# High power ultrasound treatment of crushed grapes: Beyond the extraction phenomena

## Chemical effects of Ultrasound in Winemaking

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**Abstract.** The treatment of white and red crushed grapes by high power ultrasounds (US) represents an emerging technology in winemaking. In 2019, it was officially recognized by OIV through the resolution n°616-2019, and it was also approved by European Union in January 2022. The US effect on extraction mechanisms was widely studied, but more researches are needed to better understand the ultrasound effect on some specific classes of grape compounds. This research aimed to highlight at laboratory scale some specific effects of ultrasounds on some key compounds of white and red grapes. The samples were sonicated at different frequency (20-30 kHz), time (1-10 min), and power (30-90%) technological conditions used in maceration, to obtain valuable information on potential technological transferability. Valuable results were obtained regarding the release of thiols from their precursors, and the reactivity changes of unstable proteins of white wines. The experimental trails on red grape varieties allowed a maintenance of free anthocyanins and no degradative effects were highlighted. Significant and valuable effects were determined also on the tannin polymerization, with an astringency decrease.
The sonication treatment of crushed grapes showed several chemical effects that contribute to decreasing the winemaking inputs and preserving the wine quality. The process conditions must be managed related to grape variety and ripeness for a precision winemaking.

## 1 Introduction

High-power ultrasound (US) is an emerging technology recently approved by OIV (resolution n°616-2019) for the treatment of crushed grapes to improve the extraction of positive skin compounds. In January 2022, the European Union also included the ultrasound technology as an authorized treatment of crushed grapes for winemaking purposes. The US treatment must be applied in continuous mode and it could be carried out for both red and white grapes.

Ultrasound is an acoustic wave with a frequency greater than 16 kHz. Acoustic waves are the propagation of mechanical waves of pressure and displacement through a medium as longitudinal waves, exhibiting compressions (high-pressure regions) and rarefactions (low-pressure regions). The effects of power ultrasound are attributed to ultrasonic cavitation, which is the rapid formation and collapse of gas bubbles, generated by localized pressure differentials occurring over short periods of times (a few microseconds). This ultrasonic cavitation causes localized regions of intense hydrodynamic shear forces and an increase in temperature at the site of bubble collapse inducing mechanical and chemical effects [1].

The sonication effect on extraction phenomena is well known and widely reported in literature [2-4]. Several researches were carried out on different food matrices, highlighting an increase of extraction of chemical compounds [5-8], significant effects on food colloidal systems [9-11], enzyme activities [12], and fermentation microorganisms [13, 2].

Many authors pointed out significant mechanical and chemical effects of ultrasound on food proteins, such as conformational, functionality, and stability modifications [14]. Moreover, in enology, it was reported that sonication treatment can affect the chemical reactivity of wine proteins [15] and their hydrophobicity properties [16].

Other studies highlighted also valuable effects of US on the aging mechanisms of red wines [17-19], specifically considering its effectiveness in changing the chromatic characteristics and phenolic properties of red wines. Considering the anthocyanins and the red wine color, the sonication time represent the main parameters to be considered and optimized to achieve the winemaking purposes and to avoid the undesired effects related to long sonication treatments [18, 20, 21]. Sonication time lower than 10 minutes should be applied to avoid the degradation of wine color compounds. Consequently, the treatments must be appropriately modulated to control and manage the wine quality and stability [20].

The ultrasound irradiation can induce chemical reactions and generate radical species in aqueous medium, such as hydroxyl radicals, peroxyl radicals, and hydrogen peroxide [22]. The sonication of several food matrices highlighted depolymerization mechanisms on some polymeric food components, as carbohydrates, proteins and starch [23]. Interestingly, some authors have reported a constructive and destructive effect of ultrasound on polymer reactions, depending on the operating conditions adopted [24]. A formation of narrowly dispersed polymers by degradative reactions

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induced by ultrasounds, following radical-induced chain growth mechanism, has also proposed [25].

Astringency is one of the main sensorial perceptions related to red wines, that is affect by the chemical structures of tannins, especially proanthocyanidins, their polymerization degree, galloylation, B-ring trihydroxylation, and stereochemistry [26]. As previously reported, conformational arrangements and aggregation processes of larger tannins to more condensed or folded structures can "hide" the hydrophobic functional groups responsible for tannin–protein affinity [27].

The cavitation phenomena, characterized by microstreaming and turbulent forces, can induce a particle size reduction of tannins colloidal systems. During sonication, aggregates are violently agitated, colliding frontally and tangentially, resulting in smaller broken particles with a narrower size distribution [28].

The generation of radical species could also promote other chemical reactions such as activation of aromatic thiols reactions, particularly at low pH levels.

Despite the several researches, the process optimization according to grape variety and the desired organoleptic traits in the resulting wines needs deeper investigations to evaluate the effect of US on some specific chemical compounds, particularly during the crushed grapes treatments that represent the only procedure officially approved by European legislation. In order to highlight some potential interactions of ultrasound with specific grape chemical compound, such as: (1) aroma, thiols and their precursors for white wines; (2) anthocyanins and tannins for red wines.

## 2 Materials and methods

### 2.1 Samples

White grape musts of Sauvignon Blanc and wine model solutions were considered for the evaluation of aromatic thiol precursors. Instead, white wines with high protein instability were selected for the evaluation of potential interaction of cavitation phenomena with wine proteins. Finally, several red young wines with different tannins to anthocyanins ratio were considered to evaluate the ultrasound effect on color stability and some wine aging processes.

### 2.2 Ultrasound treatments

Sonication treatments were carried out with a Sonoplus HD 2200 sonifier (Bandelin electronic, Berlin, Germany) equipped with a titanium alloy, 13 mm diameter, flat-tip probe (TT13, Bandelin, Berlin, Germany). The ultrasound probe was submerged to a depth of 20-25 mm in a 250 mL beaker containing 150 mL of sample (Fig. 1). The treatments were made on grape musts, wines, and model solutions with process conditions similar to industrial scale [4]. The samples were sonicated at different frequency (20-30 kHz), time (1-10 min), and power (30-90%), to obtain valuable information on potential technological transferability. All tests were made with at least 3 replicates.

