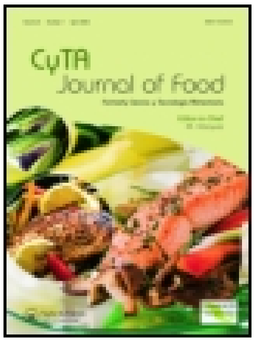

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Preliminary study of the effects of ultrasound on red wine polyphenols

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ABSTRACT

The article evaluates the effect of high-power ultrasound (20 kHz) on the phenolic structure of red wines, to study the possible applications of this innovative technique in wine aging. Different treatment conditions, with times of 1, 3 and 5 minutes and amplitudes of 51, 102 and 153 µm, were applied. In the experimental conditions the main parameters related to the evolution of red wine phenolic compounds show interesting variations; in particular, we found significant differences on tannins evaluated with some indices of tannins reactivity. On the other hand, free anthocyanins do not undergo changes due to ultrasound application. The preliminary results, which definitely need a depth, nonetheless allow us to hypothesize the application of ultrasound technology to accelerate the aging of red wines, which normally requires (needs) long times in the cellar conditions.

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Red wine; ultrasound; phenolic compounds; aging

PALABRAS CLAVE
vino tinto; ultrasonido; compuestos fenólicos; maduración

Estudio preliminar de los efectos del ultrasonido en los polifenoles del vino tinto

RESUMEN

Este estudio evalúa el efecto del ultrasonido de alta potencia (20 kHz) en la estructura fenólica de los vinos tintos, para estudiar las posibles aplicaciones de esta técnica innovadora en la maduración del vino. Se aplicaron diferentes condiciones de tratamiento con tiempos de 1, 3 y 5 minutos y amplitudes de 51, 102 y 153 µm. Bajo condiciones experimentales los principales parámetros relacionados con la evolución de los compuestos fenólicos del vino tinto mostraron variaciones interesantes; en particular, encontramos diferencias significativas en los taninos evaluados con algunos índices de reactividad de los taninos. Por otro lado, las antocianinas libres no mostraron cambios debido a la aplicación de ultrasonido. Por supuesto, los resultados preliminares necesitan profundidad, sin embargo permiten hacer hipótesis sobre la aplicación de tecnologías de ultra-sonido para acelerar la maduración de los vinos tintos, que normalmente requieren (necesitan) periodos largos en condiciones particulares en las bodegas.

Introduction

Phenolic compounds play an important role in enology, owing to their essential contribution to wine sensory properties (color, flavor, astringency and bitterness) and to their role in aging (Spranger et al., 2004).

The parameters for the evaluation of red wines coming from red grape varieties, considering the different methods of color extraction (i.e. maceration, thermovinification, carbonic maceration, pectinolytic enzymes), are strictly related to the quality and the quantity of the phenolic compounds in the raw material, but the main constraint of the technology used is the polymerization kinetic that influences the times of wine aging.

Non-enzymatic oxidative reactions produce significant sensory changes during aging. This involves the transfer of an electron (or hydrogen atom) from the oxidized compound to oxygen, or another acceptor. Regarding bottled wines, the reactions that involve molecular oxygen occur slowly (Jackson, 2008), depending on the oxygen content or the catalyzers–substratum ratio.

The temperature increases due to cavitation phenomenon, there is slight pH increase as well (Chang & Chen, 2002) and the phenolic content could significantly affect oxidative potential.

Ultrasound could promote all the reactions that occur during the aging process: oxidative reactions (involving or not molecular oxygen) and reductive reactions.

One of the most important changes during aging is a progressive increase and stabilization of the color due to copigment anthocyanin complexes, the formation of new pigments and the progressive formation of both tannin–tannin and anthocyanin–tannin complexes (Boulton, 2001; Jackson, 2008).

Several studies have literally illustrated the potential of alcoholic beverages aging by chemical methods (Boulton, 2001; Castellari, Matricardi, Arfelli, Galassi, & Amati, 2000), while very few experiments were done applying physical methods, such as ultrasound (Chang, 2005). According to Chang and Chen (2002) the application of ultrasonic waves with a frequency of 20 kHz facilitates the aging of rice wine more than the conventional one, without affecting the final quality of the product. On the opposite the application of ultrasound treatment on maize wine promotes the aging, but the final quality is not comparable with the traditional aging quality (Chang, 2004).

