wines exhibited significantly higher L* values and lower C* values compared with the Td wines. This suggests that wines fermented with *T. delbrueckii*, regardless of the strain used, had a more intense and vibrant color than the Control samples, which is related to the higher concentration of anthocyanins. Regarding the H* coordinate, the Control samples showed higher values than the Td wines, indicating a more pronounced yellowish tone. This, along with the lower concentration of anthocyanins observed, is likely associated with anthocyanin oxidation. Conversely, the Td wines exhibited the opposite trend. In terms of the a* coordinate, the Td wines had higher values compared with the Control wines, indicating a stronger red component both after CM and after AF, which could be also related to the higher concentration of anthocyanins. However, in terms of the b* coordinate, the Td wines had lower values after CM, resulting in blueish hues, which agrees with the low H* values. Nevertheless, there were no differences in the b* coordinate after AF.

3.4. Malolactic Fermentation

After the end of CM and AF, three MLF strategies were implemented: inoculation with *O. oeni* strains OoVP41 and OoCH11 and spontaneous fermentation. At the end of AF, the initial LAB concentration was low to become an MLF ($2{\cdot}10^3$ CFU/mL), and the consumption of L-malic acid was also small (Table 1), suggesting that MLF did not commence yet. The purpose of inoculating the *O. oeni* starter cultures was to observe their potential under competitive pressure of endogenous LAB.

Figure 3 shows the MLF kinetics of the three wine conditions with their respective MLF strategies. Inoculation with OoVP41 resulted in a shorter MLF duration compared with OoCH11 and spontaneous MLF. The TdP and TdV wines that underwent MLF in the presence of OoVP41, completed MLF two days earlier (8 days) than the control wines with the same MLF starter culture (10 days). Previous studies conducted under laboratory conditions [19] have also described OoVP41 as a highly efficient fermentative strain. This study further demonstrates its successful performance under competitive conditions. The effect of *T. delbrueckii* on MLF was also observed with the use of the OoCH11 starter culture. However, in this case, the MLF duration was only reduced by one day (11 days vs. 12 days) without differences between strains.

