BIO Web of Conferences **12**, 02001 (2019) https://doi.org/10.1051/bioconf/20191202001
41st World Congress of Vine and Wine

High power ultrasounds: A powerful, non-thermal and green technique for improving the phenolic extraction from grapes to must during red wine vinification

E.G. Plaza[1], R. Jurado[2], J.A. Iniesta[2], and A.B. Bautista-Ortín[1]

[1] Departamento de Tecnología de Alimentos, Nutrición y Bromatología, Universidad de Murcia, 30100 Murcia, Spain
[2] AGROVIN S.A. Alcazar de San Juan, Ciudad Real, España

Abstract. Wine color is one of the main organoleptic characteristics influencing its quality. It is of especial interest in red vinifications due to the economic resources that wineries have to invest for the extraction of the phenolic compounds. To increase this extraction, some chemical (maceration enzymes) or physical technologies (thermovinification, criomaceration, flash-expansion) can be applied. In this work, the results of the application of high power ultrasounds to the crushed grapes to increase the extraction of phenolic compounds are presented. Crushed grapes (400 kg) from the 2017 harvest were treated with ultrasound, and three different lengths of skin maceration period (2, 3 or 7 days) and the results were compared with a control vinification, where grapes were not subjected to any treatment and were skin macerated during 7 days. The wine chromatic characteristics and the individual phenolic compounds were followed during all the maceration period, at the end of alcoholic fermentation and after bottle storage. The wines made with ultrasound treated grapes presented differences with control wine, especially as regard color and total phenol and tannin content, the wines with three days of maceration time presenting similar chromatic characteristics than control wines with 7 days of maceration time.

1. Introduction

Red winemaking comprises several steps that play a crucial role during the transformation of grapes to wine and in the final quality of this wine. The most important factors generally considered by winemakers include the maceration step, the fermentation, with the proper use of relevant enological microorganisms and the aging practices. Nowadays, efforts are being devoted to the development of new practices to enhance or in some cases substitute conventional winemaking techniques. These new practices should be energetically efficient, present low effluents and implying minor use or water and additives.

Based on this, some emerging technology, among them ultrasound (US) are being tested in wineries, with different aims, such as increasing the winery productivity by reducing the production time (maceration and aging processes), allowing the winery to optimize resources and spaces [1] or the reduction of the use of SO_2 by a control of the enological microbiota.

US is a non-thermal technology characterized by mechanical effects able (1) to speed up and increase the extraction of valuable components into the resulting wine, thus improving the quality value of the product [2]. (2) to disrupt or damage the cellular membrane of either autochthonous yeasts and bacteria in grape must before primary fermentation or spoilage microorganisms in wine, thus markedly reducing the addition of SO_2, as antiseptic agent, during winemaking [3,4]. (3) promotes chemical reactions that may assist the wine aging process [1]. Therefore, the employment of this technology may provide high-quality wines in shorter time, with reduced spoilage organisms (and therefore with lower needs of SO_2 content), and higher content in anthocyanins together with pleasant flavor and full body. Moreover, they could confer added value in terms of sensorial characteristics of the resulting wine.

How are these effects achieved? The ultrasound technology is based on mechanical waves at a frequency higher than the upper limit of human hearing (>16 kHz). In the food industry, ultrasound can be divided into two frequency ranges: high frequency ultrasound (100 kHz–1 MHz) and power ultrasound (16–100 kHz). Power ultrasound has been used for many years in food technology to generate emulsions, disrupt cells and disperse aggregated materials; enzymes inactivation, to enhance drying and filtration and the induction of oxidation reactions [5]. These effects are achieved mainly due to the phenomenon of cavitation. The propagation of a high intensity sound wave in a liquid media may generate cavities in the liquid, the so-called cavitation bubbles. When the size of these bubbles reaches a critical value, they collapse generating extreme local conditions: determined temperatures up to approximately 5000 °K and estimated pressures around 50–1000 atm [6]. These hotspots create shockwave damages. Those effects can lead to fragmentation of materials and localized erosion.