Masuzawa, Ohdaira, and Ide (2000) show the ultrasound effects on phenolic compounds of red wine. From the results obtained, the authors confirm an effect of polymerization of

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polyphenolic compounds in red wine promoted by ultrasound at low sound pressure levels.

Recently, ultrasound effects on red wine physicochemical properties were investigated (Zhang, Shen, Fan, & García Martín, 2015). The authors reported that different conditions of ultrasonic treatment changed significantly the concentration of total phenolic compounds and the electrical conductivity. Therefore, this physical technology, modifying the characteristics of red wine, could be a promising method for accelerating the wine aging process (García Martín & Sun, 2013).

Ultrasound power provides high temperature and high pressure leading to the modification of chemical reactions (Suslick, 1989), such as fragmentation and subsequent recombination of polymers (Chang, 2005).

Ultrasound power is of interest since it provides a form of energy that is different from those normally used, such as heat, light and pressure (Lindley & Mason, 1987). The effects of ultrasound are believed to exist thanks to small bubbles (100 microns), which collapse into a localized 'hotspot', generating tremendous heat and pressure, shock waves, and particle acceleration in aqueous systems. This process is called cavitation and it has mainly mechanical effects at frequencies up to 20 kHz and chemical effects at higher frequencies. (Mason, 1998).

The combination of these factors (heat, pressure and turbulence) accelerates mass transfer in chemical reactions, creates new reaction pathways, breaks down and dislodges particles or even generates different products from those obtained under conventional conditions (Patist & Bates, 2008).

Ultrasound has recently been applied to evaluate the possible effect on color and flavor extraction at different stages of the winemaking process (Bates & Patist, 2010; El Darra, Grimi, Maroun, Louka, & Vorobiev, 2013; Ferraretto, Cacciola, Ferran Batllò, & Celotti, 2013): red color and aromatic compounds are localized in the skin cells and their release is facilitated by mechanical actions, disruption of tissues and cells, temperature, and alcohol.

The increase of tannins and anthocyanins concentration in wine, the main phenolic compounds responsible for flavor, color and aging, leads to wines with a higher aging potential. Therefore, the application of a polyphenol extraction technique before fermentation could enhance the further aging process (García Martín & Sun, 2013).

Ultrasound could also be used to accelerate the release of protective colloids from lees and stabilize wines in a very short time (Cacciola, Ferran Batlló, Ferraretto, Vincenzi, & Celotti, 2013; García Martín, Guillemet, Feng, & Sun, 2013). The ultrasound cavitation would be able to facilitate the release of these compounds, causing disruption of cell walls and membranes.

Our research project explores the stabilizing effects of ultrasound on tannin–anthocyanins polymers, in order to understand how to speed up the aging reactions and hence reduce the time between production and consumption.

Ultrasound, as a relatively low cost, non-hazardous and environmental friendly technology, is commonly used in the food industry (Mason, Paniwnyk, & Lorimer, 1996; Sun & Li, 2003; Zheng & Sun, 2006), hence its possible application in the wine industry might become an important technological innovation by speeding up some slow reactions required in the winemaking process.

Materials and methods

Ultrasound equipment

An ultrasonic processor (SONOPLUS HD 2200, Bandelin electronic, Berlin, Germany) with 13 mm sonotrode probe, made of titanium, was used for sonication. Samples were processed in a continuous sonication 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.

The ultrasound probe was submerged to a depth of 20 mm in a 250 mL beaker containing the sample. The amplitude levels and processing times were varied on the basis of the experiments. The final treatment temperature was measured for all the samples.

Treatment conditions

Experimental tests, carried out to assess the possible effects of ultrasound on red wines, have been set according to different work plans.

For preliminary tests (Samples 1 and 2), all treatment combinations (time and amplitude %) were analyzed with the response surface method (RSM, response surface methodology) in a model of experimental design compound central face-centered (CCF, central composite face-centered) in order to optimize the number of experiments and operating conditions, and to obtain the greatest number of data with the minimum number of samples.

