LWT - Food Science and Technology 223 (2025) 117746

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt

Influence of ultra-high pressure homogenization (UHPH) in the fermentability of Tempranillo musts by *Saccharomyces* and non-*Saccharomyces*

Carlos Escott [a,*,1], Cristian Vaquero [c,b,1], Iris Loira [b,1], Lara Tat [e], Carmen López [b,1], Carmen González [b,1], Juan Manuel Del Fresno [b,1], Buenaventura Guamis [d], Piergiorgio Comuzzo [e], Antonio Morata [b,1]

[a] Departamento de Farmacia Galénica y Tecnología Alimentaria, Sección Departamental de Farmacia Galénica y Tecnología Alimentaria, Facultad de Veterinaria, Universidad Complutense de Madrid, Avenida Puerta de Hierro s/n, 28040, Madrid, Spain
[b] EnotecUPM, Escuela Técnica Superior de Ingería Agronómica, Alimentaria y de Biosistemas (ETSIAAB), Universidad Politécnica de Madrid, Avenida Puerta de Hierro 2, 28040, Madrid, Spain
[c] Universidad Pontificia Comillas, Madrid Culinary Campus (MACC), Calle Alberto Aguilera 23, 28015, Madrid, Spain
[d] Centre d'Innovació, Recerca i Transferència en Tecnologia dels Aliments (CIRTTA), TECNIO, XaRTA, Departament de Ciència Animal i dels Aliments, Facultat de Veterinària, Universitat Autònoma de Barcelona, Bellaterra, 08193, Spain
[e] Department of Agricultural Food, Environmental and Animal Science, University of Udine, Via Sondrio, 2/A, 33100, Udine, Italy

ARTICLE INFO

Keywords:
Non-*Saccharomyces*
Winemaking
Non-thermal technology
UHPH
Microbiology

ABSTRACT

Ultra-high-pressure homogenization eliminates native microbiota in grapes. The microorganism's inactivation occurred at 300 MPa which reduce the size of all particles, including microorganisms, to the nanometric scale. Tempranillo musts were evaluated to see whether the physical-chemical and microbiological properties are optimal for conducting alcoholic fermentations using yeast starters, both *Saccharomyces* and non-*Saccharomyces*. To assess the ability of musts to be fermented, oenological parameters, aromatic volatile compounds, and chromatic properties of wines have been measured. The elimination of yeasts with UHPH treatments allowed the implantation of non-*Saccharomyces* yeast starters. For instance, *L. thermotolerans* produce 2 g/L lactic acid which avoided pigment loss when used in a consortium with *T. delbrueckii* strains. The possibility of having a sterile must without heat markers with required nutritional quality for yeast starters to be used is one of the features that makes UHPH interesting to be used in the winemaking industry.

1. Introduction

High-pressure techniques have been successfully used for the reduction or elimination of microorganisms in grape juice and other food products, especially over the past 40 years (Bañuelos et al., 2016; González-Arenzana et al., 2016; Sevenich et al., 2015). These techniques involve the use of high hydrostatic pressure (HHP), high-pressure homogenization (HPH), and, lately, ultra-high-pressure homogenization (UHPH). High hydrostatic pressure, also known as high-pressure processing (HPP), is a non-thermal process that occurs at pressures between 100 and 600 MPa and initial temperatures of 5° to 25 °C, conditions under which the process is considered a pasteurization process. If higher temperatures are used simultaneously, the process is considered high-pressure sterilization (HP-S) or high-pressure, high-temperature (HPHT) with initial temperatures of 70° to 90 °C (Turtoi, 2014). This process is performed in batches, which limits its implementation in certain food technologies, such as running liquid must prior to its fermentation in vinificators. On the other hand, HPH and UHPH are both techniques applied continuously and could be easily implemented as one processing step in winemaking before filling up the various fermentation tanks. The pressure achieved with the use of HPH is between 150 and 200 MPa, while the pressure achieved with UHPH is higher and can even

* Corresponding author.
E-mail addresses: carlos.escott@ucm.es (C. Escott), cvmiguel@comillas.edu (C. Vaquero), iris.loira@upm.es (I. Loira), lara.tat@uniud.it (L. Tat), carmen.lopez@upm.es (C. López), carmen.gchamorro@upm.es (C. González), juanmanuel.delfresno@upm.es (J.M. Del Fresno), Buenaventura.Guamis@uab.cat (B. Guamis), piergiorgio.comuzzo@uniud.it (P. Comuzzo), antonio.morata@upm.es (A. Morata).
[1] MALTA CONSOLIDER Team.

https://doi.org/10.1016/j.lwt.2025.117746
Received 9 July 2024; Received in revised form 10 December 2024; Accepted 5 April 2025
Available online 7 April 2025

reach the range of 300–400 MPa (Comuzzo & Calligaris, 2019).

HPH techniques reduce the size of particles through physical phenomena occurring during processing. In this regard, forces such as cavitation, shear, friction, turbulence, and impact are involved in the fragmentation of solid particles dispersed in liquid foodstuff (Guamis López et al., 2015). These forces are also known for their ability to eliminate spoilage yeast and bacteria, as well as to inactivate pathogenic microorganisms in liquid food matrices (Velázquez-Estrada et al., 2019). Other known effects include the inactivation of enzymatic activity associated with browning, which requires rapid processing of the juices, limiting contact with oxygen to avoid deterioration (Loira et al., 2018). The use of UHPH has been evaluated at different stages of the winemaking process (Puig-Pujol et al., 2023). In this regard, the feasibility of using this technique in processing grape juices has been demonstrated. From the elimination of yeast populations in white varieties (Bañuelos et al., 2020) to the reduction in the use of SO_2 during fermentation (Vaquero et al., 2022), UHPH may be a promising technology for the winemaking industry worldwide. Grape musts free of native microbiota and spoilage yeast or bacteria, especially white grape juice, are suitable for controlled populations of active dry yeast strains, among which non-*Saccharomyces* species have gained interest lately for their potential to increase and enhance the organoleptic profile of wines (Morata et al., 2023). Yeast species such as *Lachancea thermotolerans* may increase acidity through lactic acid production, improving the freshness perception of wines (Vilela, 2018); *Metschnikowia pulcherrima* may reduce alcohol volume and may also increase the production of volatile esters (Canonico et al., 2019); *Hanseniaspora* spp. could increase the production of certain volatile aromatic compounds through different metabolic pathways (Viana et al., 2009); and the species *Torulaspora delbrueckii*, which may have killer activity, could also increase the production of 3-ethoxy-propanol, together with other aromatic volatiles, and contribute to enhanced aromatic quality (Vaquero et al., 2021; Velázquez et al., 2019).

The possibility of having a sterile must without heat markers, with the required nutritional quality for yeast starters, is one of the features that makes UHPH interesting for use in the winemaking industry. What needs to be assessed is whether those nutritional aspects in either white or red musts are adequate for standard fermentation kinetics in terms of time and metabolite production.

1.1. Hypothesis

Native microbiota is known to be eliminated by UHPH, which may permit the implantation of yeast strains for winemaking. UHPH in musts has the potential to alter macromolecular polymer particle sizes, which could change the nutrients available for fermentation-related yeast assimilation. Thus, the purpose of these trials is to assess whether it is possible to provide non-*Saccharomyces* yeasts with better fermentative conditions so they can express metabolic features and implant more effectively than they would in untreated grape juices.

2. Materials and methods

2.1. Ultra-high pressure homogenization of musts

Vitis vinifera L. Tempranillo grapes were crushed using a pneumatic press, and the running musts were set at 15 °C. Two fractions of the musts were separated, one of which was used as a control and the other was treated by UHPH. The Tempranillo juice was processed utilizing a continuous UHPH system (60 L/h) developed by Universitat Autonoma de Barcelona under patent and manufactured by Ypsicon Advanced Technologies in Barcelona, Spain (EP2409583B1). The apparatus and the conditions are described in a previous essay (Bañuelos et al., 2020) and summarized as: intake temperature of 23–25 °C, output temperature of 13–15 °C, in-valve temperature of 78–85 °C for just 0.02 s, and flow rate of 60 L/h at 300 ± 3 MPa. The total volume that UHPH processed was 60 L.