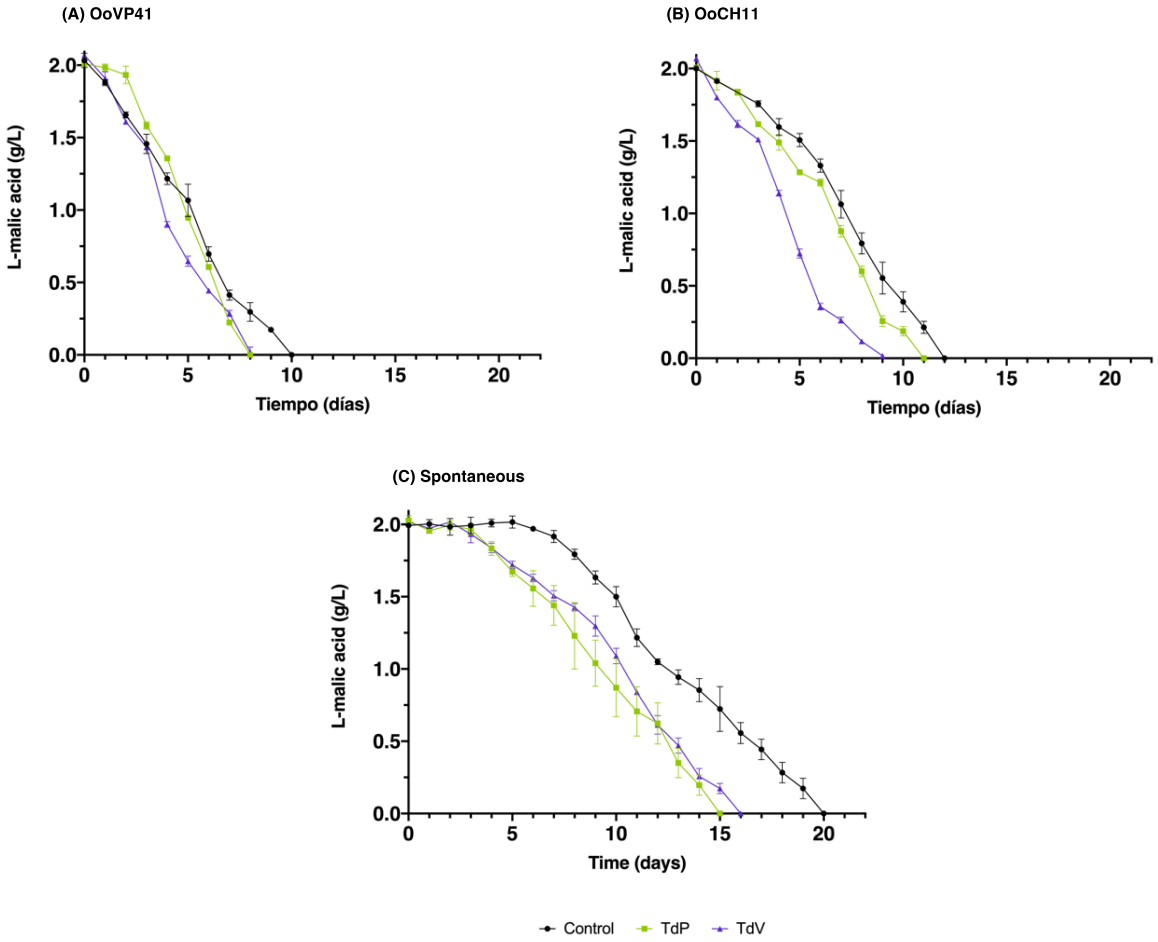

Figure 3. Consumption of L-malic acid during malolactic fermentation: (**A**) corresponds to OoVP41 MLF, (**B**) corresponds to OoCH11 MLF, and (**C**) corresponds to spontaneous MLF. Control corresponds to the Vontrol condition only fermented with Sc; TdP and TdV correspond to the fermentations with *T. delbrueckii* Prelude and Viniferm, respectively. All data are expressed as the arithmetic average of three biological replicates ± standard deviation (n = 3).

Greater differences were found in spontaneous MLF. In the case of Control wines, they took 7 days to start MLF, while TdP took 4 days and TdV took only 3 days. Regarding the total time of MLF, TdP and TdV were also shorter: TdP lasted 15 days and TdV lasted 16 days, which was notably less time than the Control, which lasted 20 days.

The presence of *T. delbrueckii* is associated with various factors that contribute to the enhanced efficiency of *O. oeni*. These factors include a reduction in inhibitory compounds such as succinic acid, SO_2, and MCFAs; the mitigation of stressful conditions, such as low pH or high ethanol content; and an increase in beneficial compounds like mannoproteins. However, in the present study, no differences were observed in ethanol levels or pH under these conditions. Therefore, the improved performance of MLF may be attributed to a reduction in MCFAs (Figure 2), which is known to be toxic to *O. oeni* [46], or an increase in ammonia and mannoproteins (Table 1). Apart from nutritional intake, the increase in mannoproteins could be associated with the reduction in MCFAs since they could absorb these lipids, thus detoxifying the media [47]. In addition, a previous study linked increased mannoprotein levels to improved MLF performance, observing that the relative expression of certain *O. oeni* genes involved in mannose uptake and other sugars was elevated in *T. delbrueckii* wines [23]. These authors also described that the metabolism of mannoproteins is more active under stressful conditions. Therefore, it is possible that in this study, the metabolism of mannoproteins was more activated due to the high concentrations of anthocyanins compared with the Control. Certain phenolic compounds can impose stress on *O. oeni*, but not all of them do. Interestingly, previous studies have reported an improvement in MLF even under higher polyphenolic conditions than the control in traditional red winemaking methods [12]. Thus, it is noteworthy that the inoculation of CM wines with *T. delbrueckii* also promotes MLF, assisting *O. oeni* with adapting to challenging conditions.