The experimental design for the tests was established with the statistical software Modde 8.0.2 of Umetrics. The experimental design provides the randomized treatment of 11 samples with three replicates of the central point (3 minutes at 60% of amplitude).

The level of amplitude varied between 30% and 90% and the time of treatment between 1 and 5 minutes; the reference sample, not subject to treatment, was evaluated separately.

The conditions for Sample 3 were 1, 3 and 5 min at 30%, 60% and 90% (51, 102, 153 μm) of amplitude and each treatment was replicated three times.

Samples

The tests have been performed on young red wines (4 months after racking) immediately after the treatment: 200 mL of wine for every sample have been treated. The wines used were the following:

Sample 1: red table wine from Veneto region;

Sample 2: red table wine, Cabernet Sauvignon, from Friuli Venezia Giulia region;

Sample 3: red DOC (Protected Designation of Origin) wine, Raboso, from Veneto region.

Analytical methods

Effects of ultrasound treatment were evaluated using the Glories' index (Glories, 1978) which show the status of phenolic compounds in red wine, mainly based on the absorbance measured using a spectrophotometer.

All the treated samples, as the reference ones, have been evaluated after the treatment for the following: total phenolics index – Abs 280 nm (Ribéreau Gayon, 1970), color intensity (Glories, 1984), and anthocyanin content (Ribéreau-Gayon & Stonestreet, 1965) (using 2% hydrochloric acid, 0.1% acidified ethanol and 20% potassium metabisulfite solutions as reagents).

In addition, hydrochloric acid index (i.e. index of maturation, which shows the proportion of polymerized phenolic compounds); ethanol index (i.e. index of smoothness, which shows the proportion of polysaccharide and phenolic compounds that combine with the saliva); gelatin index (i.e. index of astringency) (Glories, 1978); and catechins content (Zironi, Buiatti, & Celotti, 1992) were analyzed.

Statistical analysis

An analysis of variance (ANOVA) with 95% confidence level was carried out for each variable response on Samples 1 and 2 in order to test the model significance and its suitability obtained using Modde 8.0.2 Umetrics software.

The results obtained from the tests replicated three times (Sample 3), instead, have been analyzed with ANOVA through the Statistica for Windows 7.0.

Results and discussion

The addition of mechanical and chemical energy to the sample causes some modifications in wine: by comparing Samples 1 and 2 with Sample 3; it is noted that the ultrasound applied to wines with different composition leads to different results.

Since the temperature rises during the treatment, resulting into possible changes on the treatment effects and final wine quality, this parameter was monitored and recorded during the treatments. The increase in temperature from the standard 20°C is related to treatment times and power (Figure 1). The temperature increased to a maximum of 43.5°C for Sample 1, 38.1°C for Sample 2 and 43.1°C for Sample 3.

However, the increases of temperature are not to be considered a problem in a potential future industrial application; the increase that could occur in a few minutes of treatments doesn't cause a loss of wine color quality, as found by Galvin' studies (1992). Besides, it has to be considered that it is possible to carry out the treatment in controlled temperature conditions, if necessary, to avoid undesirable increases of wine temperature.

The effects of the different treatments on color and predisposition to accelerated aging of the young red wines treated are reported in Tables 1 and 2.

It is interesting to notice that no negative consequences on the anthocyanins, either loss of color, appeared after the treatment. Free anthocyanins are not modified, thus confirming their chemical stability under the treatment conditions applied. The stability of free anthocyanins is very important because they can combine later with tannins to obtain stable macromolecular complexes.

The most interesting results have been achieved with tannic compounds. The catechin content analysis shows an increase in values on all samples; the treatment probably promotes the liberation of the monomeric catechins from tannins with different effects on their reactivity (tannins reactivity). Statistical parameters of the response surface model present a good correlation coefficient for catechins (r^2 = 0.8989, r^2 Adj. = 0.7977) for the Sample 1 (Table 3).

The relationship between independent and dependent variables has been illustrated by the three-dimensional representation of the response surfaces and the two-dimensional contours generated by the model. Different shapes of the contour plots indicated different interactions between the variables and, particularly in Figure 2, it is possible to observe a non-regular response regarding catechins depending on time and amplitude.