2.2. Culture media and yeast population counts

The yeast strains used in this experimental setup were all isolated at the Food Technology Laboratory (enotecUPM, ETSIAAB, UPM, Madrid) except for the species *Torulaspora delbrueckii* strain Biodiva TD291 (Lallemand, Montreal, Canada) and the species *Hanseniaspora vineae* strain Hv (Facultad de Química, Universidad de la República, Uruguay). In this way, the species *Saccharomyces cerevisiae* strain 7VA, *Lachancea thermotolerans* strain L3.1, *Hanseniaspora vineae* strain Hv, *Metschnikowia pulcherrima* strain M29, and *Torulaspora delbrueckii* strain TD291 were all grown in culture media (1 % yeast extract – 2 % bacterial peptone – 2 % D-glucose) at a constant 24 °C, for 48 h prior to their use as yeast starters for either sequential or pure fermentation.

The yeast populations in both untreated and UHPH-treated musts were assessed in selective media plates prepared with CHROMagar (Laboratorios Conda, Madrid, Spain). This selective media is used to isolate fungi and to differentiate the population based on the color that different colonies adopt after the enzymatic reaction taking place through their metabolic expression. YEPD-agar media were also plated to count total populations.

2.3. Micro-fermentation trials

The micro-fermentation tests were conducted in 250 mL capacity ISO flasks. Two different musts were utilized in total, one of which was Tempranillo without any treatment and one must that was treated with UHPH as per section 2.1's instructions. Each of the two musts was subjected to sequential fermentation with five different yeast strains with the species *Saccharomyces cerevisiae* (see section 2.2). Each of these five fermentative consortiums was: *L. thermotolerans* → *S. cerevisiae* (T-L3.1), *H. vineae* → *S. cerevisiae* (T-Hv), *T. delbrueckii* → *S. cerevisiae* (T-Td), *M. pulcherrima* → *S. cerevisiae* (T-M29), and a pure fermentation with *S. cerevisiae* (T-7VA) used as control trial. The sequential fermentation was started on day 8 to ensure that all first-phase strains had enough time to produce and accumulate lactic acid and other volatile compounds at the laboratory-scale fermentation volume respectively. The same trials were performed with the same fermentative consortiums, but using UHPH-treated musts (e.g., TU-L3.1 and TU-Hv). All fermentations were carried out in triplicate and spanned until sugar depletion which did not exceed 21 days in any case. After the fermentations were completed, 5 mL of each wine triplicate were filtered with 0.45 μm MCE syringe membranes (Branchia, Dismadel, Madrid, Spain) in order to perform the analytical characterization. The remaining volume was clarified with cellulose filter sheets K800 for wine sensory analysis.

2.4. Oenological parameters

General oenological parameters were determined in grape juices and finished wines using FTIR spectrometry (FOSS, Barcelona, Spain) and a Y25 enzymatic analyzer (Biosystems, Barcelona, Spain). The analysis comprised the determination of amino nitrogen, ammonia, total sugars, and organic acids (tartaric acid and malic acid) in grape juices, while organic acids (malic acid, tartaric acid, lactic acid, acetic acid), residual sugars (glucose and fructose), ethanol, and pH were measured in finished wines. Additionally, pH values were measured with a GLP 21 Crison Instruments (Hach Lange Spain, S.L.U., Madrid, Spain). Five mL samples were filtered with 0.45 μm membrane and the trapped $CO_2$was removed with agitation.

2.5. Volatile compounds by HS-SPME-GC-MS

The method reported by Tat and colleagues (Tat et al., 2005) was slightly modified to determine volatile compounds (VOCs) using

SPME-GC-MS. A GCMS-QP2020 NX GC-MS system (Shimadzu, Kyoto, Japan) with a 2800T autosampler (HTA S.r.l., Brescia, Italy) was utilized. The samples were prepared as follows: 20 mL glass vials containing 10 mL of filtered wine were filled with 3 g of NaCl and sealed with PTFE/silicon septa. An internal standard of 100 μL of ethyl heptanoate (0.106 g/L in ethanol) was also added. A 2 cm long, 50/30 μm DVB/Carboxen/PDMS fiber (Supelco, Bellefonte, PA, USA) was used for SPME, and it was heated to 40 °C for 15 min. Before microextraction, vials were pre-conditioned in the autosampler for 15 min to enable the samples' thermal equilibration. The splitless time for injections was 60 s. The ion source was set at 200 °C, and the injector and transfer line had respective temperatures of 250 °C and 240 °C. Helium was the carrier gas, flowing at a linear pace of 36 cm/s. Based on the gradient published by Voce et al. (Voce et al., 2019), compounds were separated using a J&W DB-Wax capillary column, 30 m × 0.25 mm i.d., 0.25 μm film thickness (Agilent Technologies Inc., Santa Clara, CA, USA). The process of identifying volatile compounds involved recording electron impact mass spectra at 70 eV and comparing the resulting mass spectra and retention times with standard compounds, or comparing the mass spectra with those found in the mass spectrum libraries Wiley 6 and NIST 107. On the basis of n-alkane retention periods, linear retention indices were also computed and compared to those documented in the literature. Using the internal standard method, semi-quantitative analysis was carried out with a response factor of 1.00.

2.6. Anthocyanin content

Anthocyanins were determined using high-performance liquid-chromatography with diode array detector (HPLC-DAD) from the series 1200 (Agilent, Palo Alto, CA, USA). According to the procedure outlined by Escott et al. (Escott et al., 2017), the solvents A (water/formic acid 95:5 v/v) and B (methanol/formic acid 95:5 v/v) were utilized in a reverse-phase Poroshell 120 C18 column (Phenomenex, Torrance, CA, USA) with particle size of 2.7 μm and size of 50 × 4.6 mm. After filtering each sample through a 0.45 μm membrane, 20 μL of the mixture was injected. Malvidin-3-O-glucoside was used as a calibration curve to determine concentrations ($r^2 = 0.9999$, LOD = 0.1 mg/L).

2.7. Color parameters

The color parameters were determined with the apparatus DNA Phone Smart Analysis - Wine (Parma, Italy). The parameters comprised the CIELab coordinates: lightness (L), green-red (a), and blue-yellow (b) components, as well as the CIELChuv coordinates: chroma (C) and hue (h). The samples were placed in 1 mm path length polymethyl methacrylate cuvettes. Prior to the analysis, the samples were filtered using 0.45 μm methyl-cellulose membranes.

2.8. Total Polyphenol Index (TPI)

A UV–visible spectrophotometer 8453 from Agilent Technologies™ (Palo Alto, CA, USA) was used to determine the Total Polyphenol Index (TPI) by acquiring the absorbance at 280 nm. For TPI determination, it was necessary to dilute the samples 100 times. The measurement was done in 1 cm path length quartz cuvettes. The value of the TPI was the result of multiplying the absorbance read at 280 nm by the dilution factor.

2.9. Statistics

ANOVA and the least significant difference (LSD) test were used to analyze differences and determine the means and standard deviations. PCA and all calculations were performed with the program PC Statgraphics v.5 (Graphics Software Systems, Rockville, MD, USA). The significance was set to $p < 0.05$.

3. Results and discussion

Ten wines were obtained from the two original musts, one UHPH-treated and one used as a control, through sequential fermentation with four different consortiums of yeast strains and a pure fermentation with the *S. cerevisiae* strain. These wines were filtered and analyzed using several analytical techniques and the results were compared hereafter to determine whether the use of UHPH improved the fermentation in terms of strain implantation, sugar depletion, alcohol content, volatile production, and their influence on color.

