INNOVATIONS ON RED WINEMAKING PROCESS BY ULTRASOUND TECHNOLOGY

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1. Introduction

Polyphenols play a fundamental role in enology, especially in the color and flavor of red wines. This class of compounds comes from different part of grapes and they have different chemical structure and, consequently, different chemical properties and reactivity. Anthocyanins and tannins are the most abundant and important grape polyphenols, as they great influence the color, taste and maturation potential of the wines. Anthocyanins are the main compounds responsible of color of red young wines, but they are unstable and their content decrease along wine aging due to degradation and stabilization reactions (He et al., 2012). Tannins, especially proanthocyanidins, are the main compounds affecting astringency and bitterness perception.

The maceration step is an extraction process of the grape components, with a special focus on polyphenols. Generally, in typical red winemaking, 30-50% of the polyphenols end up in wine, and the last 50-70% remain in grape tissues. Every phenol class and every given compound show different extraction rate and coefficient, depending on three main factors: location, polarity and molecular size (Waterhouse et al., 2016).

Several procedures and techniques can be used to enhance the extraction rate and yields: enzymatic reactions, heat treatments, time extensions, mixing and others. Other chemical and physical technologies have been studied to enhance the extraction of grape components, such as ultrasound, microwave, pulsed electric fields, high pressures, and others (Morata et al., 2015; Tartian et al., 2017).

Ultrasound has been considered one of the most promising technique to be applied on winemaking processes (Plaza et al., 2019). Recently, the International Organization of Vine and Wine has officially approved the ultrasound treatment of crushed grapes to promote the extraction of their compounds (OIV, 2019).

The high intensity and low-frequency ultrasound waves can induce physical and mechanical effects on biological and molecular structures, and chemical effects through the formation of high reactive radical species (Bhargava et al., 2021).

In wine research, this technology has been studied for several purposes in winemaking processes, such as the extraction of phenolic compounds during maceration (Bautista-Ortìn et al., 2017), the extraction of aroma precursors (Roman et al., 2020), replacing the preservatives addition (Clodoveo et al. 2016), management of wine microbiology (Jiranek et al., 2008), and valorization of winery by-products (Natolino et al., 2020; Romero-Dìez et al., 2019). In addition, ultrasound is also regarded as the most promising technique for accelerating the wine aging process (Tao et al., 2014; Garcìa-Martin et al., 2013), specifically considering its effectiveness on changing the chromatic characteristics and phenolic properties of red wines (Tiwari et al., 2010; Ferraretto et al., 2016).

Despite the several studies on ultrasound technology, none of the over mentioned applications has been scaled up at the industrial level. After laboratory study and

optimization, it is generally desirable that a process be directly transferable to an industrial-scale production environment. To do that, it is essential to make sure that all processing conditions remain the same: this will ensure that the final product quality is unchanged while the productivity rate is increased (Peshkovsky, 2017). Several approaches can be adopted for a successful scale-up process. One of them is a direct method using a pilot-scale plant to simulate the operative conditions and process efficiency at the production level.

The present work resumes the pluriannual researches carried out at laboratory and pilot-scale, aimed to study the effect of ultrasounds during different steps of the red winemaking process, using respectively: a static laboratory sonifier and a pilot plant at continuous mode. The effect of the main ultrasound process parameters was studied at laboratory scale, on anthocyanins, tannins, and color stability indices of two young red wines.

A number of technological experiments at pilot-scale level were carried out on different grape cultivars from different wineries and producing areas, to study properly the potential effectiveness of ultrasounds on the winemaking process. Potential effects of ultrasound on some chemical reactions, involved during wine aging, have been considered.

2. Material and methods

2.1. Laboratory scale trials

Two young red wines (WA and WB) from different wineries of the Valpolicella region (Italy) were considered for the laboratory scale trials. All the experiments were carried out in an ultrasonic sonifier (Sonoplus model HD 2200, Bandelin electronic, Berlin, Germany) equipped with a titanium alloy flat tip probe (13 mm diameter) (TT13, Bandelin, Berlin, Germany) (**Figure 1**). Samples were processed in a continuous mode at a constant frequency of 20 kHz. The energy input was controlled by setting the amplitude of the sonicator probe; the total nominal output was 200 W. An ice bath was adopted to avoid the increase in temperature of up to 35°C, which was continuously monitored.