These results suggest the difficulty in dealing with these phenolic compounds, which play an important role for tannin structure and wine color.

A significant increase of catechins was observed only for high values of amplitude and longer times of treatment. In the others conditions the results highlight the interaction between variables and accordingly the need of ultrasound treatments with specific combinations of amplitude and time based on the result expected.

On the basis of this result, we could suppose different treatment conditions depending on the ratio between the polyphenols and mainly in function of the tannic structure of the wine, closely related to the content of catechins.

The two-dimensional contours relative to color intensity (Figure 3a) and hydrochloric index (Figure 3b) of Sample 2

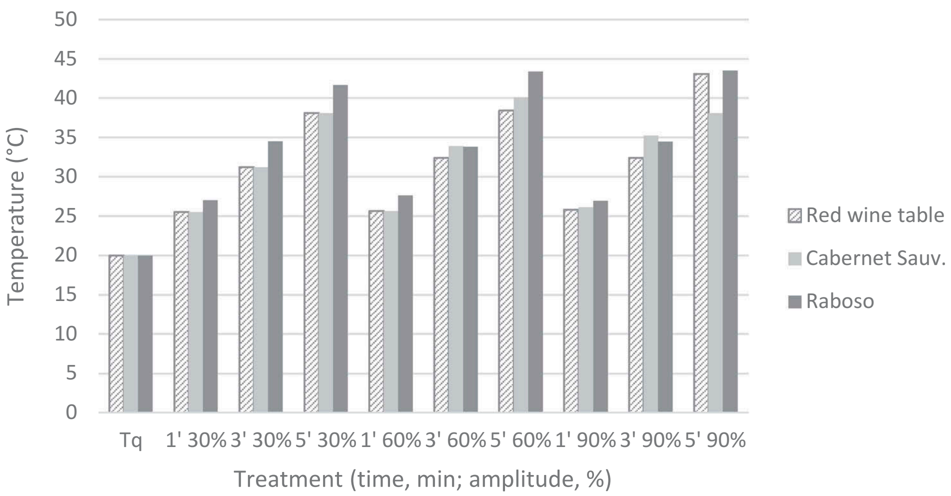

Figure 1. Temperature (°C) trend on ultrasound-treated samples and on reference sample (Tq), for Samples 1 (red table wine), 2 (Cabernet Sauvignon) and 3 (Raboso).

Figura 1. Tendencia de la temperatura (°C) en las muestras tratadas con ultrasonido y en la muestra referencia (Tq), para la Muestra 1 (vino tinto de mesa), 2 (Cabernet Sauvignon) y 3 (Raboso).

Table 1. Effect of ultrasound power on color and phenolic compounds of Sample 1 (red table wine) (analyzed by experimental design; means ± standard deviation (DS) ($n = 3$) for the central point). au, absorbance unit.

Tabla 1. Efecto de la potencia de ultrasonido en el color y los compuestos fenólicos de la Muestra 1 (vino tinto de mesa) (analizada con un diseño experimental; promedios ± desviación estándar (DS) ($n = 3$) para el punto central). au = unidad de absorción.

Sample 1 Red table wine	tq	1′ 30%	3′ 30%	5′ 30%	1′ 60%	3′ 60%	5′ 60%	1′ 90%	3′ 90%	5′ 90%
Color intensity (au)	5.957	6.168	6.035	6.294	5.992	6.006 ± 0.008	6.117	5.873	5.947	6.206
Anthocyanins (mg/L)	208	228	223	219	229	220 ± 10.7	216	218	228	230
EtOH index (%)	20	24	12	11	13	17 ± 2.6	20	12	9	13
HCl index 7 h (%)	33	44	39	39	39	39 ± 3.6	44	37	44	39
HCl index 24 h (%)	5	12	13	13	11	14 ± 3.3	13	13	12	14
Gelatin index (%)	24	23	18	16	16	15 ± 3.4	24	25	22	15
Catechins (mg/L)	316	324	329	318	341	331 ± 6.0	341	326	329	342

Table 2. Effect of ultrasound power on color and phenolic compounds of Sample 2 (Cabernet Sauvignon) (analyzed by experimental design; means ± standard deviation (DS) ($n = 3$) for the central point). au, absorbance unit.