3.1. Yeast population counts

The initial population of native yeasts was determined for treated and untreated musts as shown in Figs. S1–A. There were no counts for UHPH-treated must as the treatment eliminated the native yeast completely. This observation was previously noted and confirmed the efficacy of this technology in eliminating native microbial colonies (Bañuelos et al., 2020). On the other hand, there were 1.0E+06 CFU/mL in untreated Tempranillo musts. UHPH is capable of eliminating living microorganisms when friction, shear, cavitation, turbulence, and impact forces are applied to the liquid must at the homogenization valve (Suárez-Jacobo et al., 2010). The high population in the untreated Tempranillo must is likely related to the 24-h storage at 15 °C before the UHPH treatment. Once each inoculum was added to the musts in the fermentations flasks, the counts were done every second day during the first fermentative stage until sequential inoculation with *S. cerevisiae* (7VA). As seen in Figs. S1–B, the yeast population counts in Tempranillo musts were higher for those untreated juices. The native microbiota presents in the musts at the inoculation time increased the number of counts. Despite this, there is a more rapid decrease in the population counts in untreated musts, suggesting a more tumultuous fermentation phase compared to the treated musts, which had more constant colony counts over these six days. The population curve corresponds to a succession of yeast species from low-alcohol potential to high-alcohol potential until sugar depletion. The populations reached in treated musts did not show the increase observed in the untreated counterpart, as there was no succession of different alcohol potential species.

3.2. Enological parameters

The initial composition of the Tempranillo musts is shown in Table S1. There is no evidence of modification in the enological composition of the juices due to the homogenization process. The initial fermentative conditions, physical-chemical wise, are similar for treated and untreated musts as previously reported (Loira et al., 2018). Although all fermentations are considered finished as the concentration of sugars was depleted to below 2 g/L in most cases (Table 1), there is a slightly higher concentration of residual sugars in most wines elaborated with UHPH-treated Tempranillo musts. A correlation between sugar depletion and the homogenization process could be evaluated as an effect of the technology applied in this matrix. In terms of alcohol (% v/v), the wines produced with Tempranillo, although the results seem similar for all the fermentations, show slight differences, which in some cases are statistically significant. The average values obtained for the fermentations with untreated must were between 14.5 and 15.0 % v/v ethanol, while the average value for the UHPH-treated must were between 13.3 and 13.6 % v/v (see Table 1). In the case of treated musts, the pairs TU-Hv and TU-L3.1 had larger statistical differences between the samples with treated must and with the untreated samples, showing the lowest concentration of ethanol of all. These results are in line with the slightly higher concentration of residual sugars in those fermentations as the fermentations kinetics were slower than in the untreated must. Nonetheless, the concentration of residual sugars detected in untreated samples does not account for the slight differences observed in ethanol content. Similar observations with Cabernet Sauvignon treated with

Table 1
Physicochemical characterization of finished wines elaborated with untreated, and UHPH-treated Tempranillo musts. Enological parameters, volatiles, anthocyanins and chromatic parameters are given for all wines produced. Values are means with standard deviations for n = 3. Values in the same line that share the same letter do not differ significantly ($p < 0.05$). FTIR, GC-FID, HPLC-DAD, and DNA color space were used for the analyses.

Parameter		T-7VA	TU-7VA	T-L3.1	TU-L3.1	T-Td	TU-Td	T-M29	TU-M29	T-Hv	TU-Hv
Ethanol	%v/v	14.9 ± 0.4a	13.9 ± 0.5bc	14.9 ± 0.1a	13.3 ± 0.4c	14.9 ± 0.2a	13.8 ± 0.3bc	15.0 ± 0.1a	13.9 ± 0.1bc	14.5 ± 0.3 ab	13.6 ± 0.3c
pH		3.9 ± 0.0abc	4.0 ± 0.0a	3.9 ± 0.0bc	3.7 ± 0.0d	3.9 ± 0.0abc	3.9 ± 0.0c	3.9 ± 0.0abc	4.0 ± 0.0a	3.9 ± 0.0abc	4.0 ± 0.0 ab
Total acidity	g/L	4.0 ± 0.2bc	3.5 ± 0.1bc	4.2 ± 0.1bc	5.3 ± 0.8a	4.2 ± 0.1bc	3.4 ± 0.1c	3.8 ± 0.1bc	3.6 ± 0.1bc	4.4 ± 0.0 ab	3.7 ± 0.6bc
Volatile acidity	g/L	0.2 ± 0.0bc	0.3 ± 0.0b	0.3 ± 0.0b	0.1 ± 0.1cd	0.3 ± 0.0b	0.1 ± 0.0d	0.3 ± 0.0 ab	0.3 ± 0.0 ab	0.3 ± 0.0 ab	0.4 ± 0.1a
Malic Acid	g/L	1.5 ± 0.1 ab	1.4 ± 0.0 ab	1.6 ± 0.2a	0.8 ± 0.2c	1.7 ± 0.1a	1.2 ± 0.1bc	1.5 ± 0.0 ab	1.3 ± 0.1 ab	1.7 ± 0.1a	1.4 ± 0.4 ab
Lactic Acid	g/L	0.2 ± 0.1b	0.3 ± 0.1b	0.2 ± 0.0b	2.5 ± 1.3a	0.1 ± 0.0b	0.0 ± 0.0b	0.1 ± 0.1b	0.2 ± 0.1b	0.4 ± 0.1b	0.5 ± 0.6b
Residual sugars	g/L	1.1 ± 0.1b	1.3 ± 0.1b	1.2 ± 0.2b	1.0 ± 0.1b	1.1 ± 0.1b	0.8 ± 0.1b	1.2 ± 0.1b	1.3 ± 0.1b	1.4 ± 0.2b	2.9 ± 1.4a
Total volatiles	µg/L	1407 ± 78.0a	846 ± 39.3cd	1253 ± 86.7b	755 ± 66.3de	1411 ± 93.7a	782 ± 11.5de	1418 ± 75.7a	941 ± 29.9c	1414 ± 47.1a	710 ± 17.9e
Esters[a]	µg/L	709.1 ± 84.4 ab	347.6 ± 62.7d	559.1 ± 53.7c	131.9 ± 28.3ef	689.8 ± 52.8b	121.5 ± 2.7f	773.3 ± 36.0a	372.8 ± 49.5d	679.4 ± 33.7b	210.2 ± 21.0e
Higher alcohols	µg/L	598.4 ± 26.6a	433.4 ± 30.7d	610.6 ± 31.6a	579.5 ± 32.6 ab	620.7 ± 43.2a	621.1 ± 9.8a	548.8 ± 35.3b	493.7 ± 16.3c	621.1 ± 11.0a	425.4 ± 9.1d
Acids[b]	µg/L	95.3 ± 11.9b	59.5 ± 2.7d	77.9 ± 6.1c	37.7 ± 4.2e	94.2 ± 5.6b	33.3 ± 3.3e	92.0 ± 6.9b	69.2 ± 4.4cd	109.7 ± 6.7a	70.0 ± 5.7cd
Carbonyl and lactones[c]	µg/L	3.7 ± 1.8bc	4.8 ± 0.3abc	5.0 ± 0.4abc	5.2 ± 1.5 ab	6.0 ± 0.8a	5.2 ± 0.7 ab	3.9 ± 1.1bc	4.2 ± 0.6bc	3.5 ± 1.0c	3.7 ± 0.4bc
Terpens[d]	µg/L	0.2 ± 0.0 ab	0.2 ± 0.0bc	0.1 ± 0.0c	0.3 ± 0.1 ab	0.2 ± 0.1bc	0.2 ± 0.1 ab	0.2 ± 0.1bc	0.3 ± 0.1 ab	0.2 ± 0.0 ab	0.3 ± 0.1a
Nor-isoprenoids[e]	µg/L	0.3 ± 0.0bc	0.3 ± 0.1bc	0.3 ± 0.0bc	0.4 ± 0.1a	0.4 ± 0.2 ab	0.3 ± 0.0bc	0.2 ± 0.1c	0.3 ± 0.0bc	0.3 ± 0.1abc	0.2 ± 0.0c
Total anthocyanins	mg/L	173.1 ± 0.7bc	174.9 ± 3.5b	124.7 ± 19.6d	172.2 ± 3.6b	145.7 ± 22.9c	195.5 ± 5.7a	165.4 ± 5.3b	167.0 ± 4.2b	166.8 ± 10.2b	169.2 ± 3.5b
Vitisins	mg/L	7.9 ± 0.4cd	7.9 ± 0.4cd	12.8 ± 0.8a	9.9 ± 0.8b	7.7 ± 0.6cd	6.7 ± 0.1e	7.4 ± 0.1de	6.8 ± 0.1e	7.9 ± 0.3cd	8.4 ± 0.0c
TPI	%	15.2 ± 0.8c	16.4 ± 0.8bc	15.5 ± 0.4c	15.9 ± 0.5c	15.0 ± 0.2c	15.9 ± 1.0c	15.5 ± 1.0c	18.7 ± 0.3a	16.0 ± 0.6c	17.4 ± 1.5 ab
Chroma		45.3 ± 2.1a	48.6 ± 1.1abc	43.3 ± 1.7abc	42.3 ± 0.8bc	43.5 ± 3.7abc	42.1 ± 2.0bc	40.7 ± 1.3c	46.9 ± 1.4 ab	42.2 ± 0.9bc	45.4 ± 2.5abc
Hue	°	30.4 ± 1.7abc	26.9 ± 1.5c	28.6 ± 0.6bc	34.2 ± 3.7a	29.8 ± 1.5abc	32.3 ± 1.2 ab	29.2 ± 1.0bc	34.0 ± 0.7a	27.9 ± 0.3bc	30.9 ± 1.5abc
Luminosity		58.1 ± 2.4 ab	48.5 ± 2.0c	61.7 ± 1.6a	51.7 ± 4.8bc	62.2 ± 3.0a	56.9 ± 1.5 ab	64.2 ± 2.9a	51.9 ± 1.8bc	63.7 ± 2.8a	57.8 ± 2.8 ab

