Use of Ultra-High Pressure Homogenization processing in winemaking: control of microbial populations in grape musts and effects in sensory quality.

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Abstract

Ultra-high pressure homogenization (UHPH) is a fast and efficient technique that can sterilize fluid foods at low temperatures or even under cooling conditions. A white must (*Vitis vinifera* L.) was processed at 300 MPa (inlet temperature 20 ºC, in-valve temperature 98 ºC, outlet temperature 25 ºC, and time in valve 0.02 s) and their performance was compared with two untreated controls, a must that underwent a spontaneous fermentation (without SO_2 addition) and another must that was sulfited with 35 mg/L of total SO_2 and inoculated with the same *Saccharomyces cerevisiae* yeast as the UHPH-treated must. UHPH treatment led to the total elimination of grape microorganisms considering an initial population of $1x10^6$ CFU/mL in average of wild yeasts and fungi in must, and approximately $7x10^3$ CFU/mL of background bacteria. In a parallel assay, UHPH-processed must without yeast inoculation showed absence of fermentation for eight days at 18 ºC. The musts treated with UHPH showed a lighter appearance (10%) before fermentation compared to the control. The triangular test verified the existence of sensory differences between the wines obtained and the preference tests showed that the judges found the wine obtained from the UHPH-treated must more fruity (3.5/5 compared with 1.5-2 in controls) and with better aroma.

Industrial relevance

UHPH is an interesting way to process the must before fermentation allowing the reduction of sulfite addition while controlling wild and spoilage microorganisms.

Keywords: Ultra-High Pressure Homogenization (UHPH), grape must, wine, sulfites reduction, microbial control

1. Introduction

Pressurization technologies have been used to process must and crushed grapes to control wild grape microorganisms at low temperatures. High hydrostatic pressure (HHP) applied at 400-600 MPa for 10 min eliminates yeast counts of $1x10^4$ CFU/mL in grape must; however, it is less efficient in the elimination of lactic acid bacteria (Morata et al., 2017). It also shows some effects on skins that favor the extraction processes during grape maceration (Morata et al., 2015). However, the main drawback is that it is a batch process that is difficult to use in winemaking (Morata et al., 2017). Ultra-high pressure homogenization (UHPH) is also a high pressure technology, but operates in continuous mode. Antimicrobial effect is produced when fluid is pumped at 200-600 MPa and go through a "special valve" before expansion. During the process, microorganisms and colloidal particles suffer both strong shear forces and impact, which not only cause the complete destruction of living microorganisms but also spores (Amador Espejo, Hernández-Herrero, Juan, & Trujillo, 2014), thus producing sterilization. All particles are reduced to a range size of 100-300 nm (Zamora & Guamis, 2015). The mechanical effect due to the hypersonic speed reached in the valve and the subsequent depressurization produce an intense fragmentation of cells and particles. The only requirement for processing by UHPH is that the particles in the fluid must be less than 500 μm in size. Both HHP and UHPH can be referred to as cold pasteurization treatments that are sensory-protective because the processes do not affect covalent bonds; pigments, aroma (Bermúdez-Aguirre & Barbosa-Cánovas, 2011; Oey, Van der Plancken, & Van Loey, 2008). In UHPH, the exposure time to the peak process temperature is less than 0.2 seconds, and therefore, without significant thermal repercussion (Ypsicon, 2018).

Industrial UHPH equipment are currently available with capacities up to 50.000 liters/hour based on modular systems. Moreover, power consumption is approximately 50% lower than HPP (Ypsicon, 2018).

The use of UHPH or HHP in must processing is a clear alternative to the use of sulfites to control wild spoilage microorganisms that can also affect the fermentation performance and sensory quality of the wine (Morata et al., 2015; Puig, Olmos, Quevedo, Guamis, & Mínguez, 2008). That is especially interesting when modern fermentation biotechnologies are used such as fermentation with non-*Saccharomyces* yeasts or co-inoculation using yeast and bacteria mixtures to perform simultaneous malolactic and alcoholic fermentations (Bañuelos et al., 2016).

UHPH at 150 MPa has been also described as a way to accelerate biological ageing processes like ageing on lees (Comuzzo et al., 2015, 2017). The homogenization effects by high pressure produce the lysis and de-polymerization of yeast cell walls releasing polysaccharides and mannoproteins that affect mouthfeel improving wine softness. Moreover, UHPH can be used to modulate the autolytic capacity of yeast starters used to age sparkling wines (Patrignani et al., 2013).

The aim of this work was to check the effectiveness of UHPH in the control of wild microorganisms in grape musts and evaluate the enological and sensory parameters of the wines obtained after fermentation with *Saccharomyces cerevisiae* compared to control wines produced from sulfited must or spontaneously fermented must.

2. Materials and Methods

2.1 Must preparation

Grapes from *Vitis vinifera* L. variety "Hondarribi zuri" were pressed using a pneumatic press and running must was settled at 4 ºC. Clean must was separated in three batches: i) sulfited at 35 mg/L of total SO_2, ii) UHPH processed and iii) untreated. UHPH sterilization was performed using a continuous device (150 L/h) patented by UAB (EP2409583) and manufactured by Ypsicon Advance Technologies (Barcelona, Spain) working at 300±3 MPa, an inlet temperature of 20°C, valve temperature of 98°C reached at 0.02 s, and outlet temperature of 25°C (detailed temperatures and pressures included as supplementary material). Initial must parameters are described in **Table 1**.

2.2 Fermentations and microbial counts

Fermentations were performed in 2-L flasks with 1.8 L of must in triplicate at 18 ºC. Fermenters were inoculated with 50 mL starters of a 24-hours culture in YPD broth (10 g/L yeast extract, 20 g/L peptone, 20 g/L glucose. Supplied by Conda, Madrid, Spain) containing $1x10^8$ CFU/mL. The population in the fermenters after inoculation was checked by plating being $1x10^5$ CFU/mL. The yeast strain used was *Saccharomyces cerevisiae* 7VA (enotecUPM, Spain).

As control, a parallel assay was performed in which another three batches of each processing method (sulfited, UHPH and non-treated) were placed in 100-mL vials with 50 mL of must and allowed to ferment with the wild population. These flasks were sealed with Müller valves and the fermentation development was monitored gravimetrically recording weight losses by the release of CO_2. Each fermentation was performed in triplicate and isothermally controlled at 18 ºC.