### 2.3 Analytical determinations

#### *2.3.1 Thiols precursors*

The thiol precursors were determined using an Acquity Ultra Performance Liquid chromatographer (UPLC Waters Corporation, Milford, MA, USA) coupled with a Xevo TQ MS mass spectrometer (Waters Corporation, Milford, CT, USA) according to the method and conditions reported by Larcher et al. (2013) [29]. Quantification of volatile thiols was performed using a Varian 450 gas chromatograph (Middelburg, The Netherlands) equipped with Varian 300 triple quadruple mass spectrometer (Walnut Creek, CA, USA), using specific conditions according to Larcher and collaborators (2015) [30].

#### *2.3.2 Wine protein*

Wine proteins were precipitated from 4 mL of wine sample, adding 20 mL of ethanol (96% v/v). Subsequently, 10 mL of the obtained solution was subjected to centrifugation, the ethanol was completely removed, and the proteins were dissolved in 1 mL of milli-Q water. HPLC analysis was performed on an LC-2010 AHT liquid chromatographic system (Shimadzu, Kyoto, Japan), equipped with an integrated autosampler and UV–VIS detector.

#### *2.3.3 Surface Electric Charge (SEC)*

The surface electrical charge (SEC) was determined with a particle size detector (Mütek PCD 03, Mutek Analytical GmbH, Herrsching, Germany), a titration organic cationic polydiallydimetylammonium chloride (PolyDADMAC) solution was used to quantify the negative surface electrical charge [31].

#### *2.3.4 Cold tannin test (CTT)*

The samples were filtered in a 0.45 μm syringe filter; subsequently, 100 μL of chestnut tannin–ethanol solution (5% w/v) was added. Turbidity was measured using a AL250T-IR turbidimeter (Acqualytic, Dortmund, Germany) before and after the tannin solution addition.

#### *2.3.5 Heat stability test (HT)*

Ten milliliters of sample were filtered through 0.45 μm filters and sealed in test tubes with screw caps. The tubes were heated at 80◦C for 30 min [32, 33]. Afterward, the sample was left to cool at room temperature and then the turbidity was measured [34].

#### *2.3.6 Colloidal Particle Size (DLS)*

Particle size was determined with a Nicomp 380 ZLS Nanoparticle Size Analyzer (Particle Sizing Systems, Santa Barbara, CA) equipped with a 10 mW He–Ne laser at a wavelength of 633 nm. Measurement occurred at 90◦C from the incident beam and gave an estimation of the particle mean diameter distribution, expressed in nanometers. DLS measurements were performed at 20◦C for a period of 5 min.

### *2.3.7 Astringency index*

Astringency evaluation is based on the reactivity of tannins against bovine serum albumin (BSA).

### *2.3.8 Anthocyanin determination*

The total anthocyanins content was determined as reported by Ribereau-Gayon and Stonestreet [35]. Moreover, a qualitative analyze of anthocyanins was performed on a LC-2010 AHT liquid chromatographic system (Shimadzu, Kyoto, Japan), equipped with an integrated autosampler and UV-VIS detector. Compounds were separated on a 5 µm packed, 150 × 4.6 mm Zorbax Eclipse Plus C18 column (Agilent Technologies, Santa Clara, CA, USA) thermostated at 25° C.

### *2.3.9 Color Indices*

HCl index and polymerized pigments index were determined according to Glories method [36].

### *2.3.10 Statistical analysis*

All experiments were performed in triplicate. Minitab 17 software (Minitab Inc., State College, PA, USA) was used for statistical analysis by one-way analysis of variance ANOVA, with Tukey's HSD multiple comparison) with the level of significance set up at $p = 0.05$.

### *2.3.11 Mathematical modelling*

The analytical parameters, which were significantly influenced by ultrasound treatments, were correlated with the absorbed energy density (AED). The trends obtained were mathematically described using three different models: power law function, logistic, and Peleg's model. [37].

**Figure 1.** Ultrasound device for laboratory test.

## 3 Results

### 3.1 Effect on key compounds of white wines

### *3.1.1 Aromatic thiol precursors*

The pre-fermentative phases of white wines with high content ofthiols and precursors are essential to preserve the aroma potential developed by yeasts during fermentation. The effect of ultrasound at 20 kHz for 5 minutes was studied on a model solution in order to avoid any possible interferer and to highlight the specific effect of US on thiol precursors.

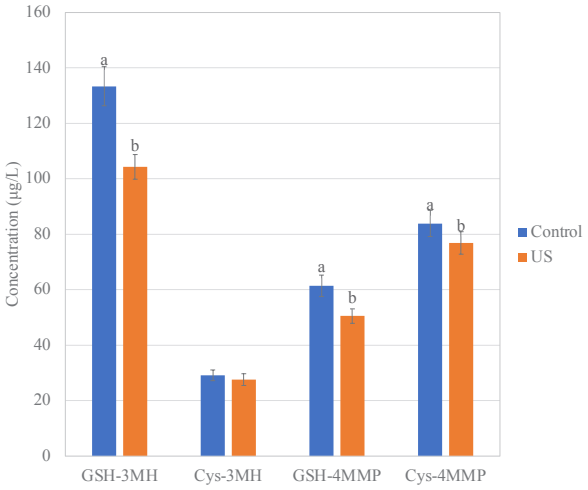


**Figure 2.** Concentration of thiols precursors in the model solution of control and treated samples (US). Different letters indicate mean values statistically differentiated.

Figure 2 shows the mean values of 3-S-glutathionyl mercaptohexan-1-ol (GSH-3MH), 3-S-cysteinyl marcaptohexan-1-ol (Cys-3MH), 3-S-glutathionyl mercapto-4-methyl-pentan-2-one (GSH-4MMP), and 3-S-cysteinyl mercapto-4-methyl-pentan-2-one (Cys-4MMP) concentration in the model solution of control and sonicated samples (US).