[a] Ehtyl esters. higher alcohol acetates and fatty acid ethyl esters.
[b] Fatty acids. branched acids and acetic acid.
[c] γ-butyrolactone.
[d] Linalool.
[e] β-damascenone.

UHPH and fermented with 7VA were reported (Vaquero et al., 2022). Still, this effect was not only observed for L3.1, but also for 7VA strains belonging to the species *L. thermotolerans* and *S. cerevisiae* respectively, and these results are not in line with the observations made with *Vitis vinifera* L. variety "Hondarribi zuri" also inoculated with 7VA (Loira et al., 2018). In terms of total acidity, expressed as tartaric acid equivalents (g/L), there are statistically significant differences between treated must fermented with L3.1 and the rest of fermentations. The reason is the accumulation of lactic acid in these fermentations with higher concentratins in the treated juice where the starter thrived since no competitive species were in the must at inoculation time. Similar results are obtained with other non-thermal technologies such as pulsed light when the species *L. thermotolerans* is used to compare untreated grape juices with treated musts (Escott et al., 2021). Another difference with statistical significance was the case of Hv which had higher acidity in the untreated must compared to its counterpart with treated must, due to higher levels of residual malic acid after fermentation was completed. Because of this metabolic performance, the pH values also showed statistical differences for those fermentations with L3.1 as the inoculated yeast in the initial fermentative phase, with the lowest values observed (pH 3.5). These results correspond to microfermentations which may vary from large-scale trials. The influence of dissolved oxygen, volume, and shape of the tank could eventually modify the outcomes.

3.3. Volatile profile

According to the results obtained from the analyses of volatile compounds by HS-SPME-GC-MS the volatile compounds were grouped into six different families of chemicals: esters, carbonyls and lactones, higher alcohols, acids, terpenes and nor-isoprenoids. Fig. 1 shows the media plots for the first four groups: esters, carbonyl and lactones, higher alcohols, and acids. These four plots separate the chemicals into those conditioned by the UHPH-treatment (esters and acids), and those strongly dependent on the strain (carbonyl/lactones and higher alcohols). In the first group for esters, the mean values show statistical differences for most of the pairs evaluated with the LSD method (Table 1), nonetheless, there is a relevant difference in the must of origin and the esters formed. The samples produced from untreated must have produced more esters than the global average value (CL = 459.47 µg/L; Fig. 1A), while, on the contrary, UHPH-treated musts have formed less than the low detection limit (LDL) which is 378.04 µg/L. The acids

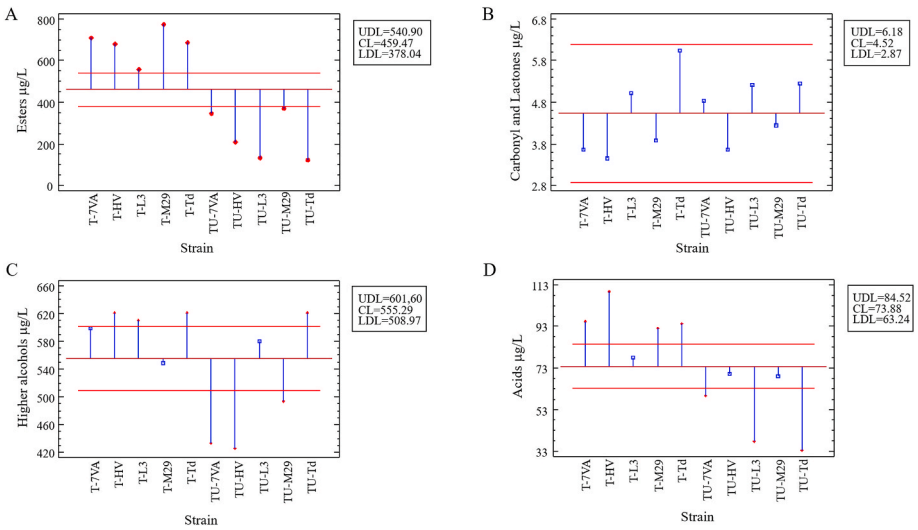

Fig. 1. Analysis for mean plots for volatiles composition. A) Esters, B) Carbonyl compounds and lactones, C) Higher alcohols, and D) Acids. UDL = upper decision limit, CL = grand mean or overall mean, ad LDL = lower decision limit.