Microbiological analyses were performed in musts after UHPH treatments and in wines at the end of fermentation. Serial decimal dilutions in saline peptone (0.85% NaCl with peptone at 0.1%) were pour-plated (1 mL) in selective media for total aerobic bacteria

and lactic acid bacteria and 100 µL were spread-plated for yeasts. The media were: Glucose chloramphenicol agar (GCA) incubated aerobically during 4 days at 25 ºC (yeast); synthetic lysine agar (Oxoid, Hampshire, UK) for non-*Saccharomyces* counts (Heard & Fleet, 1986); PCA supplemented with nystatin (50 mg/L) after sterilization, and incubated during 3 days at 30 ºC (aerobic bacteria); MRS agar supplemented with nystatin (50 mg/l) after sterilization and incubated during 4 days at 30 ºC in anaerobic conditions in a jar under CO_2 atmosphere (lactic acid bacteria). GCA and MRS media were purchased from Pronadisa (Barcelona, Spain).

2.3 Enological parameters by Infrared spectroscopy

The equipment OenoFoss™ (FOSS Iberia, Barcelona, Spain) using Fourier transform infrared spectroscopy (FTIR) was used to identify and quantify major compounds such as residual sugars, organic acids, total and volatile acidity (Urbano-Cuadrado, Luque De Castro, Pérez-Juan, García-Olmo, & Gómez-Nieto, 2004). This technique also determines pH value.

2.4. Analysis of organic acids and residual sugars

Lactic acid, malic acid and residual sugars were measured enzymatically (Peynaud, Blouin, & Lafon-Lafourcade, 1966) using an Y15 enzymatic autoanalyzer (Biosystems, Barcelona, Spain).

2.5. Ethanol quantification

Ethanol was analyzed by liquid chromatography with refractive index detection (LC-RI) using a Waters e2695 apparatus (Milford, Massachusetts, USA) equipped with a 2414 Refractive Index Detector. Analyses were performed using a Phenosphere XDB C18

column (4.6 x 150 mm, 5-µm particle size) (Phenomenex, Torrance, CA, USA). The solvent was Milli-Q water (in isocratic mode) at 0.4 mL/min. The temperature was set at 30 ºC both in the column and in the detector. Calibration was performed using an external ethanol standard (Panreac, Barcelona, Spain). Samples were injected after filtration through 0.45-µm cellulose methyl ester membrane filters (Tecknokroma, Barcelona, Spain). The injection volume was 2 µL.

2.6. Analysis of volatile compounds by gas chromatography with flame ionization detection (GC-FID)

Volatile compounds were determined using an Agilent Technologies 6850 gas chromatograph (Network GC System) equipped with an integrated flame ionization detector (GC-FID). A DB-624 column (60 m x 250 µm x 1.40 µm) was used. The following compounds were used as external standards for calibration (r^2>0.999): acetaldehyde, diacetyl, acetoin, methanol, 1-propanol, 1-butanol, 2-butanol, isobutanol, 2-methyl-1-butanol, 3-methyl-1-butanol, hexanol, 2-phenylethyl alcohol, 2-phenylethyl acetate, 2,3-butanediol, ethyl acetate, isoamyl acetate, isobutyl acetate, ethyl butyrate and ethyl lactate. 4-Methyl-2-pentanol was used as internal standard. All compounds were purchased from Fluka (Sigma–Aldrich Corp., Buchs SG, Switzerland). The injector temperature was 250 ºC, and the detector temperature 300 ºC. The column temperature was 40 ºC (5 min), rising linearly by 10 ºC/min until 250 ºC; this temperature was then held for 5 min. Hydrogen was used as carrier gas. The injection split ratio was 1:10, the in-column flow rate 2.2 L/min, and the detection limit 0.1 mg/L. One-hundred microliters of internal standard (500 mg/L) were added to 1-mL test samples and filtered through syringe membrane filters (pore size 0.45-µm) (Teknokroma, Barcelona, Spain). They

were then placed in 2-mL glass vials sealed with a PTFE/silicon septum. One microliter of this filtrate was injected into the GC apparatus.

2.7 Color measurements and phenols

The color of wine has been determined by the use of a UV-visible (UV-Vis) spectrophotometer 8453 from Agilent Technologies™ (Palo Alto, CA, USA) with a photodiode array detector and the use of a 1-cm path length quartz cuvette. The absorption at three different wavelengths (420 nm, 520 nm and 620 nm) was used to compare color intensity and hue in all wines after fermentation was complete.

Total polyphenol index (TPI) was measured after dilution 1:10 with milli-Q water in 1-cm path length quartz cuvette at 280 nm. Hydroxycinnamic acids were also estimated by measuring the absorbance at 320 nm in the same conditions.

2.8 Determination of Polyphenol oxidase (PPO) activity

Polyphenol oxidase (PPO) activity was determined according to the method described by Cano, Hernandez, & De Ancos (1997) with slight modifications. PPO determination consisted on mixing 3 mL of a solution based on a 0.07 M catechol solution and 0.05 M sodium phosphate buffer (pH 6.5) with 150 µL of the sample. The absorbance change was spectrophotometrically monitored (UV2310, Dinko Instruments Ltd., Barcelona, Spain) at 420 nm during 10 min at 25 ºC.

2.9 Antioxidant activity: FRAP assay

The reducing antioxidant power by the ferric reducing ability of plasma (FRAP) method was used according to a modified version of Benzie & Strain (1996). A daily FRAP

reagent was prepared by mixing 25 mL of 0.3 mM acetate buffer (pH 3.6) with 2.5 mL of 10 mm TPTZ solution in 40 mM HCl and 2.5 mL of 20 mM Ferric chloride ($FeCl_3.6H_2O$). After heating the FRAP reagent to 37 ºC, 900 µL of the reagent were allowed to react with 30 µL of sample and 90 µL of water. Readings were taken after 8 min at 37 ºC at the wavelength of 593 nm against an acetate buffer blank. Quantification was based on the standard curve ranged from 0-1000 µM of Trolox. Antioxidant capacity was expressed as mM of TE (Trolox Equivalents).