Samples of wine A (WA) were sonicated for 3 min at two levels of amplitude (40 and 80%) to assess the amplitude effect. Instead, samples of wine B (WB) were sonicated at fixed amplitude (80%) but at different levels of sonication time (tUS) (1, 3, and 5 min).

2.2. Pilot scale trials

The technological experiments were carried out on three grape samples (Raboso, Amarone and Lagrein), at different wineries and producing areas. A prototype ultrasound plant (**Figure 1**), developed and built by TMCI Padovan (Vittorio Veneto, Treviso, Italy), was used in continuous mode at the end of the crusher destemmer. An appropriate Y-valve was placed between crusher-destemmer and sonication time, in order to split into equal and homogeneous aliquots and to ensure the comparison of untreated and sonicated samples. All the trials were carried out at fixed frequency (27 kHz), amplitude (100 %), and sonication time (2 min). Subsequently, the same operative protocols and vinification conditions were adopted for untreated and sonicated samples.
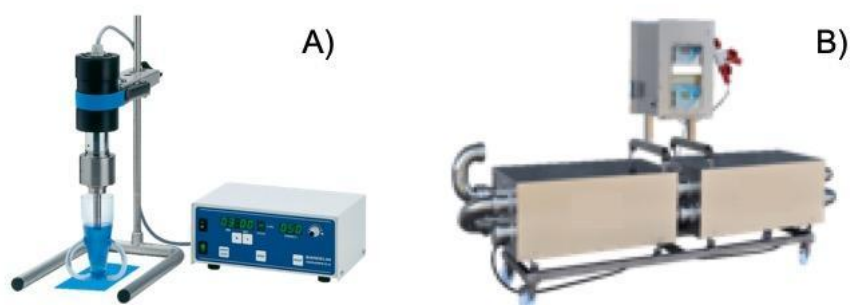
Figure 1. Laboratory (A) and Prototype (B) ultrasound plant.

A technological experiment was also carried out on a Merlot wine, before the aging period. The same ultrasound plant was used at the same frequency (27 kHz) and amplitude (100 %), but at different sonication times (3, 10, and 20 min).

2.3. Analytical determinations

The effects of ultrasounds on the main phenolic compounds at laboratory scale were evaluated by spectrophotometric methods. Anthocyanin, Tannins, Flavan-3-ols contents, and color intensity were determined using the methods reported by Ribereau-Gayon et al. (1965), Bate-Smith (1954), Zironi et al. (1992), and Glories (1984), respectively. Anthocyanin profile was also measured using an HPLC method (Morata et al., 2006), with slight modifications.

The sonication of crushed grapes by prototype plant was evaluated by the determination of optical densities at 280, 420, 520 and 620 nm (Glories, 1984), anthocyanin content (Ribereau-Gayon et al., 1965) and anthocyanin oxidability indices (Celotti et al., 2006). The optical densities of untreated and sonicated Raboso and Amarone samples were monitored along all maceration period (7-8 days).

Untreated and sonicated Merlot wine samples were evaluated along 45 days of aging by several analytical indices: anthocyanin content (Ribereau-Gayon et al., 1965), color intensity (Glories, 1984), polymerized pigments index (Glories, 1978), and HCl index (Glorie, 1978).

2.4. Sensorial analysis

A sensorial evaluation of untreated and sonicated samples of Lagrein variety was carried out by a selected panel of 10 judges. Several descriptors regarding aroma, taste and flavour were considered. Judges scored the magnitude of each attribute from 1 to 7 where 1 was "low" and 7 was "high".

2.5. Statistical analysis

All laboratory experiments were performed in triplicate, and the results were expressed as mean ± standard deviation. 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 $p < 0.05$.