The data show a significant decrease in thiol precursors, differently from the results of our previous researches which highlighted the significant extractive effect of ultrasound from white grape skins [4]. The decrease of thiols precursors was higher on average on glutathionylated than on cysteinylated forms, and it could be due to a degradation or a chemical modification.

The breakage of the compounds would be possibly related to the radical and electrontransfer processes generated by the high energy and excited state species induced by cavitation phenomena. The mechanism of the cleavage and the degradation products is not known yet and specific studies should be performed. However, in case the precursors cleavage occurs, the release of 3MH and 4MMP should represent the most important reaction products in winemaking.

Figure 3 shows the concentration of 3-mercaptohexan-1-ol (3MH) and 4-mercapto-4-methyl pentan-2-one (4MMP) in the model solution in control

and treated (US) samples after 5 min of sonication. The experimental results showed a significant effect of high-power ultrasounds on the release of the volatilethiols from their precursors after treatment. US showed a positive effect on the sensorial properties of the treated samples, however the operating conditions should be appropriately modulated considering the initial grape status.

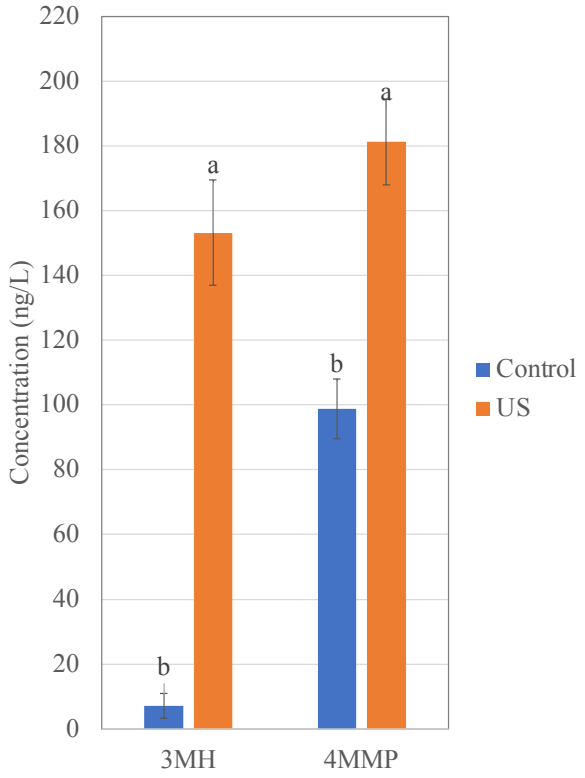


**Figure 3.** Concentration of 3MH and 4MMP in the model solution in control and treated sample (US) after 5 minutes of sonication. Different letters indicate mean values statistically differentiated.

On a molar basis, the release ratio of 3MH and 4MMP due to sonication, with respect to the net decrease of each precursor is on average 1%. The results could be affected by the possible degradation of the volatile thiols within the cavitation bubbles or following the radical's formation. It is necessary to investigate this phenomenon further to be exploited in winemaking within the optimization of ultrasound treatment to enhance the grape thiol potential. The formation of radical species would be strongly dependent on the process variables and on the matrix composition, such as pH [38, 39].

Previous researches reported that the ratio between volatile thiols in wine and grape precursors in musts is very low [40] and the biogenic origin of the aroma molecules present in wines from precursors is only explicable for a 50% [41]. Consequently, it could be interesting to better understand the effect of US on thiolsprecursors, that could allow enhancing the tropical aromas of wines through specific winemaking protocols.

Considering the effectiveness of US on the white grape maceration as innovative technique [2-4], it can be used also as a valuable tool for the management of aromatic thiols. Specific process conditions allowed the release of the volatile forms of 3MH and 4MMP from their precursors linked to cysteine and glutathione. The sonication effect on thiol precursors could highlight a potential application of US in the management of volatile compounds of aromatic varieties and their sensorial potentiality, related to several enological purposes.

### *3.1.2 Proteins*

White wines can be affected by several sensory faults inducing a decrease of wine quality, consumer unpleasantness, and economic losses. Among them, the formation of protein haze is one of the main defects to be managed, and several enological adjuvants and practises can be adopted.

Previous experimental investigation pointed out potential effects of ultrasounds on the protein stability of white wines [42]. In the present work some aspects of physio-chemical properties of wine colloidal status and the HPLC quantification of haze related proteins were considered and deeply investigated.

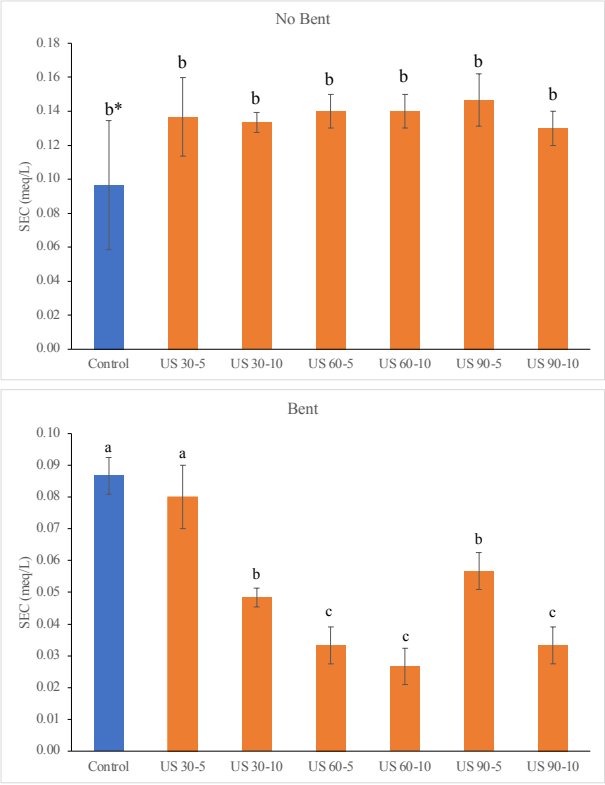


**Figure 4.** Surface electrical charge (negative) in untreated and treated (US) samplesat different amplitude (30, 60, 90) and sonication times (5, 10). Different letters indicate mean values statistically differentiated.