These phenomena may be useful in enology since it may help some of the main winemaking processes such as the maceration process. Red wine vinification implies the maceration of grape skins with the must, this step being one of the most important processes of this type of vinification. One of the effects of ultrasound useful to reduce the winemaking time is its ability to promote the breaking of the cell, improving the matter transfer. The permeabilization of cell membranes to the intra-cellular substances may be favored. The mechanical activity of US supports the diffusion of solvents into the tissue, speeding up the process of soaking crushed grapes [2].

It is commonly assumed that to achieve a good and stable wine color and a desirable varietal aroma a certain length of skin maceration is needed to promote the extraction of anthocyanins (responsible of the wine red color and located inside the cells in the skin), tannins (located in skin and seeds, their presence is necessary for stabilizing the unstable anthocyanins, they need longer maceration time than anthocyanins) and aroma compounds (also mainly located in the skin cells).

However, and especially in large wineries, and when harvest occurs only during a very short period of time, the capacity of the winery, regarding maceration tanks may be exceeded. In this case, the winery can be forced to reduce the maceration time and, as a consequence, the quality of the wine and its potential for ageing can be compromised. To limit this problem some strategies have been used to shorten the maceration time but maintaining color and wine quality such as the use of maceration enzymes [7], or physical technologies such as thermovinification [8]. Ultrasound, being a clean, eco-friendly and energetically very efficient technology, it may be an useful technique to accelerate the extraction of compounds from skin cells to must, during winemaking, reducing the maceration time.

Related to this [9] studied the effects of ultrasound, mechanical stirring and commercial enzyme preparations *Vitis vinifera* Cabernet Sauvignon must extraction. They found that ultrasounds improved the extraction yield, anthocyanin content, color and total soluble solids, while the combined techniques of ultrasound + mechanical stirring with enzyme improved yield, antioxidant activity and color extraction.

[10] compared three alternative technologies, electric pulses, thermomaceration and ultrasound. They found the best chromatic results when electric pulses were used although the ultrasound and thermomaceration treatments also permitted to enhance the phenolic, anthocyanin, and tannin contents of wines, as well as to obtain the higher color intensity comparing to the untreated samples. Similarly [5]. reported an improvement in the extraction of polyphenolic substances, with a reduction in the duration of classic maceration using US.

It is clear that the use of ultrasound attracts a lot of interest in the enological field, however [2] did a literature review where it can be seen that almost all the previous studies have been done with laboratory equipment. We could only find two studies where a medium-scale system, suitable for working in small wineries, were used [11, 12]. Our previous studies [11] reported that the wines made with ultrasound-treated grapes showed differences with the control wine, especially regarding total phenol content and tannin content. [12] also worked at winery scale to study the polyphenols extraction during winemaking of three red grape cultivars grown in southern Italy (Primitivo, Nero di Troia and Aglianico). They found differences due to variety, however, their system presented some problems and in order to overcome a skin screen effect in the US system, grapes had to be processed after diluting the grapes with previously extracted juice (1:1 w/v).

In this paper, we focused our attention on the application of a small-scale power ultrasound system for treating crushed Tempranillo and Monastrell grapes, looking for a reduction of the maceration time needed for the extraction of phenolic and volatile compounds and to find out if a varietal effect may also exist.

2. Material and methods

2.1. Grapes

Monastrell and Tempranillo red grapes were harvested from vineyards in the province of Murcia (Spain) and they were transported the same day to the winery for their processing.

2.2. Winemaking

The grapes (400 kg) were destemmed and crushed. The crushed grapes were treated with a pilot scale power ultrasound system that could treat 400 kg of crushed grapes per hour. The system operated at 2500 W and 28 kHz frequency, with a power density of 8 W/cm^2. A batch of crushed grapes was not treated (control vinification). 10 kg stainless-steel small tanks were filled with the control and ultrasound treated crushed grapes. Total acidity was corrected to 5.5 g/L and selected yeasts were added (Viniferm CT007, Agrovin SA, Spain, 20 g of dry yeast/100 kg of grapes). Three different skin maceration times were tested for the sonicated grape: 2 (SW48h), 3 (SW72h) and 7 (SW7d) days, whereas the control vinification had a skin maceration time of 7 days (CW). All vinifications were done in triplicate. During the macerative fermentation, the cap was punched down twice a day. At the end of this period, the wines were pressed. After alcoholic fermentation was finished, wines were cold stabilized and bottled. Must and wines were analyzed at the end of alcoholic fermentation and after two and five months in the case of Tempranillo or 12 months, in the case of Monastrell wines.