3. Results and discussion

3.1 Laboratory-scale trials

In the initial step, several ultrasound treatments were carried out at a laboratory scale in order to evaluate the effect of acoustic cavitation on the main polyphenol compounds, involved in winemaking process. Two red young wines were considered as model systems, and they were sonicated at different levels of amplitude (40 and 80%) and time (1, 3, and 5 min). Figure 2 shows the results of anthocyanins, flavan-3-ols, tannins content, and color intensity of untreated and sonicated samples.
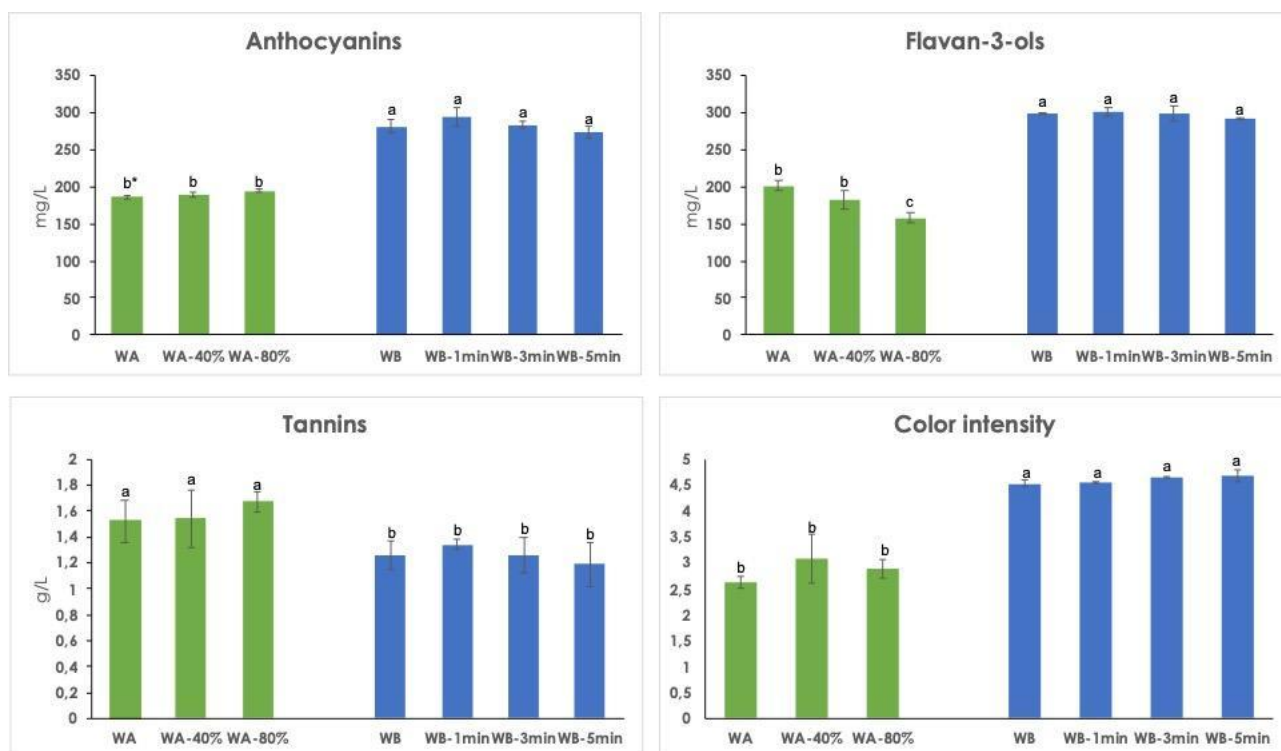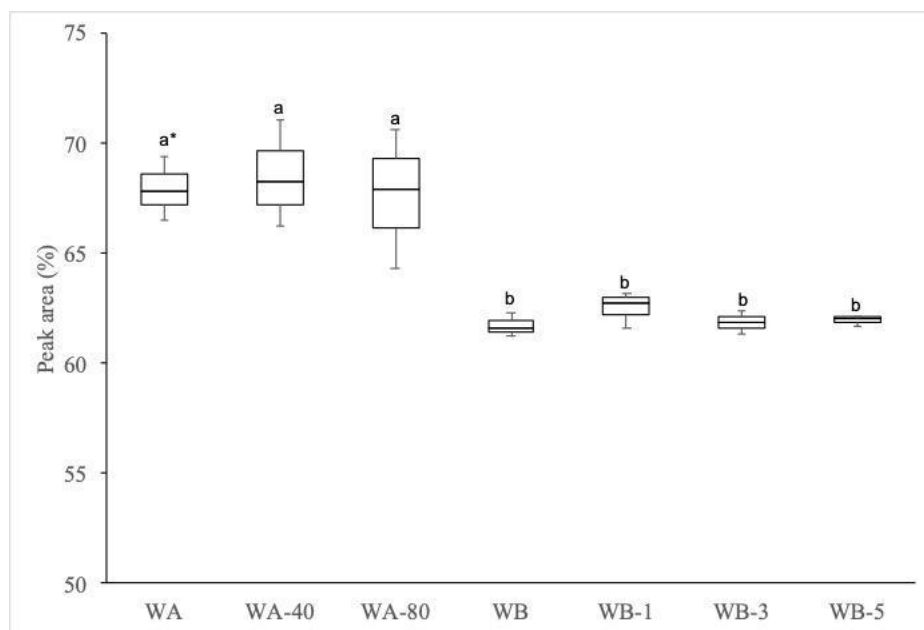