2.10 Sensory evaluation

A preference test was developed to assess the quality of the wines. A panel of nine experienced tasters (age range: from 30 to 60 years old, 4 women and 5 men) evaluated the wines. The blind tasting took place in the tasting room of Chemistry and Food Technology Department, Universidad Politécnica de Madrid, provided with fluorescent lighting and presenting samples in random order. The wines (20 mL/tasting glass) were served at 20 ± 2 ºC in three different standard odor-free wine-tasting glasses. Briefly, the panelists used a scale from 0 to 5 to rate the intensity of different attributes (0 = attribute not perceptible, 5 = attribute strongly perceptible). Each panelist also provided an overall impression of the wines produced, taking into account olfactory and taste features, including any defect.

2.11 Statistical analysis

Means and standard deviations were calculated and differences examined using ANOVA and the least significant difference (LSD) test. All calculations were made using PC Statgraphics v.5 software (Graphics Software Systems, Rockville, MD, USA).

Significance was set at $p<0.05$. Fermentations of each treatment proceeded in triplicate and chemical and microbiological analyses were done of each replicate. These data were used in the statistical treatments.

3. Results and discussion

After must treatments, a higher limpidity with very low size colloidal particles in UHPH processed must was observed. However, sulfited must showed bigger colloidal particles and phase separation in a liquid fraction and pectin fragments in the bottom of the fermenters. This is explained because of the intense homogenization by UHPH processing producing nanoparticles lower than 300 nm of molecular size (Zamora & Guamis, 2015).

3.1 Antimicrobial effect of UHPH

UHPH has demonstrated higher efficiency in controlling spores (Amador Espejo et al., 2014) and bacteria than discontinuous HHP processes because of the intense shear forces to which the liquid is subjected when it crosses the valve and undergoes a strong decompression. Microbial analyses were focused in wild microorganisms typically found in grapes and must (fungi, *Saccharomyces* and non-*Saccharomyces* yeasts and lactic acid bacteria). Yeast and bacteria counts were similar in sulfited and untreated musts. *Saccharomyces* yeasts were in average $1x10^6$ CFU/mL (**Figure 1a**), non-*Saccharomyces* in lysine media in the same value, and the bacterial counts measured in PCA and MRS were in average $7x10^3$ CFU/mL. No yeasts were detected in the must processed by UHPH (LOD 10 CFU/mL) (**Figure 1a**). Hence, at the end of fermentation, only the inoculated yeast can be found in UHPH-processed must, with a total absence of non-*Saccharomyces*

(**Figure 1b**) which shows the effectiveness of the treatment. Conversely, non-*Saccharomyces* wild yeasts can be found at 2 log CFU/mL in the sulfited must. Therefore, the wild yeasts remain in the must throughout the entire fermentation. The presence of non-*Saccharomyces* yeasts was more noticeable in the non-treated must, being higher than 3 log CFU/mL at the end of fermentation. Vegetative bacteria populations were also eliminated from the must by the UHPH treatment (LOD 1CFU/mL), and remained undetected at the end of fermentation (**Figures 2a, b**). This result highlights the intense antimicrobial effect of UHPH for all the microbial groups analyzed, even considering the high initial microbial load in the must. HHP treatments have previously demonstrated high efficiency in yeasts control at 400 MPa-10 min, but some residual populations of bacteria still remain even at 550 MPa-10 min (Morata et al., 2015). The use of UHPH allows winemakers to avoid the use of SO_2 to control apiculate yeasts and bacterial populations, but also facilitates the implantation of non-*Saccharomyces* starters when the use of these unconventional yeasts is desired to improve the sensory profile. This agrees with the same application previously described for HHP processing (Bañuelos et al., 2016). By using UHPH processing, it is possible to reduce the SO_2 doses only to the suitable levels to control oxidations due to the total antimicrobial effect produced by continuous pressurization. Moreover, the use of emerging antioxidants such as glutathione (GSH) opens new possibilities to strongly reduce SO_2 in wines (Kritzinger, Bauer, & Du Toit, 2013).

To evaluate how is the evolution of these wild populations over time, 50 mL of each must (not inoculated) were left to ferment during 8 days. A fast evolution of the populations in the sulfited must was observed, with strong fermentation on day 3 and reaching approximately 10 %v/v of ethanol in 8 days (**Figure 3**). The untreated control showed a slower fermentation, probably because the absence of sulfites promoted a greater

development of non-*Saccharomyces* yeasts with lower fermentative power, thus reaching only 5 % v/v of ethanol in the same period. The fermentation did not occur in the UHPH-treated must as the weight of the fermenters remained at the initial value during the 8 days of the trial (**Figure 3**). The lack of fermentation for 8 days supports the absence of viable but non-culturable yeasts that sometimes can be detected when microorganisms are processed by discontinuous HHP (Lado & Yousef, 2002)

3.2 Enological parameters in must before fermentation

The sugar content in the musts is typical of Txacoli wines (**Table 1**), normally ranging between 9-12 % v/v in alcoholic degree because of the early harvest that gives them their distinctive sensory profile. Consequently, the acidity is high and, correspondingly, the pH is quite low (3.2-3.3) in these musts. The levels of yeast assimilable nitrogen (YAN) compounds, α-amino nitrogen and ammonia, were a little bit lower in the musts dosed with SO_2, but the levels found were enough for a correct fermentation. It has been described that 150 mg/L is a suitable YAN value to avoid sluggish or stuck fermentations (Henschke & Jiranek, 1993). The absence of volatile acidity is considered an indicator of grape health, because normally it increases when undesirable bacteria grow uncontrollably before the alcoholic fermentation. In this case, the grape/must quality is quite good (**Table 1**). Similarly, gluconic acid is not detected, which corroborates the quality of the must. It is used as an indicator of fungal developments, since it is produced by *Botrytis cinerea*'s metabolism (Cinquanta et al., 2015).