The wine proteins show a positive surface electrical charge, among the other colloids. The Surface Electrical Charge (SEC) determination can be used to determine the protein reactivity. As reported in Figure 4, no significant differences can be highlighted between untreated and sonicated samples, before the bentonite addition. Moreover, the increase of amplitude and sonication didn't induce significant analytical changes. Despite no significant changes, the US

treatment before bentonite addition showed an increase trend of negative electrical charge and, consequently, a probable decrease of protein. The sonication of wine samples after bentonite addition showed a significant decrease of negative electrical charge, which could indicate higher protein reactivity.

High-power ultrasound can affect the chemical structure and reactivity of wine haze-related proteins. Significant changes of protein reactivity could be useful on the wine protein stability management, in order to minimize the winemaking inputs and adjuvants amounts.

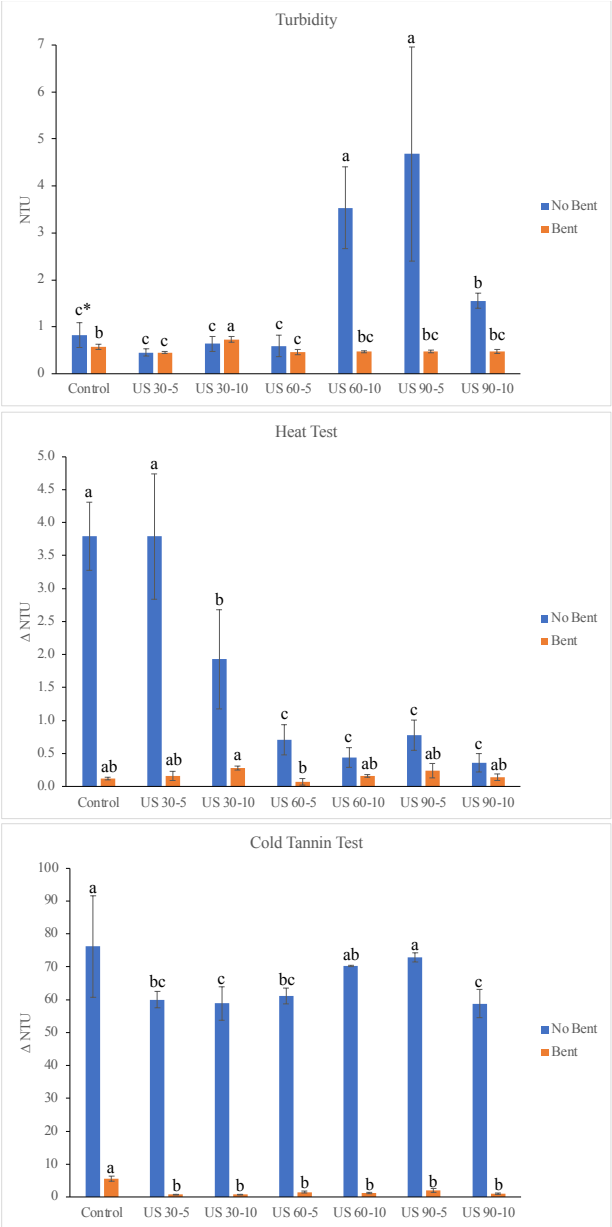


**Figure 5.** Effect of amplitude (30, 60, 90) and sonication time (5, 10) on turbidity, heat test and cold tannin test. Different letters indicate mean values statistically differentiated between treatment (control *vs* US).

Further analytical determinations can be considered to evaluate potential effects of sonication on wine protein stability. Figure 5 reports the results in term of turbidity, heat stability test (HT), and cold tannin test (CTT) of untreated (WB) and sonicated samples of Wine B before (No Bent) and after (Bent) bentonite addition. Sonication before bentonite addition showed an increase in turbidity within the increase in amplitude and sonication time. The turbidity results indicated also that there is a possible combination effect of sonication and bentonite treatment: sonication at fixed conditions could precipitate some chemical compounds, which could be subsequently eliminated by bentonite. The heat stability test (HT) is the most reliable method to predict protein haze and sediment formation in the bottle during storage [43]. The increase of amplitude and sonication time decreases the HT values. Sonication at 90% amplitude and 10 min showed a turbidity of 0.36, which is comparable to the turbidity measured after bentonite addition to the untreated sample (0.12). The sonication treatments could alter the reactivity and some functional properties of wine proteins, as reported by other authors on other food matrices [44, 45]. The Cold Tannin test (CTT) results highlighted significant differences and a potential synergic effect of ultrasound and bentonite on the stabilization of protein haze. Ultrasounds can affect the exposure level of hydrophobic tannin-binding sites for the main wine protein classes (chitinases and TLPs), which is an important property that influences the stability, conformation, and functionality of the proteins [14, 16]. The sonication didn't allow a complete protein stability of white wines. However, the wine protein reactivity can be affected by ultrasound treatment, at different levels of amplitude and time, according also to wine variety. The different behaviors of protein stability pointed out after the sonication treatments were also evaluated by an HPLC analysis of haze-related proteins. The experimental results of two white wines (WA and WB) were reported in Table 1. The HPLC analysis of two wines showed different qualitative protein profiles. As expected, bentonite addition at 10 g/hL removed all the haze-forming proteins and they were not detected. Sonication treatments did not significantly affect the qualitative protein profile of either white wine, but they induce a significant decrease of TLPs and CTs amounts, particularly at amplitude above 60%. The sonication at 90% and 10 min decreased the total protein content of Wine B from 81mg/L (untreated sample) to 51 mg/L. Considering the mechanisms of wine protein haze formation, several possible strategies can be applied for preventing wine haze that would either reduce or eliminate the need for bentonite [46-48]. As revealed by experimental results, it is important to adopt an integrate approach with multiple technologies and practices to achieve a wine protein stabilization and, contemporary, a preservation of wine sensorial qualities.