(Fig. 1D) show a similar behavior to the esters as some of these chemicals are products of the esterification produced between acetic acid, fatty acids and branched fatty acids, and ethanol. The tendency is to accumulate fewer acids in the wines elaborated from UHPH-treated musts. This could be explained by an alteration in the structure of fatty acids during the nanofragmentation carried out in the valve of the UHPH process as it has been seen that high-pressure homogenization (HPH) is capable of modifying the composition of fatty acids in the membrane of yeast cells after the stress caused by damage to the integrity of such structures and, in some cases, producing the collapse of some areas or whole cell destruction (Serrazanetti et al., 2015). UHPH uses higher energy, which would increase the changes caused in these organic molecules present in the musts. In the case of the groups of volatiles mostly influenced by strain metabolism, there are carbonyls and lactones as well as higher alcohols. These chemicals are produced during fermentation, and through the Ehrlich pathway from the metabolism of yeast (Tufariello et al., 2021), and in certain cases, their formation may be affected by other factors such as fermentation temperature, dissolved oxygen, the grapes, and others (Muñoz et al., 2006). The plot for carbonyl and lactones (Fig. 1B) and the plot for higher alcohols (Fig. 1C) evidence this phenomenon. *L. thermotolerans* and *T. delbrueckii* are prone to accumulating slightly higher concentrations of acetoin and γ-butyrolactone which together account for more than 4.5 µg/L for both species. The redox potential when fermenting untreated musts may also play an important role in this metabolism. In the case of UHPH treated musts the concentration of these compounds, as well as higher alcohols, could be lower as the oxidative capacity is increased by a higher concentration of antioxidant compounds. The production of acetoin and higher alcohols is prone to forming as the aeration is increased or the redox potential is lower (Romano & Suzzi, 1996). The last two groups are terpenes and nor-isoprenoids, these compounds are related to flowery, muscat or fruit aromatic descriptors, in particular linalool to the first two descriptors, and β-damascenone to the latter one (Culleré et al., 2019). According to Hernández-Orte, these compounds, as well as other aromatic molecules, need the presence of precursors in the must and fermentation to take place (Hernández-Orte et al., 2008). They also observed the influence that different genera have in the synthesis of these compounds, which explains the strain-dependent relation of starters and the accumulation of these volatile compounds. Nonetheless, the relation between genus and UHPH treatments is not yet clear as some species, expected to stand out from the rest of the strains for producing more terpenes or nor-isoprenoids, are changing their performance depending on whether the must was treated or not (Table 1). A deeper study on the effect of UHPH on the overall composition of musts could be done, as terpenes and nor-isoprenoids precursors are not prone to modifying their structure during ultra-high-pressure homogenization (Puig-Pujol et al., 2023).

The overall concentration of aromatic volatile compounds in each of the fermentations is also shown in Table 1. It can be observed that, together, both volatile compounds of metabolic origin and those whose concentration has been influenced by UHPH treatment, correspond to values between 700 and 950 µg/L. These values are significantly lower than those observed in untreated musts. With average values between 1250 and 1420 µg/L. The differences are, as previously discussed, coming from higher alcohols, esters of acetic acid such as ethyl acetate, and fatty acids and branched fatty acids, whose concentration is higher in wines produced from untreated musts. As per the organoleptic impact of these aromas, the higher alcohols would provide a vinous characteristic aroma when the olfactory thresholds have been clearly exceeded. Nonetheless, they would also contribute positively to the perception of fruitiness in wines or to mask the negative character of animal aromas caused by ethyl phenols when fatty acids are in higher proportion (Culleré et al., 2019). A reduction in the concentration of higher alcohols is not expected to necessarily affect the aroma profile of wines negatively.

In summary, Figs. 2 and 3 show PCA charts illustrating the influence of the total volatiles, whether the musts were treated with UHPH or not. In the first case, Fig. 2 plots the results observed for the analysis of both conditions together. The two factors explain the 82.37 % variability of the experimental setup in this PCA chart where there is a clear separation of wines produced from untreated must labeled “Control” to the left and the wines from UHPH-treated musts to the right (Fig. 2 – up). A second PCA chart would explain the higher total concentration of volatile compounds that contributed positively to the split produced in both sub-groups. The same values were used to get an output done only using the wines produced with a specific must. The results for the untreated musts are shown in Fig. 3A, while the results for the UHPH treated musts are shown in Fig. 3B. The first PCA, with a 78.23 % variability explained in two factors, corresponds to wines from untreated musts. There is an absolute separation between wines fermented with *L. thermotolerans* from the rest when considering just factor 1. In this case, the difference is mainly produced by 1-butanol, 2-ethyl-1-hexanol, γ-butyrolactone, 2 and 3-methyl-1-butanol, and 2-phenylethanol that contributed greatly to the volatiles of these fermentations. On the other hand, the PCA shown in Fig. 3B, with 78.23 % variability explained in the same two factors, shows three differentiated groups of wines in terms of aroma.

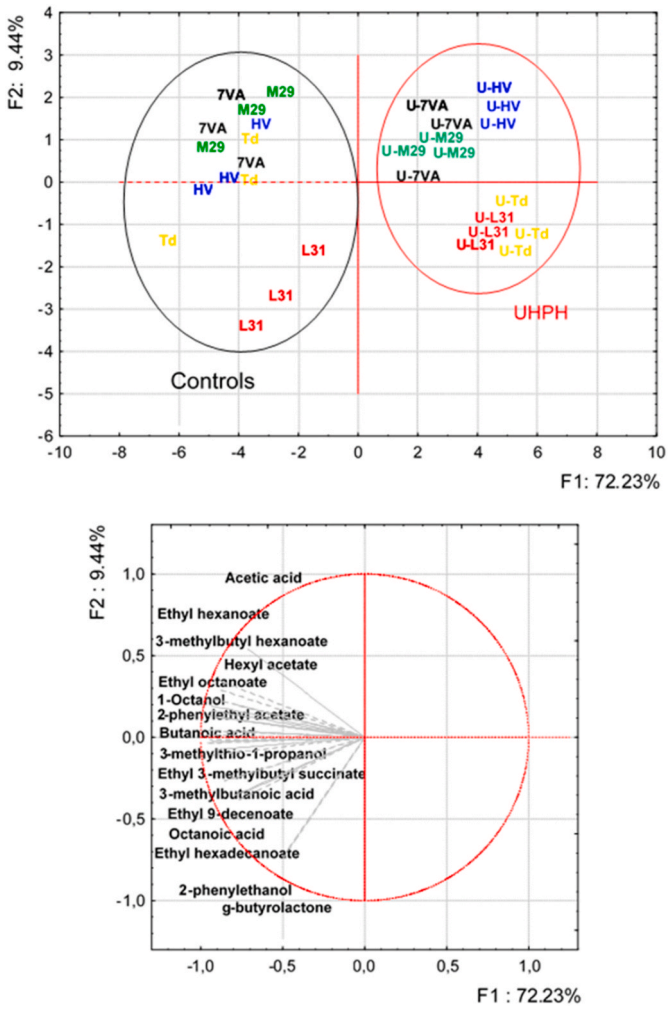

Fig. 2. Analysis of volatile compounds with PCA plots of SPME-GC-MS data for both musts, controls and UHPH (U) musts, fermented by *S. cerevisiae* (Sc7VA), *L. thermotolerans* (L31), *T. delbrueckii* (Td), *H. vineae* (Hv) and *M. pulcherrima* (M29).

The wines produced with UHPH-treated musts inoculated with *L. thermotolerans* and *T. delbrueckii* (to the left of the chart), were greatly contributed by 2-ethyl-1-hexanol, 1-butanol, γ-butyrolactone, 2 and 3-methyl-1-butanol, and 2-phenylethanol; the wines fermented with *H. vineae* (to the top), were contributed mainly by ethyl octanoate and 2-phenylethyl acetate; and the wines located to the right of the chart, fermented with *M. pulcherrima* and pure *S. cerevisiae*, were described by ethyl esters of fatty and branched fatty acids mainly. In this regard, non-*Saccharomyces* yeast strains are often associated with having a positive impact on wine aroma as they possess enzymatic activity able to increase the complexity of aroma profile by increasing 1) terpene concentration (King & Dickinson, 2000), 2) ethyl and acetate esters (Tristezza et al., 2016), 3) reducing volatile acidity and ethyl phenols (Binati et al., 2020), or 4) increasing thiols through regulated β-lyase activity in pre-fermentative stages (Zott et al., 2011).