3.3 Enological parameters in wine after fermentation

After fermentation, wines from sulfited must reached an alcoholic strength of 10 % v/v ethanol and about 9 % v/v in both the UHPH treatment and the untreated control, as expected, according to the initial amount of sugars (**Table 2**). All fermentations finished with very low levels of residual sugars (below 0.2 g/L). The concentrations of malic acid (above 2 g/L) and the absence of lactic acid indicate that malolactic fermentation did not occur. The low levels of acetic acid (0.2 g/L in UHPH and sulfited treatments) indicate the purity of the alcoholic fermentation (Loira et al., 2014). However, in control fermentation without SO_2, the values were a little higher than normal (**Table 2**), probably because of the greater population of bacteria and non-*Saccharomyces* yeasts that remain uncontrolled in absence of SO_2 (**Figures 1b and 2b**). This is a typical situation in spontaneous fermentations with predominance of apiculate yeasts at the beginning of fermentation (Fleet, 2003). The content of glycerol was higher in wines from sulfited must because of the binding effect of SO_2 on acetaldehyde which delays its reduction to ethanol and increases the production of glycerol (Wang, Zhuge, Fang, & Prior, 2001).

3.4 Fermentation volatiles by GC-FID

Sulfited wines showed the highest levels of volatile compounds (**Table 3**), mainly due to the concentration of higher alcohols, which are undesirable because they normally produce a winey aroma typical of low quality wines. Non-treated and especially UHPH-processed wines had lower values of higher alcohols, allowing thus to show fruity or varietal smells with better aromatic repercussion. Higher concentrations of esters can be observed in wines from must processed by UHPH or untreated. These compounds commonly produce fruity smells in wines and therefore increase the aromatic complexity. Ethyl acetate is an ester that produces complexity but it can be defective at high

309 concentration. Normal values in wines are between 30-80 mg/L, but it produces spoilage
310 notes when present in concentrations higher than 150 mg/L (Zoecklein, Fugelsang,
311 Gump, & Nury, 1995). All techniques showed suitable values of ethyl acetate, but slightly
312 higher and with a bigger standard deviation in the wines from the untreated must,
313 probably because of the greater populations of bacteria and non-*Saccharomyces* yeasts.
314 Especially interesting were the values of isobutyl acetate and isoamyl acetate related to
315 fruits like pear and banana (Loira et al., 2015) that were undetected in the wines from the
316 sulfited must and with higher concentrations in the wines from the UHPH-treated must.
317 Similar behavior is showed by 2-phenylethyl acetate (rose petal smell) (Molina et al.,
318 2009) with higher concentrations in non-treated fermentations but also in UHPH
319 processed. In non-treated fermentations, the levels of volatile compounds can be favored
320 by the presence of non-*Saccharomyces* yeasts (Ciani, Comitini, Mannazzu, & Domizio,
321 2010; Viana, Belloch, Vallés, & Manzanares, 2011; Viana, Gil, Vallés, & Manzanares,
322 2009). Ethyl butyrate with fruity profile and ethyl lactate with toffee descriptors were also
323 found in higher concentrations in the wines obtained from the UHPH or non-treated
324 musts.

325 It is also remarkable that UHPH processed musts after fermentation showed a lower
326 concentration of hexanol than in the non-treated fermentations and especially in the
327 sulfited musts (**Table 3**). Hexanol is responsible for the herbaceous or grassy hints in
328 wine aroma (Peinado, Moreno, Bueno, Moreno, & Mauricio, 2004) which negatively
329 affect sensory quality. Concerning defective buttery notes, levels of acetoin were similar
330 in all fermentations regardless of the must treatment and within the suitable values.

331

332 **3.5. Enzymatic and antioxidant activity**

Enzymatic activities were measured in sulfited and UHPH-treated musts. A higher degree of inactivation for PPO due to UHPH treatment was achieved compared to SO_2 must. Considering 100 % of PPO activity the value given by sulfited must, UHPH sample diminished up to 90 % their activity. Suárez-Jacobo et al., (2012) reported complete inactivation of PPO in apple juice treated at 300 MPa. Grape juices containing PPO enzymes are more prone to suffer oxidative reactions, trigger darkening reactions during winemaking and thus decreasing the quality of the final wine (Hendrickx, Ludikhuyze, Van den Broeck, & Weemaes, 1998).

Slight differences were found between sulfited and UHPH musts when determined antioxidant activity by the FRAP assay. Sulfited samples reached values of 1.83 ± 0.36 mM of TE (Trolox Equivalents) while UHPH-treated must obtained 2.67 ± 0.41 mM of TE. This indicated better antioxidant capacity in musts with UHPH treatment. The antioxidant activity of a must or a wine is largely dependent on its phenolic content and composition, as different compounds and their combinations exhibit varying degrees of activity (Salaha, Kallithraka, Marmaras, Koussissi, & Tzourou, 2008). Although no differences were observed in TPI data between must with SO_2 and UHPH-processed (**Figure 4**), UHPH treatment could produce changes in the molecules of the matrix (amino acids, peptides, sugars among others) that could affect this activity.

3.6 Color, phenols and sensory evaluation

Higher color intensity was measured in non-treated wine probably by browning oxidative processes because of the absence of SO_2 (**Figure 4a**). As expected, the lowest values were reached in the must processed with sulfites. UHPH wine showed intermediate values with significant differences ($p<0.05$) regarding non-treated and sulfited wine. No significant differences were found in the tonality of all the wines (**Figure 4a**). Sulfited wine and

UHPH-processed wine showed similar values of TPI and a slightly higher value was found in the untreated wine (**Figure 4b**). Higher values of hydroxycinnamic acids (HCAs) were observed in UHPH wine and lower concentrations in untreated samples, probably by the mechanical effect of UHPH processing. Maybe due to inactivation of PPO by UHPH.

As for the sensory analysis, UHPH wine was better evaluated in global quality, but especially in aromatic profile, and described as fruitier by the panelists than either untreated or sulfite added wines (**Figure 5**). This is in accordance with the higher values of esters found in UHPH wines (**Table 3**) compared to sulfited wines. Tasters were able to detect lower color intensity in the sulfited wine. However, no significant differences ($p<0.05$) were detected in tonality between UHPH and sulfited wines in agreement with the spectrophotometric analysis. The sulfited wine was described as more herbaceous than either untreated or UHPH-processed wines, what is also in accordance with the higher hexanol concentrations observed by GC-FID (**Table 3**). The untreated wine showed higher reduction hints.