2.3. Analytical determinations

2.3.1. Spectrophotometric parameters

Color intensity (CI) was calculated as the sum of absorbance at 620, 520 and 420 nm. Total and polymeric anthocyanins were determined spectrophotometrically [13]. Total phenols (TP) were calculated by measuring wine absorbance at 280 nm. Total tannins were determined by the methyl cellulose method [14].

2.3.2. Determination of proanthocyanidins by phloroglucinolysis

Wine samples were prepared by an optimization of the method described by [15] and the detailed methodology can be found in [16]. The analyses of proanthocyanidins were done by depolymerizing the molecule using the phloroglucinol reagent and the depolymerized samples were analyzed by HPLC. Proanthocyanidin cleavage products were estimated using their response factors relative to (+)-catechin, which was used as the quantitative standard. These analyses allowed determination of the

Table 1. Chromatic characteristics of Tempranillo wines at the end of alcoholic fermentation, and after 2 and 5 months in the bottle.

Samples	CI	TP	TA (mg/L)	PA (mg/L)	TT (mg/L)
EAF					
Control	12.8b	72.4b	583.8b	47.7b	2119.4bc
SW 48h	9.2a	39.1a	385.5a	19.0a	1292.1a
SW 72h	10.8ab	71.6b	551.4b	38.5b	1999.6b
SW 7d	12.5b	81.4c	687.4c	41.9b	2212.8c
2mb					
Control	14.3a	67.1b	507.2b	46.2b	2110.2b
SW 48h	10.2a	38.5a	341.1a	22.9a	1303.7a
SW 72h	12.7a	65.8b	456.0b	45.7b	2024.3b
SW 7d	14.1a	74.5c	638.7c	41.4b	2184.4b
5mb					
Control	13.5c	50.5c	240.1a	50.3c	2301.1c
SW 48h	8.14a	30.2a	218.9a	30.0a	1145.8a
SW 72h	12.1b	43.9b	281.3a	40.6b	1871.3b
SW 7d	13.9c	55.0c	356.3b	45.5c	2239.6c

CI: color intensity, TP: Total phenols, AT: total anthocyanins (mg/L). PA: polymeric anthocyanins (mg/L). TT: total tannins by the methyll cellulose method (mg/L).
EFA: end of alcoholic fermentation. mb: months in bottle. SW sonicated wines. Different letters within the same column and for each time indicate significant differences $p < 0.05$.

Table 2. Compositional data of Tempranillo wine tannins at the end of alcoholic fermentation.

Samples	TT	mDP	%G	%EGC
Control	660.0b	4.1b	3.2a	15.2b
SW 48h	529.8^{a}	3.7a	2.8a	15.1b
SW 72h	574.5a	4.5c	2.6a	12.9a
SW 7d	652.2b	4.2b	3.4b	14.3ab

TT: total tannins by phloroglucinolysis method (mg/L).
mDP: mean degree of polymerization. %G: percentage of galloylation.
Different letters within the same column indicate significant differences $p < 0.05$.

total proanthocyanidin content, the apparent mean degree of polymerization (mDP) and the percentage of each constitutive unit. The mDP was calculated as the sum of all subunits (flavan-3-ol monomer and phloroglucinol adducts, in moles) divided by the sum of all flavan-3-ol monomers (in moles).

2.3.3. Isolation of wine volatile compounds by SPME

A divinylbenzene-carboxen-polydimethylsiloxane 50/30 micras (DVB/CAR/PDMS) fiber was used. The conditions of the SPME extraction process and GC/MS analysis can be found in [17].

3. Results and discussion

3.1. Tempranillo wine characteristics

The results regarding the effect of the sonication of crushed grapes on the chromatic and phenolic characteristics of Tempranillo wines can be found in Tables 1 and 2.