**Table 1.** Protein values by HPLC of untreated and treated (US) wines. Different letters indicate mean values statistically differentiated.

| Sample | Amplitude | Time | Wine A | | Wine B | |
|---|---|---|---|---|---|---|
| | | | Total | CTs | TLPs | CTs |
| | | (min) | (mg/L) | (mg/L) | (mg/L) | (mg/L) |
| Control | - | - | 22.23 b* | n.d. | 80.24 a | 0.69 b |
| US1 | 30 | 5 | 24.47 a | n.d. | 75.13 b | 0.79 a |
| US2 | 30 | 10 | 22.46 b | n.d. | 67.62 c | 0.63 c |
| US3 | 60 | 5 | 15.53 de | n.d. | 63.18 d | 0.43 f |
| US4 | 60 | 10 | 17.47 c | n.d. | 55.64 e | 0.49 e |
| US5 | 90 | 5 | 14.30 e | n.d. | 50.40 f | 0.52 e |
| US6 | 90 | 10 | 17.36 cd | n.d. | 50.83 f | 0.58 d |

## 3.2 Effect on key cOmpounds of red wines

### *3.2.1 Anthocyanins*

The ultrasound treatments didn't affect the anthocyanin composition and the color intensity of two red young wines (WA, WB). Experimental results indicate that sonication preserved the phenolic compounds, especially anthocyanins, but the operative conditions should be appropriately modulated. The ultrasound conditions adopted were the optimal for the treatment of crushed grapes to increase the extraction of skin compounds.

**Table 2.** HPLC analysis of anthocyanins of young red wines at different amplitude and sonication times. Different letters indicate mean values statistically differentiated.

| Compound | Amplitude | | | Time | | | |
|---|---|---|---|---|---|---|---|
| | 0% | 40% | 80% | 0 min | 1 min | 3 min | 5 min |
| Delphinidin-3-monoglucoside | 1.60 c* | 3.15 b | 3.21 b | 3.97 a* | 3.94 a | 3.88 a | 4.15 a |
| Cyanidin-3-monoglucoside | 0.46 b | 0.68 b | 0.69 b | 0.98 a | 0.93 a | 0.95 a | 0.99 a |
| Petunidin-3-monoglucoside | 5.03 a | 5.00 a | 4.96 a | 5.51 a | 5.10 a | 5.52 a | 5.30 a |
| Peonidin-3-monoglucoside | 7.65 b | 9.88 a | 9.55 a | 10.39 a | 10.75 a | 10.30 a | 10.94 a |
| Malvidin-3-monoglucoside | 67.95 a | 68.51 a | 67.65 a | 61.73 b | 62.53 b | 61.85 b | 61.97 b |
| Vitisin A | 1.14 b | 1.32 b | 1.33 b | 2.17 a | 2.13 a | 2.27 a | 2.19 a |
| Petunidin-3-monoglucoside acetyltated | 0.55 b | 0.66 b | 0.55 b | 0.73 a | 0.71 a | 0.74 a | 0.69 a |
| Peonidin-3-monoglucoside acetylated | 1.35 a | 1.34 a | 1.91 a | 2.12 a | 1.64 a | 2.00 a | 1.41 a |
| Malvidin-3-monoglucoside acetylated | 7.37 a | 7.99 a | 7.01 a | 6.39 ab | 6.15 ab | 6.41 b | 6.12 b |
| Delphinidin-3-monoglucoside p-coumarylated | 2.82 a | 2.48 a | 2.57 a | 2.21 a | 2.25 a | 2.14 a | 2.23 a |
| Malvidin-3-monoglucoside p-coumarylated | 4.13 a | 4.26 a | 4.39 a | 3.24 a | 3.32 a | 3.37 a | 3.43 a |
| Malvidin-3-monoglucoside vinylphenol | n.d. | n.d. | n.d. | 0.15 a | 0.16 a | 0.19 a | 0.18 a |
| Malvidin-3-monoglucoside vinylphenol acetylated | n.d. | n.d. | n.d. | 0.41 a | 0.37 a | 0.39 a | 0.39 a |

Table 2 shows the HPLC analysis of anthocyanins of untreated and sonicated samples, at different levels of amplitude (0, 40, and 80%) and sonication time (0, 1, 3, and 5 min). The high-power ultrasound did not affect the anthocyanin profile of the red wines.Significant changes can be highlighted only for the delphinidin-3-monoglucoside, but this compound represents only 1-3% of the total anthocyanin content, and the main compounds are not affected by ultrasound treatments. Indeed, as reported in the table, the malvidin-3-glucoside, the main anthocyanin of red wines, is not affected by sonication at different levels of amplitude and time.

Qualitative profiles of glycosidic and acetylated anthocyanins did not change, and no significant differences can be highlighted between untreated and sonicated samples, as confirmed by the one-way analysis of variance.

The anthocyanins preservation, observed at the adopted amplitude and time ranges, allows wide operative conditions on the treatment of crushed grapes according to grape cellular maturity.

Sonication time is one of the main parameters to be considered and optimized to achieve the desired winemaking targets and, at the same time, to avoid undesirable effects due to longer irradiations [21].

Contrary, other authors [49] reported that sonication at high amplitude and longer time can promote the degradation of anthocyanins of red grape juice, and their degree of degradation was specific to each individual anthocyanin. Higher sonication times up to 20 min have been tested on commercial red wines and unacceptable limits of chromatic and sensory properties were achieved. As reported by Ferraretto and Celotti [20], sonication treatment should be accurately modulated to control and manage the quality and stability of wines.