4. Conclusions

UHPH is a fast and effective technique to remove wild microorganisms in grape must facilitating the implantation of yeast starters and the use of new biotechnologies such as the sequential fermentations with non-*Saccharomyces* yeasts. Moreover, compared with previous reported results, UHPH shows better effectiveness against lactic acid bacteria than traditional HHP. The processed must can be fermented with lower sulfite levels.

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Conflict of interest

The authors declare that there is no conflict of interest.

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514 **Tables**

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Table 1. Enological parameters of musts sulfited, processed by UHPH or untreated before fermentation. Values are means with standard deviations, n=3. Values with the same letter in the same row are not significantly different ($p<0.05$). Analyses were performed by FTIR.

	Must with SO_2	UHPH processed	Non treated
Sugars (g/L)	169.9 ± 1.1c	151.9 ± 0.3b	147.1 ± 2.2a
TSS (ºBrix)	16.1 ± 0.2c	14.6 ± 0.1b	14.2 ± 0.2a
Total acidity (g/L)	5.9 ± 0.1a	6.6 ± 0.1b	6.5 ± 0.2b
pH	3.2 ± 0.0a	3.3 ± 0.0a	3.3 ± 0.0a
Volatile acidity (g/L)	0.1 ± 0.0b	0.1 ± 0.0b	0.0 ± 0.0a
α-amino N (mg/L)	149.9 ± 4.5a	192.3 ± 6.1b	196.8 ± 6.1b
Ammonia (mg/L)	112.6 ± 1.3a	167.4 ± 11.8b	173.4 ± 9.9b
Gluconic acid (g/L)	nd	nd	0.1 ± 0.1

nd: Not detected

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Table 2. Enological parameters of the wines produced from the musts sulfited, processed by UHPH or untreated. Values are means with standard deviations, n=3. Values with the same letter in the same row are not significantly different ($p<0.05$). Analysis of organic acids, residual sugars and glycerol were made by enzymatic tests. Ethanol was analyzed by LC-RID and pH with a pH-meter.

	Must with SO_2	UHPH processed	Non treated
Malic acid (g/L)	2.3 ± 0.0a	3.1 ± 0.0b	3.1 ± 0.0b
Lactic acid (g/L)	nd	nd	nd
Acetic acid (g/L)	0.2 ± 0.0a	0.2 ± 0.0a	0.6 ± 0.4a
Residual sugars (g/L)	0.1 ± 0.1a	0.2 ± 0.1a	0.1 ± 0.1a
Glycerol (g/L)	9.7 ± 0.1c	7.4 ± 0.1a	8.1 ± 0.1b
Ethanol (% v/v)	10.1 ± 0.1b	8.7 ± 0.1a	8.7 ± 0.1a
pH	3.1 ± 0.0a	3.1 ± 0.0a	3.1 ± 0.0a

nd: Not detected

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Table 3. Fermentative metabolites analyzed by GC-FID produced in musts sulfited, processed by UHPH or untreated after fermentation with *S. cerevisiae* (7VA). Values are means with standard deviations, n=3. Values with the same letter in the same row are not significantly different ($p<0.05$). Concentrations in mg/L.

	Must with SO_2	UHPH processed	Non treated
Acetaldehyde	94.43 ± 19.94b	32.89 ± 3.18a	29.92 ± 8.72a
Diacetyl	nd	nd	nd
Acetoin	8.05 ± 0.46a	7.82 ± 0.59a	8.65 ± 1.21a
Methanol	52.86 ± 0.85b	31.30 ± 4.34a	32.68 ± 0.95a
1-Propanol	26.20 ± 0.54a	42.40 ± 3.45c	36.33 ± 0.63b
2-Butanol	nd	nd	nd
Isobutanol	79.88 ± 3.11c	62.27 ± 3.79b	55.91 ± 2.25a
1-Butanol	nd	4.01 ± 0.08	nd
2-Methyl-1-butanol	270.15 ± 9.79c	87.04 ± 4.52a	132.07 ± 0.64b
3-Methyl-1-butanol	68.61 ± 2.04c	22.48 ± 0.53a	30.22 ± 1.01b
Hexanol	7.41 ± 0.11c	5.01 ± 0.63a	6.03 ± 0.32b
2-Phenyl ethanol	49.58 ± 2.89c	22.64 ± 1.95a	27.91 ± 0.46b
2,3 butanediol	466.30 ± 3.17c	383.77 ± 17.16b	344.38 ± 5.39a
Ethyl acetate	27.62 ± 0.88a	61.24 ± 2.84b	69.56 ± 10.78b
Isoamyl acetate	nd	5.05 ± 0.25a	5.25 ± 0.90a
Isobutyl acetate	nd	1.25 ± 0.04a	0.85 ± 0.74a
Ethyl butyrate	nd	1.59 ± 0.05a	1.54 ± 0.01a
Ethyl lactate	4.21 ± 3.65a	6.33 ± 0.09a	6.27 ± 0.14a
2-Phenylethyl acetate	nd	5.52 ± 0.06b	5.37 ± 0.09a
Higher alcohols	501.84 ± 18.24c	245.85 ± 8.59a	288.48 ± 1.00b
Esters	31.84 ± 3.72a	80.98 ± 2.71b	88.84 ± 9.22b
Total volatiles	1,155.32 ± 36.33b	782.61 ± 24.45a	792.96 ± 11.64a

nd: Not detected

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Figure captions

Figure 1. Wild yeast counts in musts sulfited, processed by UHPH or untreated before fermentation (A) and 9 days after the beginning of fermentation (B). In B, the observed yeasts are wild species together with the inoculated *S. cerevisiae*. Plating media used were GCA for total yeasts and fungus, Lysine media for non-*Saccharomyces* yeasts. Values are means with SD of 3 independent fermentations. Different letters indicate significant differences between means ($p < 0.05$). nd: not detected, LOD 10 CFU/mL.

Figure 2. Wild bacteria counts in musts sulfited, processed by UHPH or untreated before fermentation (A) and 9 days after the beginning of fermentation (B). Plating media used were PCA for aerobic bacteria, and MRS for lactic acid bacteria. Values are means with SD of 3 independent fermentations. Different letters indicate significant differences between means ($p < 0.05$). nd: not detected, LOD 1 CFU/mL.