3.5. Organoleptic Characteristics

To better understand the effects of *T. delbrueckii* treatment during CM on wines, a sensory analysis was conducted after AF and MLF. Initially, a triangle test was performed, comparing the Control wines to TdP, the Control wines to TdV, and TdP to TdV. The results revealed significant differences between the Control and TdP, as well as between the Control and TdV. However, the panel of tasters was unable to distinguish between TdP and TdV. Regarding the MLF comparison, the three MLF conditions (spontaneous, OoVP41, and OoCH11) were evaluated for each AF condition, but the tasters were unable to differentiate between the three MLF conditions in any of the wines. Consequently, a descriptive test was conducted exclusively on wines after AF.

The PCA shown in Figure 4A discriminates the three wines according to the results obtained in the descriptive test. All variables are correlated positively with *T. delbrueckii* strains, especially with TdV.

In Figure 4B, the same trend in some of the analyzed parameters is observed. For instance, TdV exhibited pronounced red fruit aroma, grass aroma, and banana aroma and higher values in overall perception. In the case of TdP, the red fruit and grass aromas were similar to those of the Control wines, while the banana aroma and overall perception were improved compared with the Control wine. Among these parameters, the banana aroma was the only one that showed a significant difference between *T. delbrueckii* wines and the Control wine (Figure 4B). Thus, it can be concluded that consumers associated a stronger banana aroma with the presence of *T. delbrueckii* during CM. It is noticeable that testers had a preference for TdV wines [48].