*Each data represents the mean of three replicates ± standard deviation
Values with different letters indicate significant differences (p<0.05).

Figure 2. Anthocyanins, Flavan-3-ols, Tannins content, and Color Intensity of untreated and sonicated young red wines.

The amplitude and sonication time didn't affect anthocyanins, tannins, and color intensity (Figure 2). Only flavan-3-ols content showed a slight decrease at the maximum level of amplitude (80 %), probably due to the chemical degradation promoted by ultrasounds as reported by Zhu et al. (2018).

Ultrasound has been well studied on extraction processes, and it is already well known that it can induce an increase in mass transfer mechanisms and extraction yields and efficiency (Chemat et al., 2017). As reported by Lukic et al. (2019), the application of ultrasound should ensure the preservation of sensory properties of wines, including color characteristics and stability. Ultrasonic waves could lead to changes in phenolic composition due to the cavitation phenomenon, which can induce high temperatures and the formation of radical species and trigger oxidation reactions, after the collapse of cavitation bubbles. High levels of amplitude generate high intense acoustic cavitation, which can induce degradation of phenol compounds (Kidak et al., 2006). In view of this, it is extremely important to select suitable ultrasound amplitudes to preserve polyphenols content and chromatic properties.

An HPLC analysis was carried out on untreated and sonicated samples to highlight any possible effect of ultrasound at different amplitudes and times on anthocyanin profiles (Figure 3 and Table 1).

*Each data represents the mean of three replicates ± standard deviation
Values with different letters indicate significant differences (p<0.05).

Figure 3. Malvidin-3-glucoside content

As reported in Figure 3, malvidin-3-glucoside, the main anthocyanin of red wines, is not affected by sonication treatments at 40 (68.51 ± 2.43) and 80% (67.65 ± 3.17) of amplitude, compared to the untreated sample (67.95 ± 1.45). The same results are reported by Zhang et al. (2016), who highlighted no changes on malvidin-3-glucoside content in wine samples after 14 and 28 min of sonication.

Also considering the minor anthocyanin compounds (Table 1 and 2), in most cases, no significant changes can be highlighted between untreated and sonicated samples. Moreover, the increase of amplitude and sonication time didn't affect the anthocyanin profiles.

It's notable that the sonication treatment should be modulated and optimized in order to enhance the extraction rate and efficiency during the grape maceration. At the same time, it is fundamental to avoid any degradation mechanisms of polyphenols to maintain the quality properties.

Compound	Amplitude		
	0%	41%	81%
Delphinidin-3-monoglucoside	1.60 ± 0.16 b*	3.15 ± 0.26 a	3.21 ± 0.19 a
Cyanidin-3-monoglucoside	0.46 ± 0.08 a	0.68 ± 0.15 a	0.69 ± 0.11 a
Petunidin-3-monoglucoside	5.03 ± 0.23 a	5.00 ± 0.21 a	4.96 ± 0.24 a
Peonidin-3-monoglucoside	7.65 ± 0.39 b	9.88 ± 1.25 a	9.55 ± 0.55 a
Vitisin A	1.14 ± 0.10 a	1.32 ± 0.11 a	1.33 ± 0.11 a
Petunidin-3-monoglucoside acetyltated	0.55 ± 0.09 a	0.66 ± 0.11 a	0.55 ± 0.06 a
Peonidin-3-monoglucoside acetylated	1.35 ± 0.17 a	1.34 ± 0.38 a	1.91 ± 0.44 a
Malvidin-3-monoglucoside acetylated	7.37 ± 0.33 a	7.99 ± 1.40 a	7.01 ± 0.37 a
Delphinidin-3-monoglucoside p-coumarylated	2.82 ± 0.16 a	2.48 ± 0.20 a	2.57 ± 0.40 a
Malvidin-3-monoglucoside p-coumarylated	4.13 ± 0.26 a	4.26 ± 0.26 a	4.39 ± 0.51 a
Malvidin-3-monoglucoside vinylphenol	n.d.	n.d.	n.d.
Malvidin-3-monoglucoside vinylphenol acetylated	n.d.	n.d.	n.d.