Figure 3. Fermentation kinetics in the musts that were sulfited, processed by UHPH or untreated when evolved under fermentation by grape wild population without yeast inoculation. Values are means with SD of 3 independent fermentations. Fermentation evolution was represented by ethanol formed (% v/v) calculated from the CO_2 loses.

Figure 4. A. Wine color intensity and hue after fermentation of the musts sulfited, processed by UHPH or untreated. **B.** Total polyphenol index (TPI) and hydroxycinnamic acid index after fermentation of the musts sulfited, processed by UHPH or untreated.

553 Values are means with SD of 3 independent fermentations. Bars of the same parameter
554 with the same letter are not significantly different ($p<0.05$).

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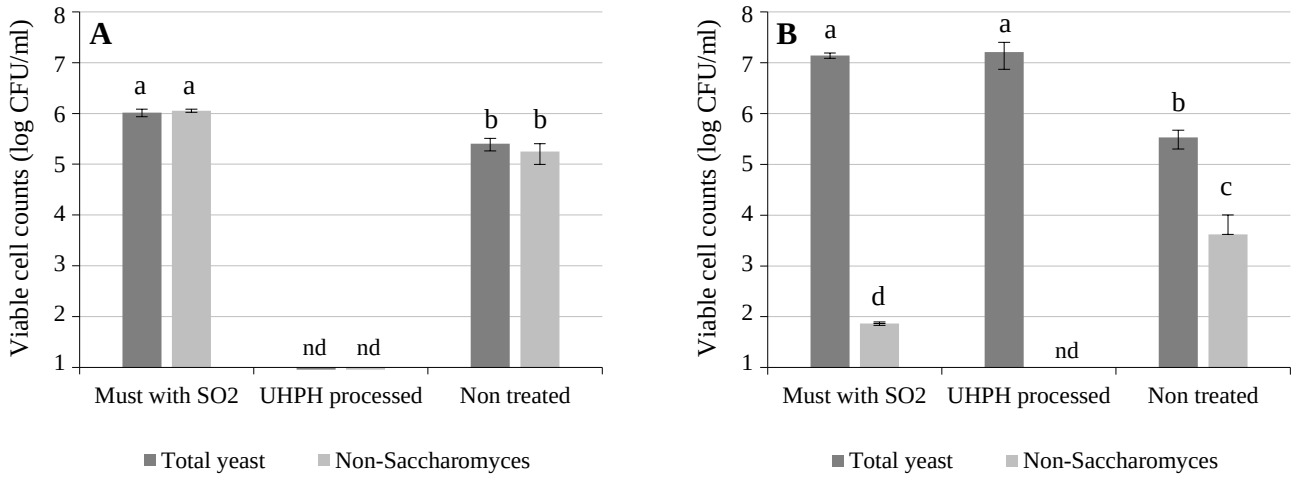
556 **Figure 5.** Sensory analysis of the wines made from the musts that were sulfited, processed
557 by UHPH or untreated. Values are means of 9 tasters. Means in the same axes with the
558 same letter are not significantly different ($p<0.05$).

559 **Figure 1.**



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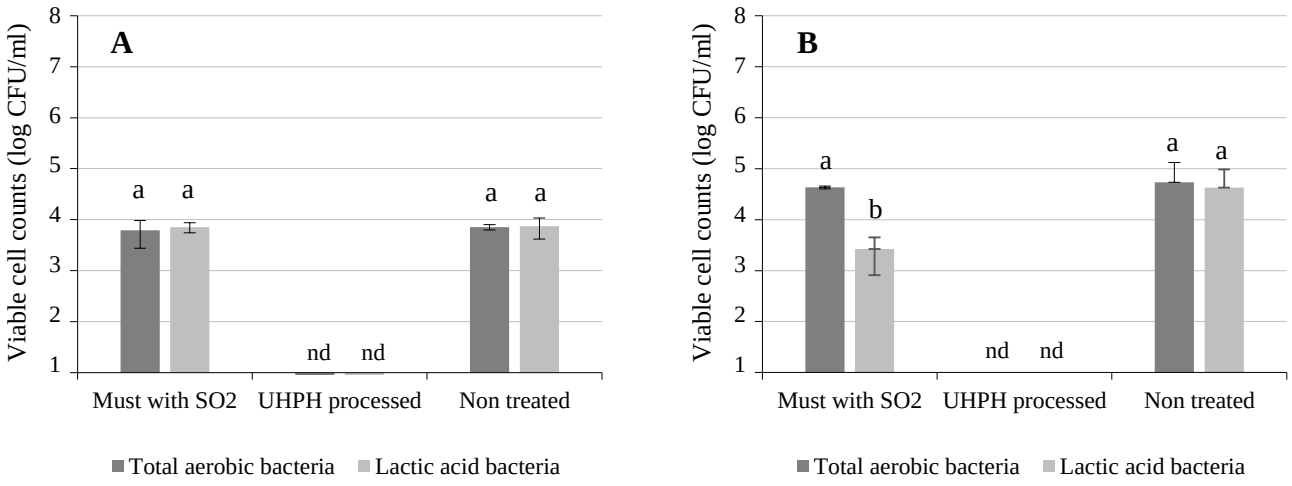
562 **Figure 2**