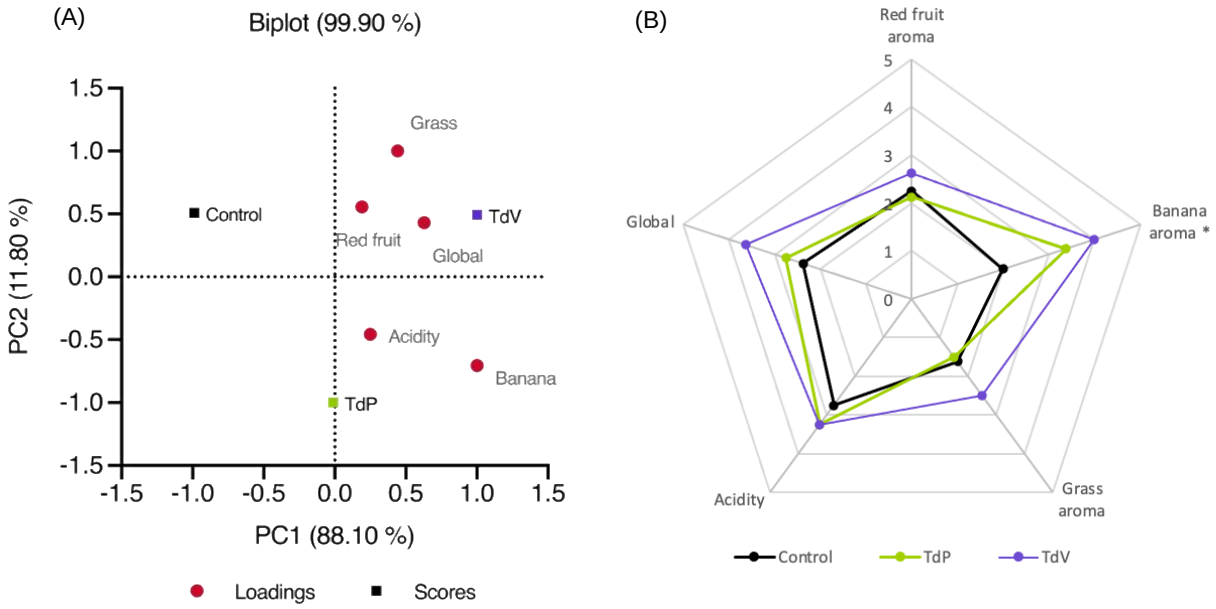

Figure 4. (**A**) Principal component analysis biplots built from the following variables: red fruit, grass, banana, acidity, and global perception. (**B**) Spiderweb diagram for sensory analysis of CM wines after AF. Asterisks (*) indicate attributes that showed significant differences (p-value < 0.05). The samples correspond to wines after alcoholic fermentation. Control corresponds to the Control condition at the end of alcoholic fermentation; TdP and TdV correspond to conditions inoculated with *T. delbrueckii* Prelude or *T. delbrueckii* Viniferm, respectively.

4. Conclusions

In this study, we investigated the impact of two *T. delbrueckii* strains (TdP and TdV) on carbonic maceration, alcoholic fermentation, MLF performance, and sensory characteristics in carbonic maceration wines. Then, three strategies of MLF were tested: inoculation with two *O. oeni* strains (OoVP41 and OoCH11) and spontaneous MLF. While the general physicochemical parameters did not exhibit significant differences among the conditions, the organoleptic parameters showed noteworthy changes with the presence of *T. delbrueckii.* When this species was introduced during CM, it resulted in wines with enhanced anthocyanin content and a distinct volatile profile. Notably, *T. delbrueckii* strains contributed to significantly higher levels of the aroma compound isoamyl acetate, a key aroma in carbonic maceration wines. The TdP strain, in particular, led to even higher concentrations of this aroma compared with the TdV strain. A sensory evaluation panel also discerned differences between the treatments, with TdV wines exhibiting more pronounced aromas of red fruit, banana, and grass.

Regarding MLF, the presence of *T. delbrueckii* significantly improved the performance, especially in cases of spontaneous MLF, where the fermentation started earlier and required less time to complete. Moreover, inoculation with OoVP41 also contributed to a two-day reduction in MLF duration. These findings underscore the potential benefits of utilizing *T. delbrueckii* strains to enhance MLF efficiency and overall sensory attributes in CM wines. In regions with increased acidity due to climate change, particularly in the new north regions, inoculation with LAB becomes crucial to ensure complete malic acid degradation and maintain the desired wine quality. This is of particular importance in the context of CM vinification. By strategically using these strains, winemakers can optimize the MLF process and enrich the sensory profile of carbonic maceration wines, providing an avenue for producing wines with enhanced quality and distinctive characteristics.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fermentation9121021/s1, Table S1. Citric and acetic acid analyzed after MLF. Control corresponds to the control fermentation; TdP and TdV corresponds to the fermentations with *T. delbrueckii* Prelude or *T. delbrueckii* Viniferm, respectively. Oo-VP41, OoCH11 and Spontaneous correspond to MLF performed with the strain Vp41, the strain CH11 and endogenous LAB. Different lowercase letters indicate the existence of significant difference between samples at the end of CM ($p < 0.05$). Different uppercase letters indicate the existence of significant difference between samples at the end of AF (p-value < 0.05). All data is expressed as the arithmetic average of three biological samples; Table S2. Total volatile compounds analyzed (mg/L). End of CM corresponds to the sampling after carbonic maceration, before to inoculate *S. cerevisiae*, and End of AF corresponds to the sampling after alcoholic fermentation. Control corresponds to the control fermentation; TdP and TdV corresponds to the fermentations with *T. delbrueckii* Prelude or *T. delbrueckii* Viniferm, respectively. All data is expressed as the arithmetic average of three biological replicates. Figure S1. Control, Td-P and Td-V yeast populations during AF. Control corresponds to the pure fermentation with *S. cerevisiae* CLOS; TdP and TdV corresponds to the fermentations with *S. cerevisiae* and CM with *T. delbrueckii* Prelude or *T. delbrueckii* Viniferm, respectively. Sc yeast populations represents *S. cerevisiae* populations, while Non-Sc indicate non-*Saccharomyces* populations. Means accompanied by standard deviations (SD) based on three replicates (n = 3).