Tabla 2. Efecto de la potencia de ultrasonido en el color y los compuestos fenólicos de la Muestra 2 (Cabernet Sauvignon) (analizada con un diseño experimental; promedios ± desviación estándar (DS) ($n = 3$) para el punto central). au = unidad de absorción.

Sample 2 Cabernet Sauv.	tq	1′ 30%	3′ 30%	5′ 30%	1′ 60%	3′ 60%	5′ 60%	1′ 90%	3′ 90%	5′ 90%
Color intensity (au)	7.169	7.825	8.027	7.973	7.836	8.035 ± 0.004	8.268	7.916	7.989	8.145
Anthocyanins (mg/L)	441	451	455	452	462	452 ± 12.1	447	446	460	455
EtOH index (%)	8	8	11	13	8	11 ± 1.8	25	11	14	13
HCl index 7 h (%)	38	60	49	48	45	45 ± 0.3	42	38	44	40
HCl index 24 h (%)	24	27	31	31	26	31 ± 0.8	33	33	28	34
Gelatin index (%)	27	29	32	26	27	27 ± 1.6	26	28	28	27
Catechins (mg/L)	401	434	423	444	413	420 ± 1.7	434	404	401	401

Table 3. Analysis of variance of the regression parameters of the predictive model for the various parameters of Sample 1 (red table wine). Significant differences ($p < 0.05$) are shown in bold.

Tabla 3. Análisis de la varianza de los parámetros de regresión del modelo previsto para los diferentes parámetros de la Muestra 1 (vino tinto de mesa). Las diferencias significativas (p < 0,05) están mostradas en negrita.

	Color intensity	Anthocyanins	EtOH index	HCl 7 h index	HCl 24 h index	Gelatin index	Catechins
F	2.91745	0.458293	1.14114	0.876225	0.899239	2.00409	8.88915
p	0.132	0.998	0.444	0.556	0.454	0.232	**0.016**
R^2	0.74476	0.0432037	0.532959	0.467017	0.473462	0.667107	0.898881
R^2 Adj.	0.489446	−0.912811	0.065918	−0.06597	−0.05305	0.334235	0.797746
Q^2	0.451348	−0.0451939	0.270434	0.119642	0.145995	0.195779	0.402307
Model validity	0.394068	0.922821	0.382411	0.208063	0.276056	0.883913	0.155895
Reproducibility	0.92319	−0.2	0.865957	0.924328	0.901695	0.195018	0.98836

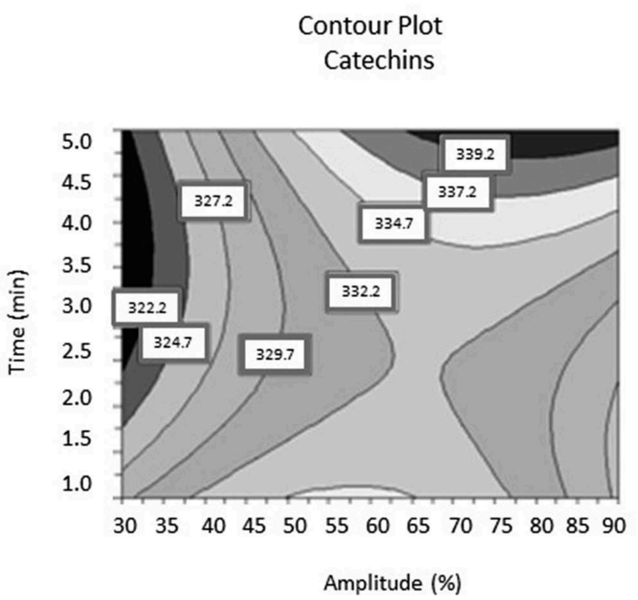

Figure 2. Response contour plot of ultrasonic amplitude and time, and their mutual interactions on the yield of catechins for Sample 1 (red table wine).