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565 **Figure 3.**

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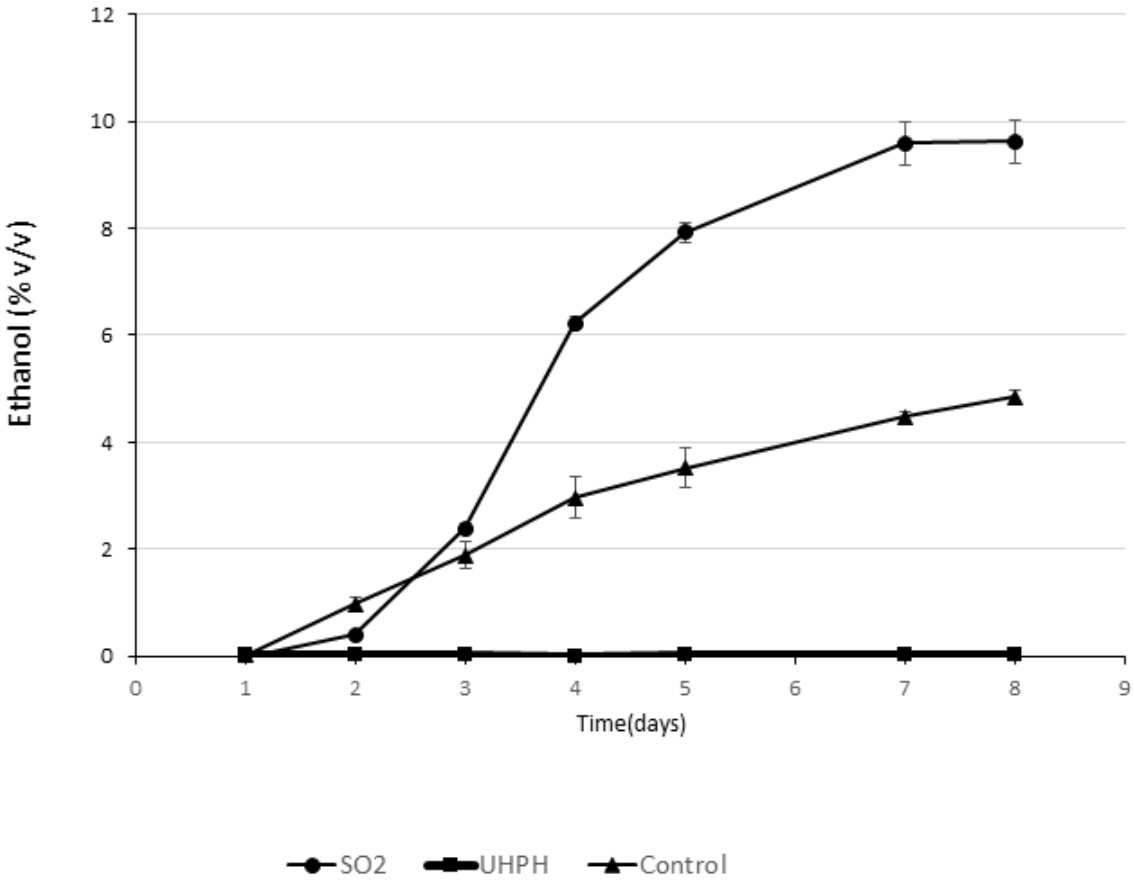

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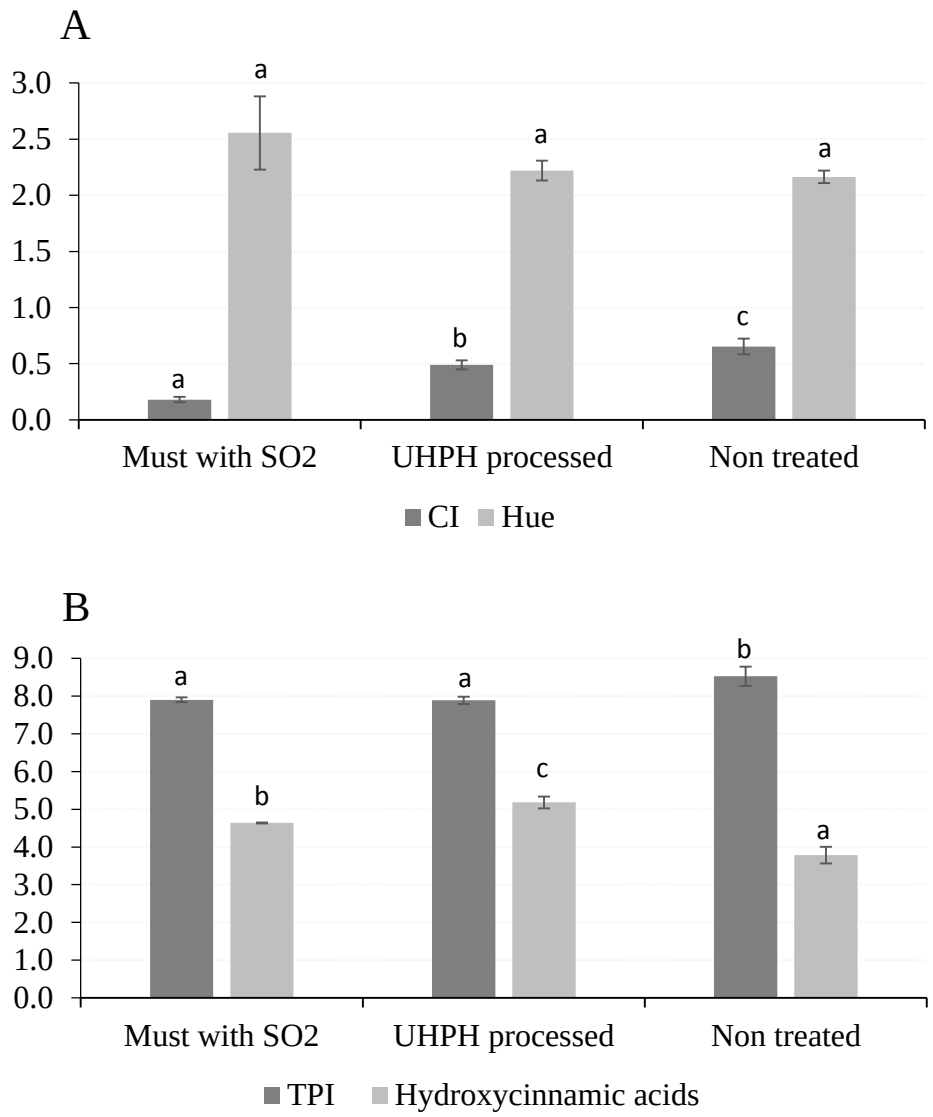
569 **Figure 4.**