Other analytical indexes of wine color can be evaluated to study the ultrasound effects. In Table 3 the anthocyaninpolymerized pigments index, HCL index, and color intensity of untreated (WB) and sonicated samples at different irradiation time (WB-1, WB-3, and WB-5). The preservation of wine color after US treatment and a better evolution of some analytical indexes along the first 30 days of aging can be pointed out. As reported in our previous work [20, 50], the US effect on the red wine color stability could be related to the interaction anthocyanins-tannins. The HCl index shows a significant increase during the first 15 days of aging, indicating an increase in the polymerization degree of tannins and a potential decrease in wine astringency. Color intensity also increase in the first 15 days, and no variations can be observed at longer time. PPI index achieve a maximum content at 15 days of storage, when a minimum values of anthocyanins and tannins were revealed. These effects may be may be due to reaction between the anthocyanin and tannins, and their involvement in copigmentation reactions that occur with a formation of new pigments and an increase in color intensity.

**Table 3.** Effect of ultrasound time and storage days on total anthocyanins, polymerized pigments index, HCL index, and color intensity. Different letters indicate mean values statistically differentiated.

| Time US (min) | $t_{stor.}$ (day) | Anthocyanins (mg/L) | P.P.I. (-) | HCl index (-) | C.I. (-) |
|---|---|---|---|---|---|
| 0 | 0 | 280.99 a* | 57.87 b | 14.22 b | 4.52 c |
| | 15 | 132.18 c | 68.95 ab | 28.51 a | 5.55 a |
| | 30 | 182.12 b | 60.57 ab | 30.98 a | 5.38 ab |
| 1 | 0 | 293.27 a | 61.37 ab | 13.13 b | 4.54 c |
| | 15 | 133.67 c | 71.89 a | 27.66 a | 5.42 ab |
| | 30 | 183.02 b | 60.45 ab | 24.27 a | 5.50 ab |
| 3 | 0 | 283.68 a | 67.55 ab | 13.68 b | 4.65 c |
| | 15 | 150.85 c | 68.11 ab | 24.74 a | 5.47 ab |
| | 30 | 187.25 b | 59.12 b | 25.16 a | 5.33 ab |
| 5 | 0 | 273.44 a | 64.53 ab | 12.59 b | 4.68 c |
| | 15 | 150.33 c | 63.14 ab | 26.20 a | 5.32 ab |
| | 30 | 190.28 b | 56.87 b | 29.84 a | 5.18 b |

Therefore, ultrasound treatments could preserve not only the initial phenolic and chromatic characteristics of the wine but also their evolution during storage. Many authors reported the US effect on phenolic degradation but also the formation of radical species that can induce positive chemical reactions for wine color stability [51].

HCl index is a quantification of the polymerized tannins and it can be related to wine astringency perception. Previous researches showed positive effect of ultrasound on polymerized tannins, with a potential decrease of wine astringency. It is well known that wine color evolution is affected by several factors, such as: wine composition, pH, and tannins-to-anthocyanins (T/A) ratio. As can be noted, wine A shows a higher T/A ratio than wine B, 8.17 and 4.58 respectively, indicating a higher ability of wine A to achieve better evolution and stabilization reactions of phenolics and chromatic properties. It is of critical importance to apply the ultrasound treatments in relation to the initial phenolic profile, in order to avoid any undesirable effects.

### *3.2.2 Tannins and astringency*

The astringency is one of the main sensorial perceptions of red wines, and it can be considered as a defect at high intensities. As a continuation of preliminary investigations on red wines [20, 50, 52] several experimental trials were carried out to study potential effects of ultrasound irradiation on wine tannins and their sensorial perceptions.

Figure 6 shows HCL index of the untreated and sonicated samples at different levels of amplitude (30, 60, and 90%) and time (2, 6, and 10 min). High-power ultrasound enhances the HCl index for all the sonicated samples, and it is possible to observe a significant increase between sonicated samples at 30% and 2 min (71.59) and at 90% and 10 min (74.25). An increase of HCl index indicates an increase of polymerization degree, phenomena that occur naturally during the red wine aging and it can be managed by specific enological practices.

In Figure 6 are depicted the results of astringency index, evaluated by the bovine serum albumin (BSA) method. As can be seen, the ultrasound treatment decreases the astringency index for all the amplitudes and sonication times. The results could indicate a potential role of ultrasound technology aimed to an astringency decrease of grape musts and wines, avoiding the use of enological adjuvants or micro-oxygenation procedures. The experimental results could be related to polymerization phenomena affect by ultrasound and widely reported in literature for several food matrices [22-25]. Morever, sonication could induce some conformational arrangements of proanthocyanidins structures or aggregation processes to more condensed or folded structures, affecting tannin–protein affinity [26, 27].

Condensed tannins are among the most abundant macro- molecules in red wine and they can aggregate and formcolloidal dispersions, with hydrodynamic diameters in magnitudes of a few hundred to over a thousand nanometers [53]. Several analytical methods can be used to monitor the colloidal status and its evolution, such as dynamic light scattering (DLS) for the determination of particle size. Figure 6 shows the mean particle size of untreated and sonicated samples at different levels of amplitude and time. It is remarkable that ultrasound treatments induced a decrease in mean particle size, from 880of untreated sample to 590 nm. During sonication the microstreaming and turbulent forces generated by cavitation phenomena induce frontal and tangential collisions of particle aggregates, generating smaller broken particles with a narrower size distribution [28, 54]. The particle size reduction could be related to conformational changes induced by ultrasound waves, as well as the decrease in astringency, but more detailed investigations are necessary.

Every technological process should be transferrable from a laboratory-scale to an industrial-scale production environment. The scaling-up process is essential to ensure that the processing conditions remain the same, as well as the final product quality, and the productivity increase [55]. The energy introduced in the system by acoustic cavitation can be a useful guide for scale-up considerations. The level of energy introduced into the system can be expressed as the acoustic energy density (AED in J/mL) and can be determined by calorimetric methods based on the temperature variations. Figure 7 shows the Astringency plotted against AED. All the analytical parameters can be mathematical well described by all the mathematical models considered, as indicated by the magnitudes of the coefficients of determination ($R^2$and $R^2$-adj) and normalized root-mean-square deviation (NRMSD). Higher values of $R^2$ and $R^2$-adj and lower values of NRMSD denote a better goodness of fit and suggest that the model represents the experimental values well.The AED parameter has been successfully adopted for the scaling up of ultrasound- and microwave-assisted extraction processes as a reference for calibrating the optimum nominal power or percentage amplitude and treatment time [56].