Figura 2. Gráfico de respuesta del contorno de la amplitud ultrasónica y el tiempo, además de sus interacciones mutuas en el rendimiento de las catequinas para la Muestra 1 (vino tinto de mesa).

(which refers to polymerized tannins with high molecular weight) showed a regular response depending on the amplitude and the time of ultrasonic treatment, with increases of color and tannin polymerization significant under experimental conditions applied.

Besides, it is interesting to observe that the results show the validity of ultrasonic treatment for few minutes and therefore the possibility to manage these parameters in the aging of red wines; in particular, the increase of HCl index related to tannins polymerization contributes to the wine astringency reduction, the sensory feature required for red wines.

The HCl index showed different results on the two samples: the ultrasound treatment which led to an increase or a decrease of these results could suggest the polymerization of the tannins (i.e. in Sample 2 the values at 24 hours were always higher than the ones at 7 hours with a good correlation coefficient – $r^2 = 0.8723$, r^2 Adj. = 0.7447) (Table 4) or a breakage of the same tannins in the case of Sample 3; it may depend on the different wine composition, including total content and of polyphenols and the ratio among different classes of molecules.

Considering the ultrasound treatment has a different effect on different wines, this approach can be useful, if

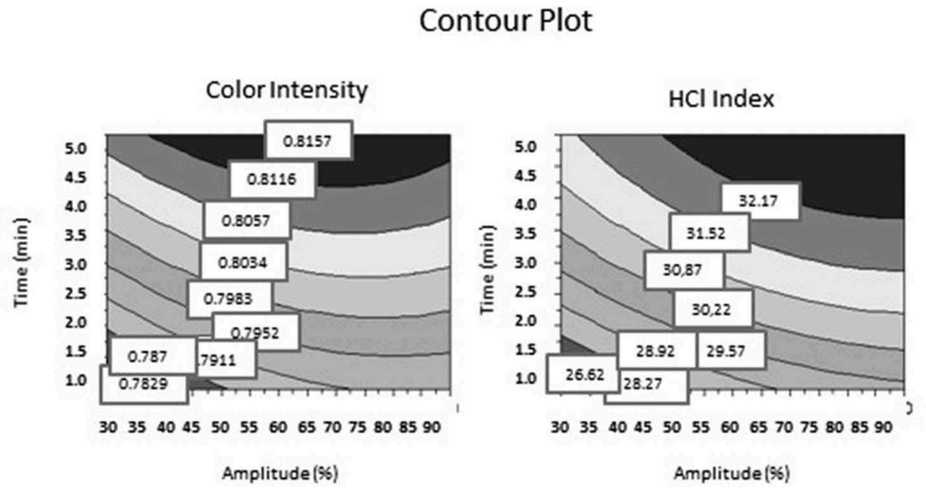

Figure 3. Response contour plots of ultrasonic amplitude and time, and their mutual interactions on the yield of color intensity (on the left) and on the yield of HCl Index (24 h) for Sample 2 (Cabernet Sauvignon).

Figura 3. Gráfico de respuesta del contorno de la amplitud ultrasónica y el tiempo, además de sus interacciones mutuas en el rendimiento de la intensidad del color (izquierda) y en el rendimiento del Índice HCl (24 h) para la Muestra 2 (Cabernet Sauvignon).

Table 4. Analysis of variance of the regression parameters of the predictive model for the various parameters of Sample 2 (Cabernet Sauvignon). Significant differences ($p < 0.05$) are shown in bold.

Tabla 4. Análisis de la varianza de los parámetros de regresión del modelo previsto para los diferentes parámetros de la Muestra 2 (Cabernet Sauvignon). Las diferencias significativas (p < 0,05) están mostradas en negrita.

	Color intensity	Anthocyanins	EtOH index	HCl 7 h index	HCl 24 h index	Gelatin index	Catechins
F	5.6199	4.87654	1.0866	0.41318	6.83351	0.348142	1.66962
p	**0.041**	0.053	0.465	0.823	**0.027**	0.864	0.294
R^2	0.848928	0.82983	0.520752	0.292379	0.872343	0.258239	0.625376
R^2 Adj.	0.697859	0.659661	0.041504	−0.41523	0.744687	0.097025	−0.0419095
Q^2	0.245413	0.246934	0.087819	0.061466	0.097025	−0.04190	−0.0843038
Model validity	0.880114	0.658403	0.832154	0.854769	0.564473	0.638687	0.765924
Reproducibility	0.641311	0.848059	0.090078	−0.2	0.922907	0.390257	0.470369

confirmed by other experiments, to manage the tannin polymerization level to be able to control the chemical and physical stability of the tannic macromolecules and their astringency.