3.4. Anthocyanins, color and TPI

Anthocyanins are responsible for the color of red wines despite not having a complex structure since these pigments, in their ionic form, absorb light in the visible spectrum at around 520 nm (Brouillard et al., 2003). Their molecular structure, one of the simplest among the anthocyanins found in the plant kingdom, is what will determine the hue, and the concentration affects the chroma values. In this regard, the difference observed in the total concentration of anthocyanins and vitisins, as well as their amounts in the finished wines can be observed in Table 1. The main anthocyanins were grouped under the label "total anthocyanins", including non-acylated or monomeric anthocyanins, acylated anthocyanins, vitisin-type anthocyanins and vinylphenolic-type anthocyanins. The values observed in this parameter suggest that the strain is more important than the UHPH treatment in terms of anthocyanins concentration (Table 1). There is only one exception with the strain TD291, where the UHPH might have had an influence in the implantation of this species as there is a statistical difference and the highest and lowest values of anthocyanins observed. In this case, *T. delbrueckii* strain TD291 had a positive impact on the concentration of anthocyanins in the wine produced from UHPH-treated must. This result correlates with the fact that *T. delbrueckii* has been reported as to adsorb less anthocyanins on the structure of the cell-wall (Minnaar et al., 2015). In the case of the untreated must, the implantation was not as successful as there is a decrease in the population of this species as it can be seen in Fig. S1. As a separate group, the vitisins are shown in an individual row since they are closely related to the evolution of the color during aging, but their formation may be related to yeast metabolism. The concentration of acetaldehyde is strain dependent and it was observed to produce moderate values for *S. cerevisiae* strains, tends to be higher for certain non-*Saccharomyces* species such as *S. pombe*, while others such as *M. pulcherrima* or *H. uvarum* strains tends to produce lower values than *S. cerevisiae* (Li & Mira de Orduña, 2017). In UHPH treated musts the implantation of starters is expected to be higher and therefore the concentration of fermentative metabolites could be associated to the metabolism of such yeast starter without competition. Conversely, when a decrease in residual acetaldehyde is caused by some non-*Saccharomyces* strains, the reduction of acetaldehyde may lead to a lower stabilization of anthocyanins through the formation of pyranoanthocyanins as a drawback, but have a positive impact by reducing SO_2 bound in wines, potentially reducing the SO2 needed as a preservative in such conditions (Li & Mira de Orduña, 2017). In this experiment, as it was already seen for total anthocyanin content, the use of UHPH does not seem to be responsible for the differences observed, but the strains used in the fermentation. In this case, the species *L. thermotolerans* produced the largest amounts of vitisins, while the lowest values were observed after fermenting the musts with the species *T. delbrueckii* and *M. pulcherrima* (Table 1).

Regarding the color expressed by the type and concentration of anthocyanins, the parameters describing the color were determined by spectrophotometry and the results are also shown in Table 1. The results indicate that the wines produced from treated must show higher hue values in general, except for *S. cerevisiae* strain 7VA, which would produce wines with more yellowish hints as it would be expected for aged wines. In this case, the increase in hue values might be related to either an increase in anthocyanin concentration after UHPH treatment as more anthocyanins express color (Morata & Guamis, 2020), or to the concentration of pyranoanthocyanins, such as vitisins and vinylphenols, which would increase the yellow fraction in wines since these molecules absorb at lower wavelengths (De Freitas & Mateus, 2011). The UHPH treatment may also play a determinant role in the evolution of the color of wines towards these hue values if the concentrations of either acetaldehyde or pyruvic acid are higher in young wines as fermentative metabolites (Marquez et al., 2013) in those cases where the starters had better implantation. In terms of chroma, the results are less consistent and have larger variability, while for luminosity, the results show a tendency to produce wines with higher values for untreated musts. As for the chroma values, there is an inversely proportional relationship to the value of luminosity; that is, the greater the chromatic value expressed by a wine, the lower its luminosity. This is because the transmittance increases as there are fewer pigments and colloidal particles in suspension and therefore affects the observed chroma value (Fig. 4). In terms of the latter parameter, the lower luminosity values obtained in UHPH-treated musts may be related to a higher stability of

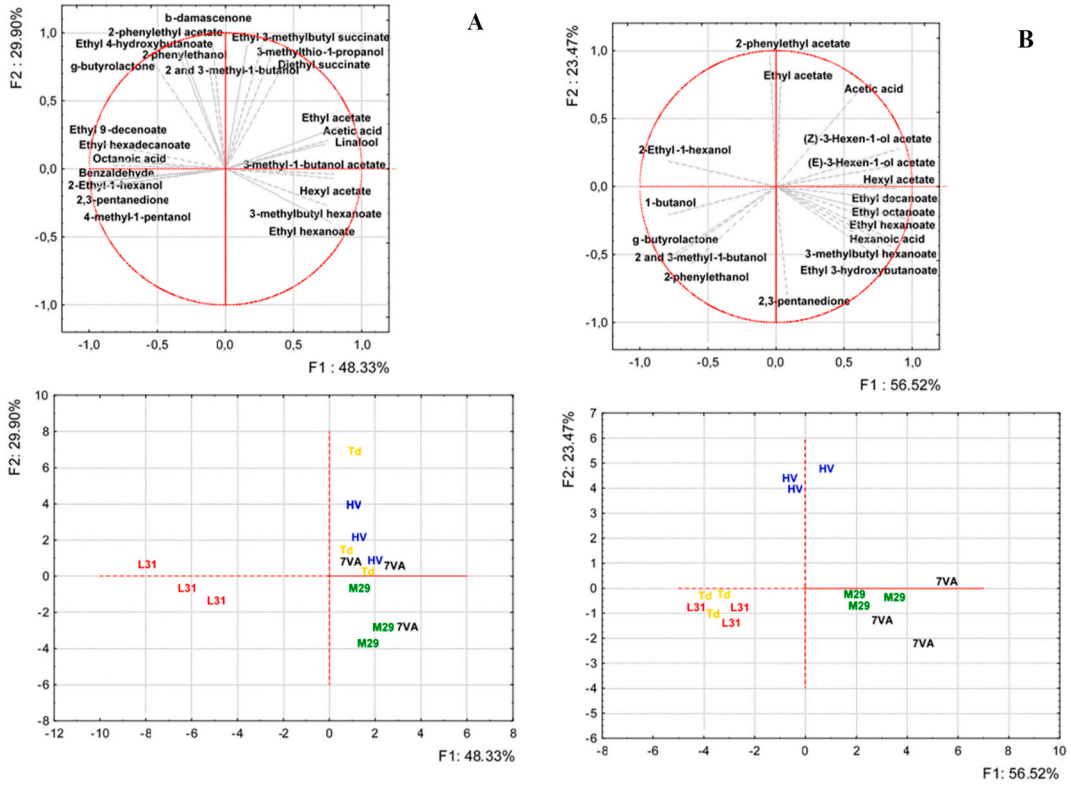

Fig. 3. Analysis of volatile compounds with PCA plots of SPME-GC-MS data in A) untreated musts fermented by *S. cerevisiae* (Sc7VA), *L. thermotolerans* (L31), *T. delbrueckii* (Td), *H. vineae* (Hv) and *M. pulcherrima* (M29); and B) in UHPH-treated musts fermented by *S. cerevisiae* (Sc7VA), *L. thermotolerans* (L31), *T. delbrueckii* (Td), *H. vineae* (Hv) and *M. pulcherrima* (M29).

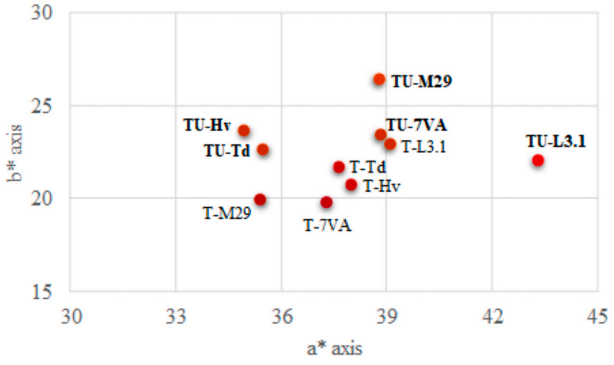

Fig. 4. CIELab parameters plot of a* and b* axis for Tempranillo wines with and without UHPH treatment. a* axis values go from negative values green to positive values magenta and b* axis go from negative values blue to positive values yellow.

the colloidal structures, including anthocyanin adducts, producing a lower transmittance. In this regard, a potential alteration of the quaternary structure of proteins in musts treated with UHPH (Morata & Guamis, 2020) would have increased the interactions with tannins/anthocyanins in suspension. On the other hand, interactions between non-stable colloidal particles would increase the removal of larger-than-colloidal-size structures which effect is observed in wines with larger luminosity values. This effect would also be responsible for having lower TPI values in wines produced from non-treated musts (Table 1). The use of UHPH may increase the antioxidant capacity of musts even if the concentration of polyphenols does not differ between untreated musts and UHPH-treated musts after the nanofragmentation (Loira et al., 2018). The changes in the structure of larger or more complex molecules after the treatment could be responsible for such changes in the antioxidant capacity.