Author Contributions: C.R.-d.-V.: investigation, methodology, and original draft preparation. J.G.: investigation, methodology, and data curation. M.P.: investigation, methodology, and data curation. A.B.: supervision, reviewing, and visualization. J.M.C.: supervision and reviewing. F.Z.: methodology and original draft preparation. C.R.: supervision, writing, reviewing, and funding acquisition. N.R.: supervision, writing, reviewing, and editing. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by grant PGC2018-503 101852-B-I00 awarded by the Spanish Research Agency. CRV is grateful for the predoctoral fellowship from the Catalan Government (2020FI).

Data Availability Statement: Data are available in Supplementary Materials.

Conflicts of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Tesnière, C.; Flanzy, C. Carbonic maceration wines: Characteristics and winemaking process. In *Advances in Food and Nutrition Research*; Academic Press Inc.: Cambridge, MA, USA, 2011; Volume 63, pp. 1–15. [CrossRef]
2. Portu, J.; Gutiérrez-Viguera, A.R.; González-Arenzana, L.; Santamaría, P. Characterization of the color parameters and monomeric phenolic composition of 'Tempranillo' and 'Graciano' wines made by carbonic maceration. *Food Chem.* **2023**, *406*, 134327. [CrossRef]
3. Antalick, G.; Perello, M.C.; de Revel, G. Esters in wines: New insight through the establishment of a database of French wines. *Am. J. Enol. Vitic.* **2014**, *65*, 293–304. [CrossRef]
4. Etaio, I.; Meillon, S.; Pérez-Elortondo, F.J.; Schlich, P. Dynamic sensory description of Rioja Alavesa red wines made by different winemaking practices by using Temporal Dominance of Sensations. *J. Sci. Food Agric.* **2016**, *96*, 3492–3499. [CrossRef]
5. González-Arenzana, L.; Santamaría, R.; Escribano-Viana, R.; Portu, J.; Garijo, P.; López-Alfaro, I.; López, R.; Santamaría, P.; Gutiérrez, A.R. Influence of the carbonic maceration winemaking method on the physicochemical, color, aromatic and microbiological features of tempranillo red wines. *Food Chem.* **2020**, *319*, 126569. [CrossRef]
6. Chinnici, F.; Sonni, F.; Natali, N.; Galassi, S.; Riponi, C. Color Features and Pigment Composition of Italian Carbonic Macerated Red Wines. *Food Chem.* **2009**, *113*, 651–657. [CrossRef]
7. Guzzon, R.; Malacarne, M.; Larcher, R.; Franciosi, E.; Toffanin, A. The impact of grape processing and carbonic maceration on the microbiota of early stages of winemaking. *J. Appl. Microbiol.* **2022**, *128*, 209–224. [CrossRef]
8. Gutiérrez, A.R.; Santamaría, P.; Olarte, C.; López-Alfaro, I.; Garijo, P.; González-Arenzana, L.; Sanz, S. Influence of microbial population on the characteristics of carbonic maceration wines. *LWT* **2022**, *166*, 113783. [CrossRef]
9. Renault, P.; Coulon, J.; de Revel, G.; Barbe, J.C.; Bely, M. Increase of fruity aroma during mixed *T. delbrueckii*/*S. cerevisiae* wine fermentation is linked to specific esters enhancement. *Int. J. Food Microbiol.* **2015**, *207*, 40–48. [CrossRef]
10. Azzolini, M.; Tosi, E.; Lorenzini, M.; Finato, F.; Zapparoli, G. Contribution to the aroma of white wines by controlled *Torulaspora delbrueckii* cultures in association with *Saccharomyces cerevisiae*. *World J. Microbiol. Biotechnol.* **2015**, *31*, 277–293. [CrossRef]
11. Chen, K.; Escott, C.; Loira, I.; del Fresno, J.M.; Morata, A.; Tesfaye, W.; Calderon, F.; Suárez-Lepe, J.A.; Han, S.; Benito, S. Use of non-*Saccharomyces* yeasts and oenological tannin in red winemaking: Influence on color, aroma and sensorial properties of young wines. *Food Microbiol.* **2018**, *69*, 51–63. [CrossRef]