* Each data represents the mean of three replicates ± standard deviation

Values with different letters within line indicate significative differences (p<0.05)

Table 1. Anthocyanin profile – effect of sonication amplitude.

| Compound	t_{US}			
	0 min	1 min	3 min	5 min
Delphinidin-3-monoglucoside	3.97 ± 0.36 a*	3.94 ± 0.30 a	3.88 ± 0.24 a	4.15 ± 0.15 a
Cyanidin-3-monoglucoside	0.98 ± 0.20 a	0.93 ± 0.18 a	0.95 ± 0.20 a	0.99 ± 0.02 a
Petunidin-3-monoglucoside	5.51 ± 0.29 a	5.10 ± 0.78 a	5.52 ± 0.14 a	5.30 ± 0.33 a
Peonidin-3-monoglucoside	10.39 ± 0.02 a	10.75 ± 0.12 a	10.30 ± 0.65 a	10.94 ± 0.28 a
Vitisin A	2.17 ± 0.07 a	2.13 ± 0.16 a	2.27 ± 0.10 a	2.19 ± 0.08 a
Petunidin-3-monoglucoside acetyltated	0.73 ± 0.03 a	0.71 ± 0.05 a	0.74 ± 0.07 a	0.69 ± 0.02 a
Peonidin-3-monoglucoside acetylated	2.12 ± 0.36 a	1.64 ± 0.68 a	2.00 ± 0.16 a	1.41 ± 0.59 a
Malvidin-3-monoglucoside acetylated	6.39 ± 0.59 a	6.15 ± 0.58 a	6.41 ± 0.20 a	6.12 ± 0.12 a
Delphinidin-3-monoglucoside p-coumarylated	2.21 ± 0.10 a	2.25 ± 0.07 a	2.14 ± 0.09 a	2.23 ± 0.05 a
Malvidin-3-monoglucoside p-coumarylated	3.24 ± 0.12 a	3.32 ± 0.05 a	3.37 ± 0.03 a	3.43 ± 0.13 a
Malvidin-3-monoglucoside vinylphenol	0.15 ± 0.02 a	0.16 ± 0.03 a	0.19 ± 0.01 a	0.18 ± 0.01 a
Malvidin-3-monoglucoside vinylphenol acetylated	0.41 ± 0.01 a	0.37 ± 0.04 a	0.39 ± 0.02 a	0.39 ± 0.03 a

* Each data represents the mean of three replicates ± standard deviation
Values with different letters within line indicate significative differences (p<0.05)

Table 2. Anthocyanin profile – effect of sonication time (t_{US}).