At the end of the alcoholic fermentation, the results showed that the only wine that presented less intensity of color than the control wine, elaborated with seven days of maceration, was the wine made from grapes treated by ultrasound and with a maceration of 48 hours, although the difference with the control wine was only three units but the difference in maceration time are five days. The color of wines made from grapes treated with ultrasounds and with a maceration time of 72 hours and 7 days did not show significant differences with the color of the control wine. After two months in bottle, the color increased in all the wines and after 5 months, this parameter slightly decreased in the wine made with sonicated grapes and 48 h of maceration time, probably due to a precipitation of colored material since total phenols and total tannins also decreased and a lower formation of stable pigment. However, wines made with sonicated grapes and 72 h of maceration showed only small chromatic differences with control wine.

With respect to total polyphenols and total tannins, only the wine made with sonicated grapes and 48 hours of maceration presented appreciable differences with control wine, the SW72h barely differed from control wine, made with untreated grapes and with a seven-day maceration.

The wine tannins were also evaluated using a chromatographic method that consists of its depolymerization with a strong nucleophile that gives us information about the characteristics of these tannins (Table 2). We could evaluate total tannin content, the mean degree of polymerization of these tannins, the percentage of galloylation and the percentage of the skin-derived subunit epigallocatechin, that can give us information on the proportion of skin tannins extracted from the grapes to must. The results showed that wines with shorter maceration time showed slightly lower concentration of depolymerizable tannins (which necessarily does not indicate a lower content of tannins. since part of wine tannins can be forming structures that do not depolymerize in the acid medium used). The average degree of polymerization of these tannins is somewhat lower in the wine with only 48 hours of maceration, it can be probably due to the fact that, at the beginning of maceration there is no ethanol in the medium and only the smallest and easily extractable tannins are extracted to the must [18].

The percentage of galloylation is lower in wines made with shorter maceration time. This may be interesting from an organoleptic point of view, since a high degree of galloylation may be related to a greater bitterness in the wine [19]. In this variety, the epigallocatechin percentage was similar in the control wine and in the wine made with sonicated grape and 48 h of maceration. Indicating an easier liberation of skin tannins due to sonication. After that, percentage decreased due to a more important contribution of seed tannins in the wine as maceration time increased.

We could observe a higher presence of suspended cell wall material in the sonicated grape musts and as stated by [11] that could help to explain why longer maceration times did not lead to a much higher tannin content since they could be adsorbed in the suspended cell walls material by a mechanism already described by [20].

The results of the semiquantitative analysis of volatile compounds in the control and the three wines made with sonicated grapes after two months in the bottle are presented in Fig. 1, where the sum of total higher alcohols, esters, fatty acids and terpenoids can be observed. The largest compounds included alcohols, monoterpenes and norisoprenoids and esters. The results showed that ultrasound treatment did not cause large differences in the wine aromatic profile. The largest concentration of higher alcohols was observed in SW48h, no differences

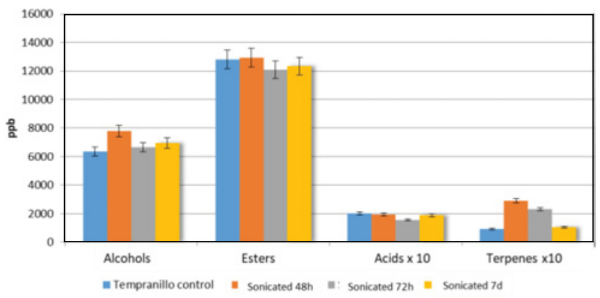

Figure 1. Concentration of different families of aroma compounds in Tempranillo wines after two months in bottle.

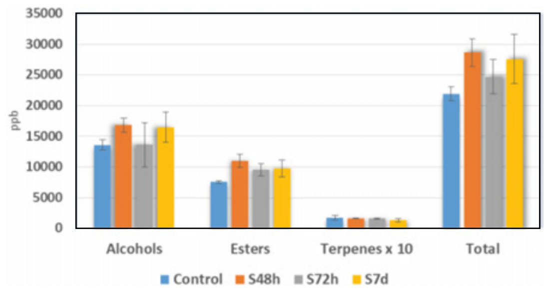

Figure 2. Concentration of different families of aroma compounds in Monastrell wines after two months in bottle.