12. Balmaseda, A.; Rozès, N.; Bordons, A.; Reguant, C. *Torulaspora delbrueckii* promotes malolactic fermentation in high polyphenolic red wines. *LWT* **2021**, *148*, 111777. [CrossRef]
13. Muñoz-Redondo, J.M.; Puertas, B.; Cantos-Villar, E.; Jiménez-Hierro, M.J.; Carbú, M.; Garrido, C.; Ruiz-Moreno, M.J.; Moreno-Rojas, J.M. Impact of Sequential Inoculation with the Non-*Saccharomyces T. delbrueckii* and *M. pulcherrima* Combined with *Saccharomyces cerevisiae* Strains on Chemicals and Sensory Profile of Rosé Wines. *J. Agric. Food Chem.* **2021**, *69*, 1598–1609. [CrossRef]
14. Bely, M.; Stoeckle, P.; Masneuf-Pomarède, I.; Dubourdieu, D. Impact of mixed *Torulaspora delbrueckii-Saccharomyces cerevisiae* culture on high-sugar fermentation. *Int. J. Food Microbiol.* **2008**, *122*, 312–320. [CrossRef]
15. Benito, S. The impact of *Torulaspora delbrueckii* yeast in winemaking. *Appl. Microbiol. Biotechnol.* **2018**, *102*, 3081–3094. [CrossRef]
16. Belda, I.; Navascués, E.; Marquina, D.; Santos, A.; Calderon, F.; Benito, S. Dynamic analysis of physiological properties of *Torulaspora delbrueckii* in wine fermentations and its incidence on wine quality. *Appl. Microbiol. Biotechnol.* **2015**, *99*, 1911–1922. [CrossRef]
17. Martín-García, A.; Balmaseda, A.; Bordons, A.; Reguant, C. Effect of the inoculation strategy of non-*Saccharomyces* yeasts on wine malolactic fermentation. *Oeno One* **2020**, *54*, 101–108. [CrossRef]
18. Ferrando, N.; Araque, I.; Ortís, A.; Thornes, G.; Bautista-Gallego, J.; Bordons, A.; Reguant, C. Evaluating the effect of using non-*Saccharomyces* on *Oenococcus oeni* and wine malolactic fermentation. *Food Res. Int.* **2020**, *138*, 109779. [CrossRef]
19. Ruiz-de-Villa, C.; Poblet, M.; Bordons, A.; Reguant, C.; Rozès, N. Comparative Study of Inoculation Strategies of *Torulaspora delbrueckii* and *Saccharomyces cerevisiae* on the Performance of Alcoholic and Malolactic Fermentations in an Optimized Synthetic Grape Must. *Int. J. Food Microbiol.* **2023**, *404*, 110367. [CrossRef]
20. Gutiérrez, A.R.; Santamaría, P.; González-Arenzana, L.; Garijo, P.; Olarte, C.; Sanz, S. Yeasts Inoculation Effect on Bacterial Development in Carbonic Maceration Wines Elaboration. *Foods* **2023**, *12*, 2755. [CrossRef]
21. De Man, J.C.; Rogosa, M.; Sharpe, M.E. A medium for the cultivation of *Lactobacilli*. *J. Appl. Bacteriol.* **1960**, *23*, 130–135. [CrossRef]
22. Quirós, M.; Rojas, V.; Gonzalez, R.; Morales, P. Selection of non-*Saccharomyces* yeast strains for reducing alcohol levels in wine by sugar respiration. *Int. J. Food Microbiol.* **2014**, *181*, 85–91. [CrossRef]
23. Balmaseda, A.; Aniballi, L.; Rozès, N.; Bordons, A.; Reguant, C. Use of yeast mannoproteins by *Oenococcus oeni* during malolactic fermentation under different oenological conditions. *Foods* **2021**, *10*, 1540. [CrossRef]
24. Ortega, C.; Lopez, R.; Cacho, J.; Ferreira, V. Fast analysis of important wine volatile compounds Development and validation of a new method based on gas chromatographic-flame ionisation detection analysis of dichloromethane microextracts. *J. Chromatogr. A* **2001**, *923*, 205–214. [CrossRef]
