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Sveučilište u Zagrebu

PREHRAMBENO-BIOTEHNOLOŠKI FAKULTET

Katarina Lukić

PRIMJENA NETOPLINSKIH TEHNIKA KAO ALTERNATIVA SUMPOROVU DIOKSIDU U PROIZVODNJI VINA

DISERTACIJA

Mentor:
prof. dr. sc. Karin Kovačević Ganić

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APPLICATION OF NON-THERMAL TECHNIQUES AS AN ALTERNATIVE TO SULFUR DIOXIDE IN PRODUCTION OF WINE

Katarina Lukić, mag. ing. techn. aliment.

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Short abstract: The aim of this study was to investigate the influence of non-thermal techniques (high power ultrasound, high hydrostatic pressure, and high voltage electrical discharge plasma – cold plasma) on the overall quality of red and white wines. The research was conducted on Cabernet Sauvignon and Graševina wines (*Vitis vinifera* L.), produced with a reduced concentration of sulfur dioxide. Optimal process parameters were determined for each technique with the aim of preserving and improving wine quality. Additionally, the influence of these non-thermal techniques in combination with the addition of antioxidants (sulfur dioxide and glutathione) was examined during 12 months of aging in bottles. Phenolic, chromatic, aroma, physicochemical and sensory characteristics of wines were analyzed. The obtained results showed that the application of different treatments resulted in different intensity of changes in wine quality. The applied techniques have shown potential in wine technology, primarily for accelerating the aging process of wine and for production of wines with a reduced concentration of sulfur dioxide.

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PRIMJENA NETOPLINSKIH TEHNIKA KAO ALTERNATIVA SUMPOROVU DIOKSIDU U PROIZVODNJI VINA

Katarina Lukić, mag. ing. techn. aliment.

Rad je izrađen u Laboratoriju za tehnologiju i analitiku vina, a dio eksperimentalnog rada proveden je u Laboratoriju za tehničku termodinamiku, Laboratoriju za tehnološke operacije i Laboratoriju za procesno-prehrambeno-inženjerstvo Prehrambeno-biotehnološkog fakulteta Sveučilišta u Zagrebu

Mentor: prof. dr. sc. Karin Kovačević Ganić

Kratki sažetak: Cilj ovog rada bio je istražiti utjecaj netoplinskih tehnika (ultrazvuk visokih snaga, visoki hidrostatski tlak i visokonaponsko električno pražnjenje plazma – hladna plazma) na cjelokupnu kvalitetu crnog i bijelog vina. Istraživanje je provedeno na vinima sorti Cabernet Sauvignon i Graševina (*Vitis vinifera* L.), proizvedenih sa sniženom koncentracijom sumporovog dioksida. Utvrđeni su optimalni procesni parametri za svaku tehniku s ciljem očuvanja i poboljšanja kvalitete vina. Osim navedenog, ispitan je i utjecaj ovih netoplinskih tehnika u kombinaciji s dodatkom antioksidansa (sumporov dioksid i glutation) tijekom 12 mjeseci starenja u bocama. Analizirane su polifenolne, kromatske, aromatske, fizikalno-kemijske i senzorske karakteristike vina. Dobiveni rezultati su pokazali da primjena različitih tretmana rezultira i različitim intenzitetom promjena u kvaliteti vina. Primijenjene tehnike pokazale su potencijal u tehnologiji vina, prvenstveno za ubrzavanje procesa starenja vina te za proizvodnju vina sa sniženom koncentracijom sumporovog dioksida.