In view of the positive effects of ultrasound on some wine analytical indices and the good fitting results of the mathematical models, it is considerable that this scale-up method could also be useful for determining the optimum operational conditions of ultrasonic systems for the astringency decrease of red wines and, generally, for the wine quality management.

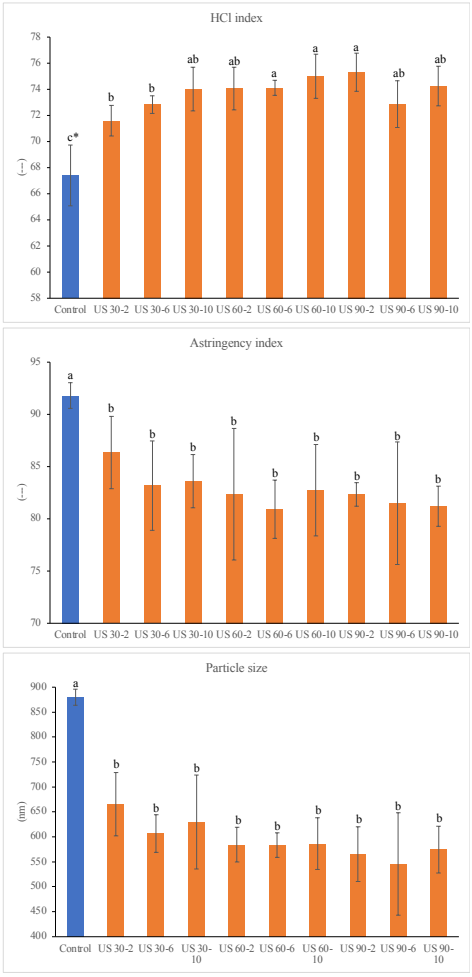


**Figure 6.** HCl index, astringency index (BSA) and particle size in wines treated with US at different time (2, 6, 10) and amplitude (30, 60, 90). Different letters indicate mean values statistically differentiated.

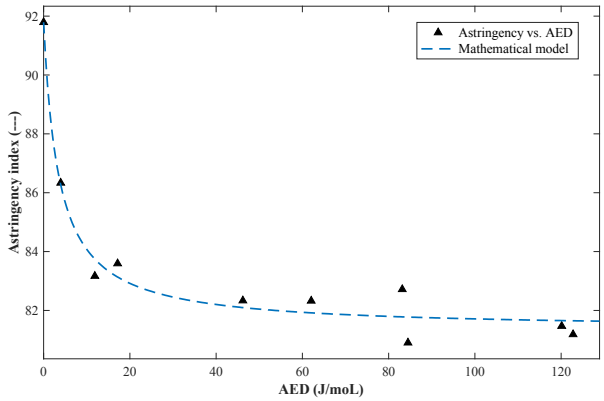


**Figure 7.** Correlation between experimental values and simulated power law model for astringency index at different level of acoustic energy densities.

## 4 Conclusions

The ultrasound technology could be applied not only to improve the extraction of grape skins compounds, but also to manage some their chemical properties related to grape variety and enological purposes. Nowadays the high-power ultrasound can be used only on crushed grapes. The present work aimed to study some valuable effects on several chemical classes of white and red grapes. The results could be useful to manage specifically the process conditions (amplitude, time, and frequency) according to grape variety.

At laboratory scale, it was highlighted a significant effect of ultrasound on thiols precursors and proteins of white grapes. Instead, on red grapes, valuable effects were pointed out on the anthocyanin and color stability during sonication treatments, and positive effects were determined on tannins polymerization with a significant decrease of astringency perception. The sonication treatment of crushed grapes showed several chemical effects that contribute to decreasing the winemaking inputs and ensuring the wine quality. The process conditions (frequency, power, and time) must be managed related to grape variety and ripeness for a precision winemaking.

Further research is already undergoing, aimed to deeply investigate directly on the crushed grapes the impact of high-power ultrasound on the most significant compounds affecting the quality and stability of red and white wines.

## Acknowledgments

TMCI-Group (Italy) for financial support.