3.2. Pilot-scale trials

3.2.1. Crushed grapes treatments
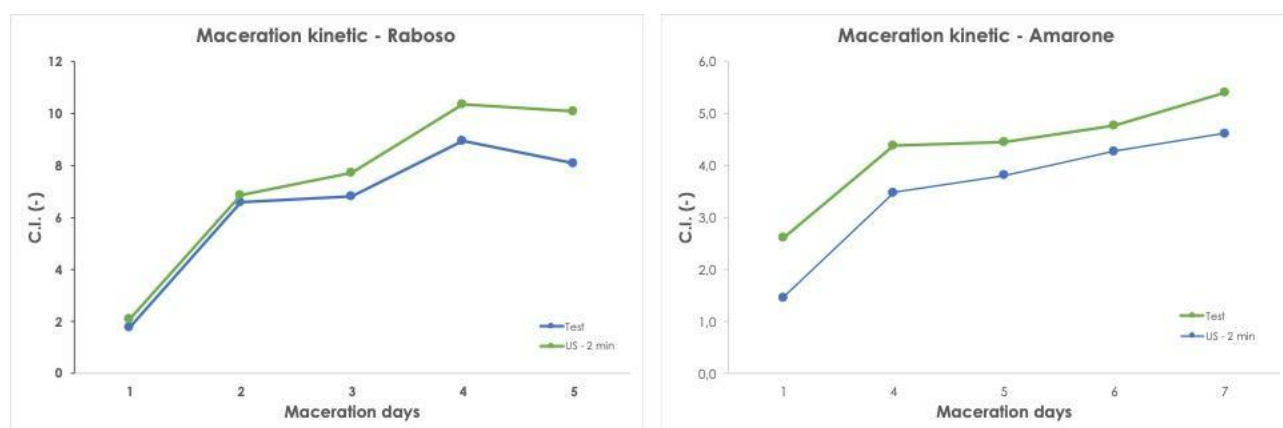
Figure 4. Maceration kinetic of untreated and sonicated samples of Raboso and Amarone.

A prototype ultrasound pilot plant was used to evaluate the effect of the cavitation phenomenon on crushed grapes at different wineries. Three different grape samples were chosen to consider different native polyphenol compositions.
In Figure 4 are depicted the maceration kinetic of untreated and sonicated samples of Raboso and Amarone, considering color intensity as quality technological parameter. During the first two days of Raboso maceration, no differences can be highlighted between untreated and sonicated samples. The kinetics starts do diverge after three days, and subsequently the sonicated sample shows higher color intensity. Also sonication of dehydrated grapes, for Amarone production, induced higher color intensity during the maceration period, which indicates a better extraction process of phenol compounds. It is notable that sonication allowed to achieve the same color intensity of untreated sample after only 3- 4 days of maceration. Ultrasound induced a potential decrease of maceration time between 40-60%. Besides economic advantages, a lower contact time with solid grapes fractions could reduce the probability of undesirable microbial growth, which can negatively affect the quality of the final product.
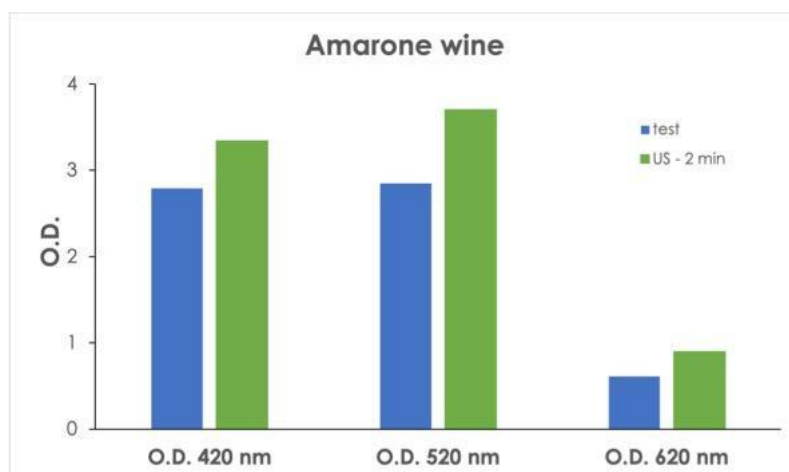
Figure 5. Comparison of Amarone wines obtained from untreated and sonicated samples.

Figure 5 shows the optical densities of untreated and sonicated samples of Amarone wine at the end of maceration time. Ultrasound treatment induced an increase of all the considered parameters. Generally, ultrasounds lead to enhancements of the extraction processes by a combination of several mechanisms: fragmentation, erosion, sonocapillary effect, sonoporation, local shear stress, and detexturation (Chemat et al., 2017). All of them induce a cell structures breakdown and higher mass transfer rates, leading to higher extraction yields and efficiency.