4. Conclusions

UHPH treatment of Tempranillo red musts has slightly altered fermentation kinetics. This change is beneficial for proper fermentation, as it results in wines without residual sugars or aromatic deviations. Some advantages observed with UHPH-treated musts include: 1) increased production of carbonyl compounds, lactones, and higher alcohols when using *L. thermotolerans* and *T. delbrueckii*, indicating a strain-dependent mechanism; 2) no impact on the final concentration of anthocyanins or vitisin formation, also strain-dependent; 3) higher IPT values, suggesting greater antioxidant capacity and better wine protection; and 4) enhanced individual metabolic expression of each yeast strain by reducing competition with native colonies. Therefore, based on the results of this study, the fermentation ability of UHPH-treated musts is comparable to that of untreated musts.

CRediT authorship contribution statement

Carlos Escott: Writing – original draft, Validation, Methodology, Investigation, Data curation, Conceptualization. **Cristian Vaquero:** Writing – review & editing, Methodology. **Iris Loira:** Writing – review & editing, Methodology, Data curation. **Lara Tat:** Investigation, Data curation. **Carmen López:** Writing – review & editing, Data curation. **Carmen González:** Methodology. **Juan Manuel Del Fresno:** Software, Methodology. **Buenaventura Guamis:** Methodology, Investigation. **Piergiorgio Comuzzo:** Validation, Software, Methodology, Investigation. **Antonio Morata:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition.

Funding

This work was supported by Ministerio de Ciencia e Innovación (MICINN), Spain [grant number PID2021-124250OB-I00] and [grant number RED2022-134388-T] from Programga Estatal de Fomento de la Investigación Científico-Técnica y su Transferencia through Red de Investigación MALTA CONSOLIDER TEAM.

Declaration of competing interest

The authors declare that they have no conflict of interest and there are not financial and personal relationships with other people or organizations that could inappropriately influence their work.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2025.117746.

Data availability

Data will be made available on request.

References

Bañuelos, M. A., Loira, I., Escott, C., Del Fresno, J. M., Morata, A., Sanz, P. D., Otero, L., & Suárez-Lepe, J. A. (2016). Grape processing by high hydrostatic pressure: Effect on use of non-Saccharomyces in must fermentation. *Food and Bioprocess Technology, 9* (10), 1769–1778. https://doi.org/10.1007/s11947-016-1760-8

Bañuelos, M. A., Loira, I., Guamis, B., Escott, C., Del Fresno, J. M., Codina-Torrella, I., Quevedo, J. M., Gervilla, R., Chavarría, J. M. R., de Lamo, S., Ferrer-Gallego, R., Álvarez, R., González, C., Suárez-Lepe, J. A., & Morata, A. (2020). White wine processing by UHPH without SO2. Elimination of microbial populations and effect in oxidative enzymes, colloidal stability and sensory quality. *Food Chemistry, 332*, Article 127417. https://doi.org/10.1016/j.foodchem.2020.127417

Binati, R. L., Lemos Junior, W. J. F., Luzzini, G., Slaghenaufi, D., Ugliano, M., & Torriani, S. (2020). Contribution of non-Saccharomyces yeasts to wine volatile and sensory diversity: A study on Lachancea thermotolerans, Metschnikowia spp. and starmerella bacillaris strains isolated in Italy. *International Journal of Food Microbiology, 318*, Article 108470. https://doi.org/10.1016/J.IJFOODMICRO.2019.108470

Brouillard, R., Chassaing, S., & Fougerousse, A. (2003). Why are grape/fresh wine anthocyanins so simple and why is it that red wine color lasts so long? *Phytochemistry, 64*(7), 1179–1186. https://doi.org/10.1016/S0031-9422(03)00518-1

Canonico, Comitini, & Ciani. (2019). Metschnikowia pulcherrima selected strain for ethanol reduction in wine: Influence of cell immobilization and aeration condition. *Foods, 8*(9), 378. https://doi.org/10.3390/foods8090378

Comuzzo, P., & Calligaris, S. (2019). Potential applications of high pressure homogenization in winemaking: A review. *Beverages, 5*(3), 56. https://doi.org/10.3390/beverages5030056

Culleré, L., López, R., & Ferreira, V. (2019). The instrumental analysis of aroma-active compounds for explaining the flavor of red wines. In A. Morata (Ed.), *Red wine technology* (1st ed., pp. 283–307). Elsevier. https://doi.org/10.1016/B978-0-12-814399-5.00020-7.

De Freitas, V., & Mateus, N. (2011). Formation of pyranoanthocyanins in red wines: A new and diverse class of anthocyanin derivatives. *Analytical and Bioanalytical Chemistry, 401*(5), 1467–1477. https://doi.org/10.1007/s00216-010-4479-9

Escott, C., López, C., Loira, I., González, C., Bañuelos, M. A., Tesfaye, W., Suárez-Lepe, J. A., & Morata, A. (2021). Improvement of must fermentation from late harvest cv. Tempranillo grapes treated with pulsed light. *Foods, 10*(6), 1416–1430. https://doi.org/10.3390/foods10061416

Escott, C., Vaquero, C., del Fresno, J. M., Bañuelos, M. A., Loira, I., Han, S., Bi, Y., Morata, A., & Suárez-Lepe, J. A. (2017). Pulsed light effect in red grape quality and fermentation. *Food and Bioprocess Technology, 10*(8), 1540–1547. https://doi.org/10.1007/s11947-017-1921-4

González-Arenzana, L., Sevenich, R., Rauh, C., López, R., Knorr, D., & López-Alfaro, I. (2016). Inactivation of brettanomyces bruxellensis by high hydrostatic pressure technology. *Food Control, 59*, 188–195. https://doi.org/10.1016/J.FOODCONT.2015.04.038

Guamis López, B., Trujillo Mesa, A. J., Ferragut Péréz, V., Quevedo Terré, J. M., Lopez Pedemonte, T., & Buffa Dunat, M. N. (2015). *Continuous system and procedure of sterilization and physical stabilization of pumpable fluids by means of ultra-high pressure homogenization (Patent No. EP 2 409 583 B1)*. European Patent Office.

Hernández-Orte, P., Cersosimo, M., Loscos, N., Cacho, J., Garcia-Moruno, E., & Ferreira, V. (2008). The development of varietal aroma from non-floral grapes by yeasts of different genera. *Food Chemistry, 107*(3), 1064–1077. https://doi.org/10.1016/J.FOODCHEM.2007.09.032

King, A., & Dickinson, J. R. (2000). Biotransformation of monoterpene alcohols by Saccharomyces cerevisiae, Torulaspora delbrueckii and Kluyveromyces lactis. *Yeast, 16*(6), 499–506. https://doi.org/10.1002/(SICI)1097-0061(200004)16:6<499::AID-YEA548>3.0.CO;2-E

Li, E., & Mira de Orduña, R. (2017). Acetaldehyde kinetics of enological yeast during alcoholic fermentation in grape must. *Journal of Industrial Microbiology and Biotechnology, 44*(2), 229–236. https://doi.org/10.1007/s10295-016-1879-7

Loira, I., Morata, A., Bañuelos, M. A., Puig-Pujol, A., Guamis, B., González, C., & Suárez-Lepe, J. A. (2018). Use of Ultra-High Pressure Homogenization processing in winemaking: Control of microbial populations in grape musts and effects in sensory quality. *Innovative Food Science & Emerging Technologies, 50*, 50–56. https://doi.org/10.1016/j.ifset.2018.10.005

Marquez, A., Serratosa, M. P., & Merida, J. (2013). Pyranoanthocyanin derivedpigments in wine : Structure and formation during winemaking. *Journal of Chemistry*, 1–35. https://doi.org/10.1155/2013/713028, 2013.