## References

1. T.J. Mason, Ultrasound in Food Processing 105 (1998)
2. J.F.G. Martin, Applications of Ultrasound in the Beverage Industry (Nova Science Publishers, New York, 2016)
3. A. Morata, C. Escott, I. Loira, F. Palomero, C. González, Antioxidants **10**, 1863 (2021)
4. E. Celotti, P. Ferrarretto, 39th World Congress of Vine and Wine, Bento Gonçalves, Brazil, **104** (2016)
5. D. Bates, A. Patist, Innovations in processing, packaging, and predictive modelling, **119** (2010)
6. L.N. Lieu, M.V.V. Le, Ultrason. Sonochem. **17**, 273 (2010)
7. N. El Darra, N. Grimi, R.G. Maroun, N. Louka, E. Vorobiev, Eur. Food Res. Technol. **236**, 47 (2013)
8. P. Ferraretto, V. Cacciola, I. Ferran Batllò, E. Celotti, Ital. J. Food Sci. **25**, 160 (2013)
9. T.J. Mason, L. Paniwnyk, J.P. Lorimer, Ultrason. Sonochem. **3**, 253 (1996)
10. F. Chemat, F.H. Zill, M.K. Khan, Ultrason. Sonochem. **18**, 813 (2011)
11. Y. Tao, D.W. Sun, Crit. Rev. Food Sci. Nutr. **55**, 570 (2015)
12. A.Osete-Alcaraz, A.B. Bautista-Ortín. P. Pérez-Porras, E. Gómez-Plaza, Foods **11**, 19 (2022)
13. S. Guerrero, A. López-Malo, S.M. Alzamora, Innov. Food Sci. Emerg. **2**, 31 (2001)
14. S.C. Van Sluyter, J.M. McRae, R.J. Falconer, P.A. Smith, A. Bacic, E.J. Waters, J. Agric. Food Chem. **63**, 4020 (2015)
15. M. Ashokkumar, J. Lee, B. Zisu, R. Bhaskarcharya, M. Palmer, S. Kentish, J. Dairy Sci. **92**, 5353 (2009)
16. M.M. Rahman, B.P. Lamsal, Food Sci. Food Safety **20**, 1457 (2021)
17. N. Masuzawa, E. Ohdaira, M. Ide, Jpn. J. App. Phys. **39**, 2978 (2000)
18. Q.A Zhang, Y. Shen, X.H. Fan, J.F. García Martín, Cyta J. Food **14**, 55 (2016)
19. V. Cacciola, I. Ferran Batllò, P. Ferraretto, S. Vincenzi, E. Celotti, Eur. Food Res. Technol. **236**, 311 (2013)
20. P. Ferraretto, E. Celotti, Cyta J. Food **14**, 529 (2016)
21. B.K.Tiwari, TRAC Trend Anal. Chem. **71**, 100 (2015)
22. T.G. McKenzie, F. Karimi, M. Ashokkumar, G.G. Qiao, Chem. A Eur. J. **25**, 5372 (2019)
23. N. Bhargava, R.S.Mor, K. Kumar, V.S. Sharanagat, Ultrason. Sonochem. **70**, 105293 (2021)
24. M. Kubo, T. Kondo, H. Matsui, N. Shibasaki-Kitakawa, T. Yonemoto, Ultrason.Sonochem. **40**, 736 (2018)
25. H. Xia, Q. Wang, Y. Liao, X. Xu, S.M. Baxter, R.V. Slone, S. Wu, G. Swift, D.G. Westmoreland, Ultrason.Sonochem. **9**, 151 (2002)
26. W. Ma, A. Guo, Y. Zhang, H. Wang, Y. Liu, H. Li, Trends Food Sci. Technol. **40**, 6 (2014)
27. J.M. McRae, J.A. Kennedy, Molecules **16**, 2348 (2011)
28. Y. Lu, N. Riyanto, L.K. Weavers, Ultrason. Sonochem. **9**, 181 (2002)
29. R. Larcher, G. Nicolini, L. Tonidandel, T. Román Villegas, M. Malacarne, B. Fedrizzi, Aust. J. Grape Wine Res. **19**, 342 (2013)

30. R. Larcher, L. Tonidandel, T. Román Villegas, T. Nardin, B. Fedrizzi, G. Nicolini, Food Chem. **166**, 56 (2015)
31. R. Ferrarini, E. Celotti, R. Zironi, Revue Française d'Œnologie – Chaier Scientifique **36**, 5 (1996)
32. M. Gabrielli, D. Fracassetti, A. Tirelli, Food Control **62**, 330 (2016)
33. J.M. McRae, V. Barricklow, K.F. Pocock, P.A. Smith, Aust. J. Grape Wine Res. **24**, 504 (2018)
34. M.V. Moreno-Arribas, M.C. Polo, Wine chemistry and biochemistry (Springer, New York, 2009)
35. P. Ribéreau-Gayon, E. Stonestreet, Bull. Soc. Chim. France **9**, 2649 (1965)
36. Y. Glories, Ph.D. Thesis, Université de Bordeaux II (1978)
37. Y.C. Cheung, K.C. Siu, J.Y. Wu, Food Bioprocess Technol. **6**, 2659 (2013)
38. T. Román Villegas, L. Tonidandel, G. Nicolini, E. Bellantuono, L. Barp, R. Larcher, E. Celotti, Foods **9**, 104 (2020)
39. T.N. Wu, M.C. Shi, Environ. Eng. Manag. J. **20**, 245 (2010)
40. G. Winter, T. Van Der Westhuizen, V.J. Higgins, C. Curtin, M. Ugliano, Aust. J. Grape Wine Res. **17**, 285 (2011)
41. H. Bonnafoux, S. Delpech, E. Rémond, R. Schneider, A. Roland, F. Cavelier, Food Chem. **268**, 126 (2018)
42. E. Celotti, T. Roman Villegas, G. Nicolini, E. Bellantuono, M. Osorio, J. Cardona, A. Natolino,LWT **147**, 111602 (2021)
43. P.R. Gogate, Ultrasound technologies for food and bioprocessing (Springer, New York, 2011)
44. F. Cosme, C. Fernandes, T. Ribeiro, L.F. Ribeiro, F.M. Nunes Beverages **6**, 19 (2020)
45. M. Ashokkumar, J. Lee, B. Zisu, R. Bhaskarcharya, M. Palmer, S. Kentish, J. Dairy Sci. **92**, 5353 (2009)
46. I. Horvat, S. Radeka, T. Plavˇsa, I. Luki´c, Food Chem. **285**, 305 (2019)
47. S. Sommer, F. Tondini, Sustainability **13**, 1860 (2021)
48. S. Vincenzi, A. Panighel, D. Gazzola, R. Flamini, A. Curioni, J. Agric. Food Chem. **63**, 2314 (2015)
49. H. Fulcrand, M. Dueñas, E. Salas, V. Cheynier, Am. J. Enol. Vitic. **57**, 289 (2006)
50. E. Celotti, S. Stante, P. Ferraretto, T. Roman Villegas, G. Nicolini, A. Natolino, Foods **9**, 1344 (2020)
51. X. Cao, C. Cai, Y. Wang, X. Zheng, J. Food Qual. **2019**, 1 (2019)
52. A. Natolino E. Celotti, LWT **154**, 1 (2022)
53. S. Li, K.L Wilkinson, A. Mierczynska-Vasilev, K.A. Bindon, Molecules **24**, 2100 (2019)
54. A.R. Jambrak, V. Lelas, T.J. Mason, G. Kreˇsi´c, M. Badanjak, J. Food Eng. **93**, 386 (2009)
55. J.M. Prado, P.C. Veggi, M.A A. Meireles, Global Food Security and Wellness (Springer, New York, 2017)
56. C.H. Chan, T.Y See, R. Yusoff, G.C., Ngoh, K.W. Kow, Food Chem. **221**, 1382 (2017)