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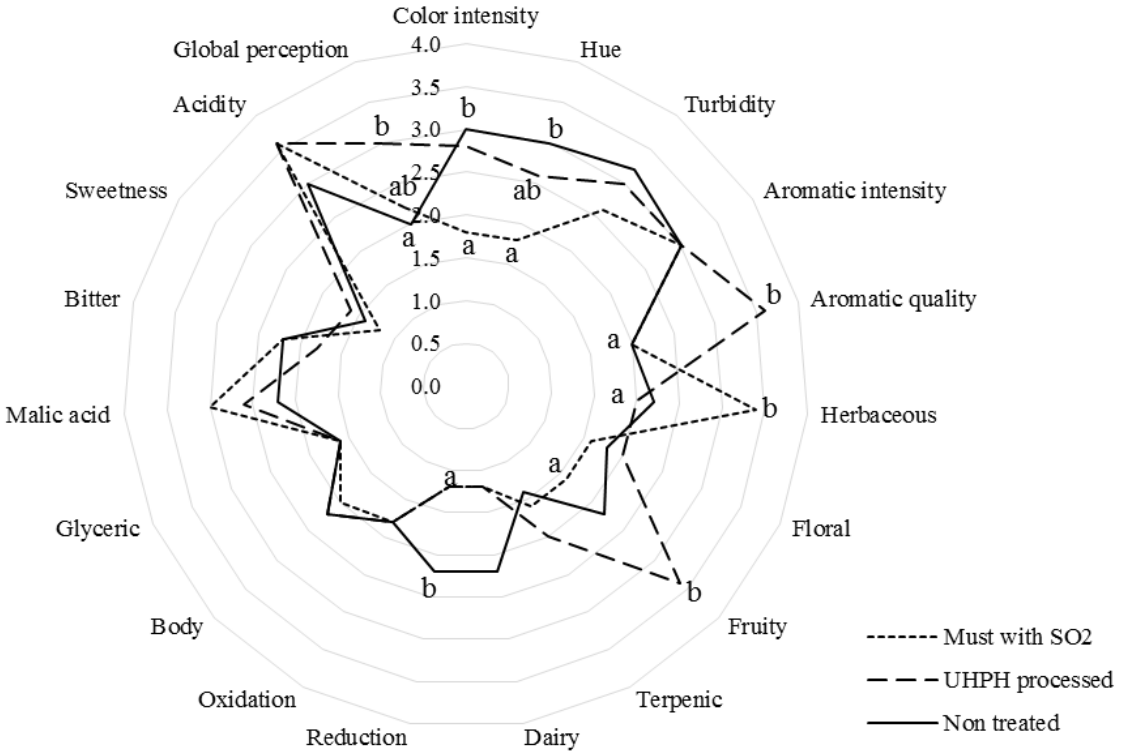
572 **Figure 5.**



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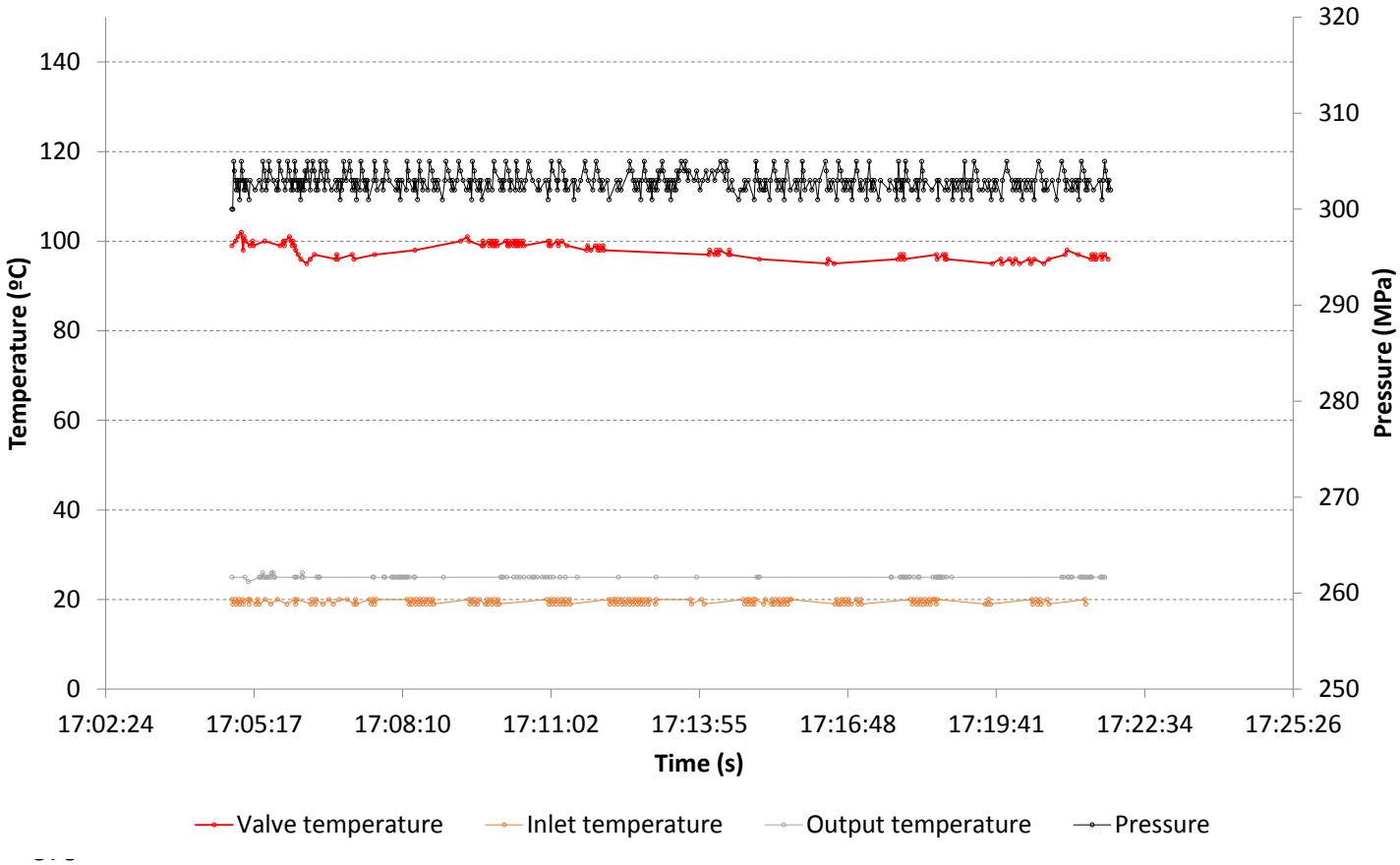
Supplementary Figure



Temperature (ºC) and pressure (MPa) in the valve during the process