Sonication treatment was carried out also on Lagrein grapes and several analytical parameters were considered (Figure 6). Despite the results obtained with Raboso and Amarone, the sonication of Lagrein grapes didn't affect the polyphenol content, oxidability index, and color intensity. Only a significant increase of anthocyanin compounds was highlighted.
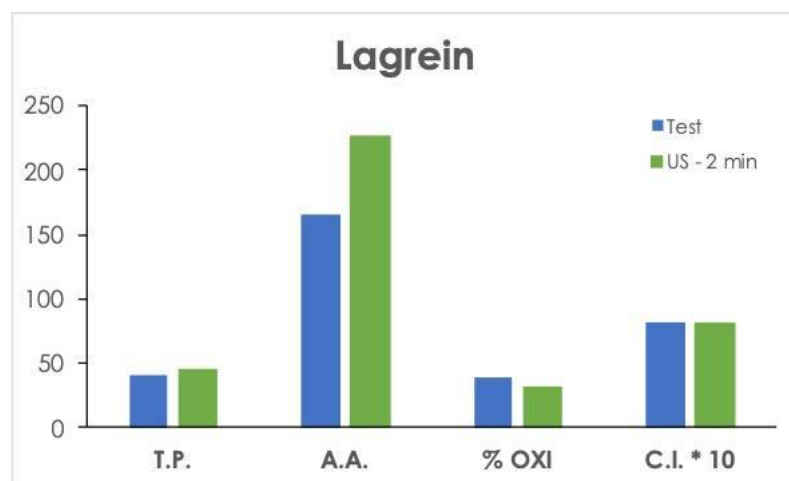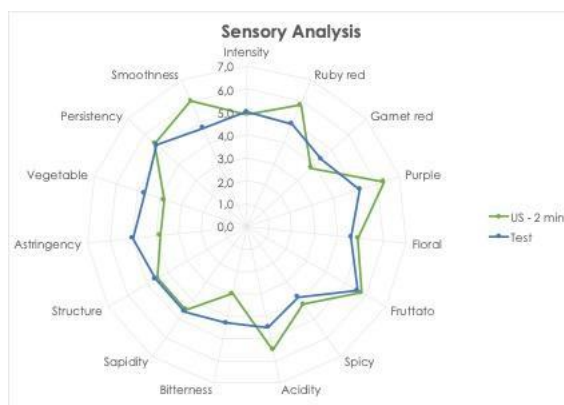

Figure 6. Comparison of Lagrein wines obtained from untreated and sonicated samples.

A sensorial analysis was also performed on Lagrein wines, obtained from untreated and sonicated samples, in order to evaluate some possible effects on organoleptic perceptions (Figure 7).

As reported, the wine obtained after sonication treatments were evaluated as less bitter and astringent, compared to the conventional one.

This result could indicate an effect of ultrasound irradiation not only on extraction mechanisms but also on chemical properties of some polyphenol classes, as tannins. Ultrasound could promote certain chemical reactions, render chemical and structural changes in wine that resemble those occurring after long periods of natural aging (Garcia-Martin & Sun, 2013).

Figure 7. Sensory evaluation of Lagrein wines obtained from untreated and sonicated samples.