25. Ayala, F.; Echavarri, J.F.; Negueruela, A.I. A New Simplified Method for Measuring the Color of Wines. I. Red and Ros Wines. *Am. J. Enol. Vitic.* **1997**, *48*, 357–363. [CrossRef]
26. Glories, Y. La couleur des vins rouges. 2ème partie: Mesure, origine et interprétation. *Connaiss. Vigne Vin* **1984**, *18*, 253–271.
27. Niketic-Aleksic, G.K.; Hrazdina, G. Quantitative analysis of the anthocyanin content in grape juices and wines. *Lebensm. Wiss. Technol.* **1972**, *5*, 163–165.
28. Roca-Mesa, H.; Delgado-Yuste, E.; Mas, A.; Torija, M.J.; Beltran, G. Importance of micronutrients and organic nitrogen in fermentations with *Torulaspora delbrueckii* and *Saccharomyces cerevisiae*. *Int. J. Food Microbiol.* **2022**, *381*, 109915. [CrossRef]
29. Contreras, A.; Hidalgo, C.; Henschke, P.A.; Chambers, P.J.; Curtin, C.; Varela, C. Evaluation of non-*Saccharomyces* yeasts for the reduction of alcohol content in wine. *Appl. Environ. Microbiol.* **2014**, *80*, 1670–1678. [CrossRef]
30. Zhu, X.; Navarro, Y.; Mas, A.; Torija, M.J.; Beltran, G. A rapid method for selecting non-*Saccharomyces* strains with a low ethanol yield. *Microorganisms* **2020**, *8*, 658. [CrossRef]
31. Ribéreau-Gayon, P.; Dubourdieu, D.; Donèche, B.; Lonvaud, A. *Handbook of Enology, Volume 1: The Microbiology of Wine and Vinification*, 2nd ed.; John Wiley & Sons Ltd.: Chichester, UK, 2006.
32. Escribano-Viana, R.; Portu, J.; Garijo, P.; López, R.; Santamaría, P.; López-Alfaro, I.; Gutiérrez, A.R.; González-Arenzana, L. Effect of the sequential inoculation of non-*Saccharomyces*/*Saccharomyces* on the anthocyans and stilbenes composition of Tempranillo wines. *Front. Microbiol.* **2019**, *10*, 773. [CrossRef]
33. Domizio, P.; Liu, Y.; Bisson, L.F.; Barile, D. Use of non-*Saccharomyces* wine yeasts as novel sources of mannoproteins in wine. *Food Microbiol.* **2014**, *43*, 5–15. [CrossRef]
34. Salinas, M.R.; Alonso, G.L.; Navarro, G.; Pardo, F.; Jimeno, J.; Huerta, M.D. Evolution of the Aromatic Composition of Wines Undergoing Carbonic Maceration Under Different Aging Conditions. *Am. J. Enol. Vitic.* **1996**, *47*, 134–144. [CrossRef]
35. Sumby, K.M.; Grbin, P.R.; Jiranel, V. Microbial modulation of aromatic esters in wine: Current knowledge and future prospects. *Food Chem.* **2010**, *121*, 1–16. [CrossRef]
36. Ruiz-de-Villa, C.; Poblet, M.; Bordons, A.; Reguant, C.; Rozès, N. Screening of *Saccharomyces cerevisiae* and *Torulaspora delbrueckii* strains in relation to their effect on malolactic fermentation. *LWT* **2023**, *112*, 104212.
37. Gombau, J.; Pons-Mercadé, P.; Conde, M.; Asbiro, L.; Pascual, O.; Gómez-Alonso, S.; García-Romero, E.; Miquel Canals, J.; Hermosín-Gutiérrez, I.; Zamora, F. Influence of grape seeds on wine composition and astringency of Tempranillo, Garnacha, Merlot and Cabernet Sauvignon wines. *Food Sci. Nutr.* **2020**, *8*, 3442–3455. [CrossRef]
38. Minnaar, P.; Nyobo, L.; Jolly, N.; Ntushelo, N.; Meiring, S. Anthocyanins and polyphenols in Cabernet Franc wines produced with *Saccharomyces cerevisiae* and *Torulaspora delbrueckii* yeast strains: Spectrophotometric analysis and effect on selected sensory attributes. *Food Chem.* **2018**, *268*, 287–291. [CrossRef]