Minnaar, P. P., Ntushelo, N., Ngqumba, Z., van Breda, V., & Jolly, N. P. (2015). Effect of Torulaspora delbrueckii yeast on the anthocyanin and flavanol concentrations of Cabernet franc and pinotage wines. *South African Journal for Enology & Viticulture, 36* (1), 50–58. https://doi.org/10.21548/36-1-936

Morata, A., Escott, C., Vaquero, C., del Fresno, J. M., Guamis, B., Loira, I., Bañuelos, M. A., Palomero, F., López, C., González, C., & Lepe, J. A. S. (2023). Improving the implantation of non-Saccharomyces yeasts in winemaking by UHPH processing. *BIO Web of Conferences, 68*, Article 02001. https://doi.org/10.1051/BIOCONF/20236802001

Morata, A., & Guamis, B. (2020). Use of UHPH to obtain juices with better nutritional quality and healthier wines with low levels of SO2. *Frontiers in Nutrition, 7*, 241. https://doi.org/10.3389/FNUT.2020.598286/BIBTEX

Muñoz, D., Peinado, R. A., Medina, M., & Moreno, J. (2006). Higher alcohols concentration and its relation with the biological aging evolution. *European Food Research and Technology, 222*(5–6), 629–635. https://doi.org/10.1007/S00217-005-0161-4/TABLES/3

Puig-Pujol, A., Roca-Domènech, G., Quevedo, J. M., & Trujillo, A. J. (2023). Application of ultra-high pressure homogenization (UHPH) at different stages of wine production. *BIO Web of Conferences, 68*, Article 02025. https://doi.org/10.1051/BIOCONF/20236802025

Romano, P., & Suzzi, G. (1996). MINIREVIEW origin and production of acetoin during wine yeast fermentation. *Applied and Environmental Microbiology, 62*(2), 309–315. http://aem.asm.org/.

Serrazanetti, D. I., Patrignani, F., Russo, A., Vannini, L., Siroli, L., Gardini, F., & Lanciotti, R. (2015). Cell membrane fatty acid changes and desaturase expression of Saccharomyces bayanus exposed to high pressure homogenization in relation to the supplementation of exogenous unsaturated fatty acids. *Frontiers in Microbiology, 6* (OCT), Article 159526. https://doi.org/10.3389/FMICB.2015.01105/BIBTEX

Sevenich, R., Rauh, C., Knorr, D., Ri, Q., Fhv, U., & Hoohqvlv, E. U. X. (2015). Inactivation of Brettanomyces bruxellensis by HHP technology. *59*(JUNE), 188–195.

Suárez-Jacobo, Á., Gervilla, R., Guamis, B., Roig-Sagués, A. X., & Saldo, J. (2010). Effect of UHPH on indigenous microbiota of apple juice: A preliminary study of microbial shelf-life. *International Journal of Food Microbiology, 136*(3), 261–267. https://doi.org/10.1016/J.IJFOODMICRO.2009.11.011

Tat, L., Comuzzo, P., Stolfo, I., & Battistutta, F. (2005). Optimization of wine headspace analysis by solid-phase microextraction capillary gas chromatography with mass spectrometric and flame ionization detection. *Food Chemistry, 93*(2), 361–369. https://doi.org/10.1016/J.FOODCHEM.2004.11.025

Tristezza, M., Tufariello, M., Capozzi, V., Spano, G., Mita, G., & Grieco, F. (2016). The oenological potential of hanseniaspora uvarum in simultaneous and sequential co-fermentation with Saccharomyces cerevisiae for industrial wine production. *Frontiers in Microbiology, 7*(MAY), 670. https://doi.org/10.3389/fmicb.2016.00670

Tufariello, M., Fragasso, M., Pico, J., Panighel, A., Castellarin, S. D., Flamini, R., & Grieco, F. (2021). Influence of non-Saccharomyces on wine chemistry: A focus on aroma-related compounds. *Molecules, 26*(3), 644. https://doi.org/10.3390/MOLECULES26030644, 2021, Vol. 26, Page 644.

Turtoi, M. (2014). Inactivation of Saccharomyces cerevisiae using new non-thermal technologies . A review. *Romanian Biotechnological Lettes, 19*(1), 8901–8909.

Vaquero, C., Escott, C., Loira, I., Guamis, B., del Fresno, J. M., Quevedo, J. M., Gervilla, R., de Lamo, S., Ferrer-Gallego, R., González, C., Bañuelos, M. A., Suárez-Lepe, J. A., & Morata, A. (2022). Cabernet Sauvignon red must processing by UHPH to produce wine without SO2: The colloidal structure, microbial and oxidation control, colour protection and sensory quality of the wine. *Food and Bioprocess Technology, 15*(3), 620–634. https://doi.org/10.1007/S11947-022-02766-8/FIGURES/10

Vaquero, C., Loira, I., Heras, J. M., Carrau, F., González, C., & Morata, A. (2021). Biocompatibility in ternary fermentations with *Lachancea thermotolerans*, other non-*Saccharomyces* and *Saccharomyces cerevisiae* to control pH and improve the sensory profile of wines from warm areas. *Frontiers in Microbiology, 12*, 656262–656278. https://doi.org/10.3389/FMICB.2021.656262/FULL

Velázquez, R., Zamora, E., Álvarez, M. L., & Ramírez, M. (2019). Using Torulaspora delbrueckii killer yeasts in the elaboration of base wine and traditional sparkling wine. *International Journal of Food Microbiology, 289*, 134–144. https://doi.org/10.1016/J.IJFOODMICRO.2018.09.010

Velázquez-Estrada, R., Hernández-Herrero, M., Guamis-López, B., & Roig-Saguès, A. (2019). Influence of ultra-high pressure homogenisation on physicochemical and sensorial properties of orange juice in comparison with conventional thermal processing. *International Journal of Food Science and Technology, 54*(5), 1858–1864. https://doi.org/10.1111/ijfs.14089

Viana, F., Gil, J. V., Vallés, S., & Manzanares, P. (2009). Increasing the levels of 2-phenylethyl acetate in wine through the use of a mixed culture of Hanseniaspora

osmophila and Saccharomyces cerevisiae. *International Journal of Food Microbiology, 135*(1), 68–74. https://doi.org/10.1016/J.IJFOODMICRO.2009.07.025

Vilela, A. (2018). Lachancea thermotolerans, the non-Saccharomyces yeast that reduces the volatile acidity of wines. *Fermentation, 4*(3), 56. https://doi.org/10.3390/fermentation4030056

Voce, S., Škrab, D., Vrhovsek, U., Battistutta, F., Comuzzo, P., & Sivilotti, P. (2019). Compositional characterization of commercial sparkling wines from cv. Ribolla Gialla produced in Friuli Venezia Giulia. *European Food Research and Technology, 245*(10), 2279–2292. https://doi.org/10.1007/S00217-019-03334-9/TABLES/5

Zott, K., Thibon, C., Bely, M., Lonvaud-Funel, A., Dubourdieu, D., & Masneuf-Pomarede, I. (2011). The grape must non-Saccharomyces microbial community: Impact on volatile thiol release. *International Journal of Food Microbiology, 151*(2), 210–215. https://doi.org/10.1016/j.ijfoodmicro.2011.08.026