Table 3. Chromatic characteristics of Monastrell wines at the end of alcoholic fermentation. and after 2 and 12 months in the bottle.

Samples	CI	TP	TA (mg/L)	PA (mg/L)	TT (mg/L)
FFA					
Control	12.1bc	53.8a	556.4b	24.6b	1367.4a
SW 48h	8.6a	52.5a	410.4a	20.9a	1556.0b
SW 72h	10.5b	62.4b	493.7b	24.3b	1808.6c
SW 7d	12.4c	68.7c	545.7b	27.5c	2163.0c
2mb					
Control	12.7b	50.4a	541.0b	30.9b	1312.0a
SW 48h	8.8a	50.3a	395.1a	24.3a	1466.4a
SW 72h	11.7b	57.6b	486.7b	30.0b	1709.3b
SW 7d	14.3c	65.6c	523.6b	36.7b	2172.5b
12mb					
Control	11.9bc	47.1a	297.8b	63.3a	1579.6a
SW 48h	10.2a	46.8a	211.7a	48.1a	1640.0a
SW 72h	10.8ab	55.4b	286.4b	50.6a	1823.9a
SW 7d	12.7c	62.9c	286.4b	76.5b	2307.1b

CI: color intensity. TP: Total phenols. AT: total anthocyanins (mg/L).
PA: polymeric anthocyanins (mg/L). TT: total tannins by the methyll cellulose method (mg/L).
EFA: end of alcoholic fermentation. mb: months in bottle. SW sonicated wines.
Different letters within the same column and for each time indicate significant differences $p < 0.05$.

Table 4. Compositional data of Tempranillo wine tannins at the end of alcoholic fermentation.

Samples	TT (mg/L)	mDP	%G	%EGC
Control	498.2c	4.5c	3.1a	21.8b
SW 48h	714.7b	3.8b	3.6b	11.0a
SW 72h	725.5b	3.6b	3.5b	10.8a
SW 7d	824.0a	3.4a	4.3c	9.6a

TT: total tannins by phloroglucinolysis method (mg/L).
mDP: mean degree of polymerization. %G: percentage of galloylation.
Different letters within the same column indicate significant differences $p < 0.05$.

could be observed in esters and fatty acids. However, the concentration of monoterpenes and norisoprenoids seemed to be favored by the sonication, especially in SW48h and SW72h and that could increase the floral notes in the wines.

3.2. Monastrell wine characteristics

The experience with the ultrasound technology was also made with Monastrell grapes, looking forward to determining if a varietal effect may also exists. The results can be observed in Tables 3 and 4.

The results are quite similar to those observed in Tempranillo wines. It can be observed that the wine made with sonicated grape and 72 hours of maceration did not differ from the control wine in color intensity, neither at the end of the malolactic fermentation nor after two or twelve months in the bottle. In addition, for this variety, the content of total phenolic compounds and tannins already reached the values observed in the control wine using only 48 hours of maceration, these values being even higher in wines made with sonicated grapes and with 72 and 7 days of maceration. It is clear that the sonication of the crushed grapes facilitated the rupture of the cellular structures and facilitated the extraction of phenolic compounds, in a much faster way than when non-sonicated crushed grapes were vinified. the differences with the control wines being larger than in the case of Tempranillo wines.

Table 4 showed the results of the analysis of tannins by the phloroglucinolysis method.

As was already observed when tannins were measured by the methylcellulose method, the wines made with sonicated grapes showed significantly higher content of depolymerizable tannins, even when only 48 hours of maceration were used. The mean degree of polymerization was lower in the wines made from sonicated grapes, as well as the percentage of EGC whereas the percentage of galloylation increased. All these data may indicate that perhaps, and for this variety, sonication seems to affect seed phenolic compound extractability, increasing the number of subunits that come from the seeds. This observation deserves a future deeper study.

The results of the analysis of the aroma compounds after two months in the bottle can be seen in Fig. 2.

Differences in volatile compounds were slightly higher than those observed in Tempranillo wines. The concentration of total aroma compounds differed from those in control wines. Although higher alcohols only differed from control wines in SW48h, the concentration of esters was higher in wines made from sonicated grapes and that could be translated into a more fruity wines.