3.2.2. Wine treatments

In view of the results of Lagrein variety, an ultrasound treatment was performed at a fixed amplitude (100%) and different times (3, 10 and 20 min) on a Merlot wine. Anthocyanin content, color intensity, polymerized pigment index and HCl index were monitored during the first 45 days of aging (Figure 8).
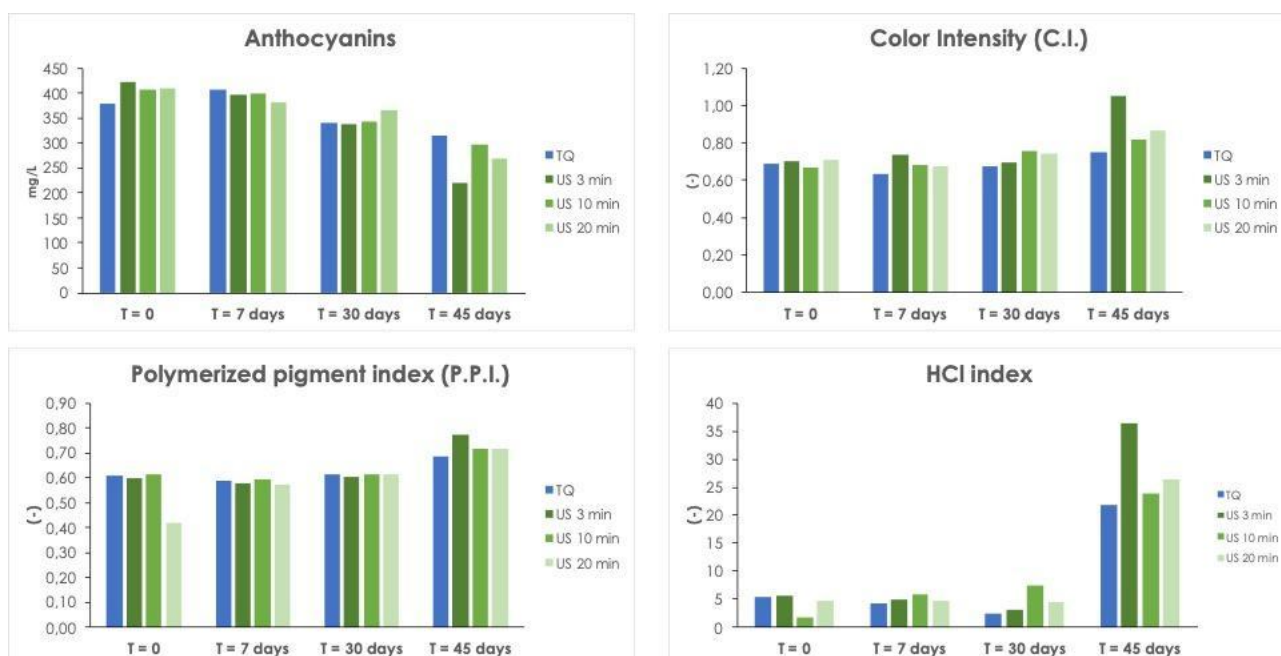
Figure 8. Evolution of anthocyanins content, color intensity, polymerized pigment index and HCl index during 45 aging days of untreated (TQ) and sonicated Merlot wines, at different sonication times (t_{US} = 3, 10, and 20 min).

Sonication treatment carried out for 3 minutes, induced a better evolution of all the analytical parameters: higher decrease of anthocyanin content, and higher increase of color intensity, polymerized pigment index, and HCl index. An increase of sonication time up to 20 minutes, which is considered as a boundary condition, didn't highlight other positive significant changes and few minutes of sonication are enough to induce positive effects of the considered parameters.

Wine aging is a long-term process during which several chemical reactions of polyphenols can occur, such as polymerizations, condensations, and others. Long aging periods are sometimes incompatible with the consumers' requirements and it represents a high cost for wineries. Several actions should be considered to accelerate the vinification processes and to ensure the quality of the wine at the same time. Ultrasound is regarded as the most promising technique for accelerating the wine aging process, specifically considering its effectiveness on changing the chromatic characteristics and phenolic properties of red wines (Garcia-Martin & Sun, 2013).

The high localized temperature and pressure, create by acoustic cavitation, induce the formation of reactive radical species, which enhance the reaction rates of existing processes or starting new reaction mechanisms (Kentish & Ashokkumar, 2010).

CONCLUSIONS

The effect of ultrasound, during different steps of the red winemaking process, was evaluated at laboratory and pilot-scale, at different amplitudes and sonication times.

Ultrasound treatments at laboratory scale preserved the initial phenolic compounds and no changes on anthocyanin profiles were highlighted. It is fundamental to optimize the operative conditions, such as amplitude and time, not only to optimize the polyphenol extraction but also to avoid degradative phenomena induced by acoustic cavitation.

Instead, the pilot-scale trials showed an increase of extraction processes and a potential decrease of maceration time between 40-60%. The decrease of maceration time has several advantages and it allows a potential decrease of microbiological risks, which are promoted by long contact time with solid grape fractions. A less contact time with grape seeds could induces also a decrease in tannins extraction and astringency perception.

Moreover, pilot-scale experiments on finished red wine showed potential positive effects also on some chemical reactions involved during the wine aging process.

Ultrasound can be considered as a sustainable technology that can be applied on the maceration step, to increase the extraction of grape compounds and reducing times, with several economic and management advantages for wineries. The sonication treatments should be opportunely modulated considering several factors, such as grape variety and oenological aims.

Ultrasound could be an alternative technology to be applied in several steps of winemaking processes, from maceration to wine aging, but more detailed researches are needed, and some of them are already underway.

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