39. Belda, I.; Conchillo, L.B.; Ruiz, J.; Navascués, E.; Marquina, D.; Santos, A. Selection and Use of Pectinolytic Yeasts for Improving Clarification and Phenolic Extraction in Winemaking. *Int. J. Food Microbiol.* **2016**, *223*, 1–8. [CrossRef]
40. Vidana Gamage, G.C.; Lim, Y.Y.; Choo, W.S. Sources and relative stabilities of acylated and nonacylated anthocyanins in beverage systems. *J. Food Sci. Technol.* **2022**, *59*, 831–845. [CrossRef]
41. Maturano, Y.P.; Rodríguez Assaf, L.A.; Toro, M.E.; Nally, M.C.; Vallejo, M.; Castellanos de Figueroa, L.I.; Combina, M.; Vazquez, F. Multi-enzyme production by pure and mixed cultures of *Saccharomyces* and non-*Saccharomyces* yeasts during wine fermentation. *Int. J. Food Microbiol.* **2012**, *155*, 43–50. [CrossRef]
42. Morata, A.; Gómez-Cordovés, M.C.; Suberviola, J.; Bartolomé, B.; Colomo, B.; Suárez, J.A. Adsorption of anthocyanins by yeast cell walls during the fermentation of red wines. *J. Agric. Food Chem.* **2003**, *51*, 4084–4088. [CrossRef]
43. Tofalo, R.; Suzzi, G.; Perpetuini, G. Discovering the Influence of Microorganisms on Wine Color. *Front. Microbiol.* **2021**, *12*, 790935. [CrossRef]
44. Rivas-Gonzalo, J.C.; Gutierrez, T.; Hebrero, E.; Santos-Buelga, C. Comparisons of Methods for the Determination of Anthocyanins in Red Wines. *Am. J. Enol. Vitic.* **1992**, *43*, 210–214. [CrossRef]
45. Fulcrand, H.; Benabdeljalil, C.; Rigaud, J.; Cheynier, R.; Moljtounet, M. A new class of wine pigments generated by reaction between pyruvic acid and grape anthocyanins. *Phytochemistry* **1997**, *47*, 1401–1407. [CrossRef]
46. Capucho, I.; San Romão, M.V. Effect of ethanol and fatty acids on malolactic activity of *Leuconostoc oenos*. *Appl. Microbiol. Biotechnol.* **1994**, *42*, 391–395. [CrossRef]
47. Lafon-Lafourcade, S.; Geneix, C.; Ribéreau-Gayon, P. Inhibition of Alcoholic Fermentation of Grape Must by Fatty Acids Produced by Yeasts and Their Elimination by Yeast Ghosts. *Appl. Environ. Microbiol.* **1984**, *47*, 1246–1249. [CrossRef]
48. Verdugo-Vásquez, N.; Orrego, R.; Gutiérrez-Gamboa, G.; Reyes, M.; Zurita Silva, A.; Balbontín, C.; Gaete, N.; Salazar-Parra, C. Trends and climatic variability in the Chilean viticultural production zones: Three decades of climatic data (1985–2015). *OENO One* **2023**, *57*, 345–362. [CrossRef]