A depolymerization could preserve tannins from colloidal precipitation risk, while its polymerization (an increase of the HCl index) would result in an astringency decrease.

The index of ethanol also led to significant results, as its changes might have supported a rearrangement, probably due to an increasing reactivity of the polysaccharidic and tannin compounds (Tables 1, 2 and 5).

The astringency, evaluated by gelatin index, of Sample 3 increases with short and mild treatment, while it is reduced producing a pleasant sensation with stronger treatment conditions, particularly in the conditions of 5 minutes at 60% of amplitude and 1, 3 and 5 minutes at 90% of amplitude (Table 5).

The different modifications of the gelatin index, directly related to astringency, might confirm the hypothesis that the ultrasound treatment conditions can be modulated depending on the tannic composition of the wine, making it possible to manage astringency perception in a short time.

It could also be interesting to study the ultrasound effect on the interactions among tannins and polysaccharides, and the consequent changes of the organoleptic characteristics.

Conclusions

From the results obtained, some indications suggest the use of the ultrasound to promote the polymerization of the phenolic compounds as the wine matures and therefore accelerate the aging process of wines. This technology can give the best result in the treatment of young, well-colored wines featured by an immature tannic structure that is still evolving.

A brief ultrasound treatment could replace or integrate the traditional aging and stabilization techniques that are often constrained by the length of the kinetics of reactions between polyphenolic molecules.

It is also necessary to further investigate on the kinetics and response times to optimize the ultrasound technology on the treatment of red wines.

Different results on different wines imply an ultrasound effect shown on disparate amounts of phenolic fractions.

The results, based on variable treatment time and percentage of amplitude, have to be considered and evaluated to set the best treatment conditions for each wine.

Acknowledgment

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Disclosure statement

No potential conflict of interest was reported by the authors.

Table 5. Effect of ultrasound power on phenolic compounds of Sample 3 (Raboso). Means ± standard deviation (DS) (n = 3); different letters represent significant differences according to ANOVA and Tukey HSD test ($p < 0.05$).

Tabla 5. Efecto de la potencia de ultrasonido en los compuestos fenólicos de la Muestra 3 (Raboso). Promedios ± desviación estándar (DS) (n = 3); las distintas letras representan diferencias significativas según los tests de ANOVA y HSD de Tukey ($p < 0,05$).

Sample 3 Raboso	tq	1′ 30%	3′ 30%	5′ 30%	1′ 60%	3′ 60%	5′ 60%	1′ 90%	3′ 90%	5′ 90%
OD 280 nm	9.915 ± 0.001cd	10.01 ± 0.007d	10.08 ± 0.001d	10.01 ± 0.014d	9.461 ± 0.025ab[c]	9.391 ± 0.014ab	9.052 ± 0.028a	9.243 ± 0.013ab	9.605 ± 0.021bcd	9.527 ± 0.015abc
EtOH index (%)	17abcd	11a	12ab	14abc	20bcd	20bcd	22d	17abcd	17abcd	22cd
HCl index 24 h (%)	51 ± 1d	53 ± 3d	48 ± 3cd	54 ± 1d	48 ± 5bcd	47 ± 2bcd	50 ± 2d	42 ± 2abc	41 ± 1ab	38 ± 1a
Gelatin index (%)	48 ± 0de	52 ± 1e	52 ± 3e	53 ± 2e	46 ± 2cde	40 ± 1bcd	39 ± 2bc	34 ± 4ab	29 ± 6a	39 ± 4bc
Catechins (mg/L)	535 ± 6a	552 ± 16ab	565 ± 19ab	557 ± 17ab	565 ± 9ab	566 ± 13ab	564 ± 8ab	557 ± 15ab	568 ± 11ab	581 ± 13b

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