4. Conclusions

We have reported the results of the application of ultrasound to the grapes just after crushing using a medium scale ultrasound device. The treatment was applied to Tempranillo and Monastrell grapes. The application of ultrasounds allowed a significant reduction of the maceration time and the obtention of high-quality wines with good chromatic characteristics. Monastrell grapes seemed to be more favored by the treatment than Tempranillo grapes and the results pointed to the fact

https://doi.org/10.1051/bioconf/20191202001

that ultrasounds not only promoted the extraction of skin phenolic compounds but also seed compounds may be affected.

References

[1] J.F. Garcia Martin, D.W. Sun, Trends Food Sci. Technol. **33**, 40 (2013)
[2] M.L. Clodoveo, T. Dipalmo, C. Rizzello, F. Corbo, P. Crupi, Inn. Food Sci. Emerg. Technol. **38**, 41 (2016)
[3] K. Aneja, R. Dhiman, N. Aggarwal, A. Aneja, Int. J. Microbiol. **20**, 1 (2014)
[4] J. Guimaraes, E. Silva, V. Alvarenga, A. Costa, R. Cunha, A. Sant'Ana, M. Freitas, M.A. Meireles, A. Cruz, Ultrason – Sonochem. **44**, 251 (2018)
[5] P. Ferrareto, V. Cacciola, I. Ferran Batllo, E. Celloti, Ital. J. Food Sci. **25**, 160 (2013)
[6] K. Ameer, H. Shahbaz, J. Kwon. Comp. Rev. Food Sci. Food Safety **16** (2017)
[7] A.B. Bautista-Ortín, A. Martínez-Cutillas, J.M. Ros-García, J.M. López-Roca, E. Gómez-Plaza, Int. J. Food Sci. Technol. **40**, 1 (2005)
[8] N. de Andrade Neves, L. Araújo Pantoja, A. Soares dos Santos, Eur. Food Res. Technol. **238**, 79 (2014)
[9] L. Dalagnol, L. Dal Magro, V. Silveira, E. Rodrigues, V. Manfroi, R. Rodrigues, Food Bio. Proc. **105**, 197 (2017)
[10] N. El Darra, N. Grimi, R. Maroun, N. Louka, E. Vorobiev, Eur. Food Res. Technol. **236**, 47 (2013)
[11] A.B. Bautista-Ortín, M.D. Jiménez-Martínez, R. Jurado, J.A. Iniesta, S. Terrades, A. Andrés, E. Gómez-Plaza, Int. J. Food Sci. Technol. **52**, 1314 (2017)
[12] G. Gambacorta, A. Trani, R. Punzi, C. Fasciano, R. Leo, G. Fracchiolla, M. Faccia, Inn. Food Sci. Emerg. Technol. **43**, 54 (2017)
[13] R. Boulton, Am. J. Enol. Vitic. **52**, 67 (2001)
[14] P. Smith, AWRI Tech. Rev. **158**, 3 (2005)
[15] J.L. Pastor del Rio, J.A. Kennedy, Am. J. Enol. Vitic. **57**, 125 (2006)
[16] N. Busse-Valverde, E. Gómez-Plaza, J.M. López-Roca, R. Gil-Muñoz, J.I. Fernández-Fernández, A.B. Bautista-Ortín, J. Agric. Food Chem. **58**, 11333 (2010)
[17] E. Gómez-Plaza, L. Mestre-Ortuño, Y. Ruiz-García, J.I. Fernández-Fernández, J.M. López-Roca, Am. J. Enol. Vitic. **63**, 394 (2012)
[18] A. Hernández-Jiménez, J. Kennedy, A.B. Bautista-Ortín, E. Gómez-Plaza, Am. J. Enol. Vitic. **63**, 57 (2012)
[19] F. Brossaud, V. Cheynier, A. Noble, Aust. J. Grape Wine Res. **7**, 33 (2001)
[20] A.B. Bautista-Ortín, R. Abdallah, L. Castro-López, M.D. Jiménez-Martínez, E. Gómez-Plaza, Int. J. Mol. Sci. **17** (2016)