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EXTENDED ABSTRACT

Sulfur dioxide (SO_2) is the most used additive in wine production due to its antioxidant and antimicrobial action, however its necessity of application is today called into question. Besides the legal restrictions and demand to increase the safety and quality of wine, there is also a growing popularity among consumers for wines with reduced concentrations of SO_2. In addition, a certain percentage of people shows and expresses sensitivity to this additive. Hence, SO_2 ended up over time on the list of allergens. Consequently, the scientific community is focused on finding new alternative solutions such as non-thermal techniques and natural antioxidants. Therefore, the aim of this doctoral thesis is to investigate the possibility of applying non-thermal techniques (high power ultrasound, high hydrostatic pressure and high voltage electrical discharge plasma – cold plasma) with the purpose of reducing the use of SO_2 in the production of high quality wines. The influence of these techniques was examined on the overall quality of Cabernet Sauvignon red wine and Graševina white wine, produced with a reduced concentration of SO_2. Additionally, the influence of these techniques in combination with the addition of antioxidants (SO_2 and glutathione) was investigated during 12 months of aging in bottles. Phenolic, chromatic, aroma, physicochemical and sensory characteristics of wines were analyzed. The optimization of each technique was performed and the optimal process parameters were determined with the aim of preserving and improving the quality of wine. Depending on the applied techniques, different process parameters were investigated. High power ultrasound included treatments with (i) an ultrasound probe and (ii) an ultrasonic bath, and the parameters examined were probe size, treatment duration, and amplitude for (i), and frequency, amplitude, treatment duration, and temperature for (ii). The tested parameters for high hydrostatic pressure were pressure and treatment duration, while for cold plasma those were the frequency and treatment duration. The long-term impact of these techniques was investigated under selected optimal conditions on wines with standard SO_2 concentration, with low SO_2 concentration and glutathione, and with low SO_2 concentration. In general, the application of different treatments resulted in different intensity of changes in analyzed parameters of wine quality. By processing the wine with milder process conditions (lower amplitude, frequency, temperature, pressure, and shorter treatment duration), a more favorable effect was achieved on the overall quality of red and white wines. Among applied techniques, high power ultrasound and high hydrostatic pressure showed a milder and more favorable effect on both wines compared to cold plasma. The changes observed during 12 months of aging in bottles, primarily in the composition of phenolic and aroma compounds and chromatic

characteristics, were inherent to wine aging, which indicates the suitability of these techniques to accelerate the aging process of wine. Furthermore, higher concentrations of antioxidants (SO_2 and glutathione) resulted in a slower decrease in the concentration of most of the analyzed compounds and sensory characteristics. Especially, HPU and HHP treated wines with standard SO_2 and low SO_2 and glutathione showed similar chemical composition, implying that these techniques in combination with glutathione and lower concentration of SO_2 could potentially preserve wine from deterioration.

Keywords: *non-thermal techniques, antioxidants, sulfur dioxide, glutathione, wine quality*

PROŠIRENI SAŽETAK

Sumporov dioksid (SO_2) je najčešće korišten aditiv u proizvodnji vina zbog svog antioksidacijskog i antimikrobnog djelovanja, međutim danas se njegova nužnost primjene dovodi u pitanje. Osim zakonskih ograničenja te zahtjeva za povećanjem sigurnosti i kvalitete vina, i među potrošačima su sve popularnija vina sa sniženom koncentracijom SO_2. Osim toga, određen postotak ljudi pokazuje i izrazitu osjetljivost na ovaj aditiv, te je on samim time uvršten na popis alergena. Slijedom navedenog, znanstvena zajednica usmjerena je na pronalazak novih alternativnih rješenja kao što su primjerice netoplinske tehnike i prirodni antioksidansi. Stoga je cilj ovog doktorskog rada istražiti mogućnost primjene netoplinskih tehnika (ultrazvuk visokih snaga, visoki hidrostatski tlak i visokonaponsko električno pražnjenje – hladna plazma) sa svrhom smanjenja upotrebe SO_2 u proizvodnji visokokvalitetnih vina. Ispitan je utjecaj navedenih tehnika na cjelokupnu kvalitetu crnog vina Cabernet Sauvignon i bijelog vina Graševina, proizvedenih sa sniženom koncentracijom SO_2. Osim navedenog istražen je i utjecaj ovih tehnika u kombinaciji s dodatkom antioksidansa (SO_2 i glutation) tijekom 12 mjeseci starenja u bocama. Analizirane su polifenolne, kromatske, aromatske, fizikalno-kemijske i senzorske karakteristike vina. Provedena je optimizacija svake pojedine tehnike te su utvrđeni optimalni procesni parametri s ciljem očuvanja i poboljšanja kvalitete vina. Ovisno o primijenjenim tehnikama ispitivani su različiti procesni parametri. Ultrazvuk visokih snaga uključivao je tretmane (i) ultrazvučnom sondom i (ii) ultrazvučnom kupelji, a parametri koji su bili ispitivani su veličina sonde, trajanje tretmana i amplituda za (i), te frekvencija, amplituda, trajanje tretmana i temperatura za (ii). Ispitivani parametri za visoki hidrostatski tlak bili su tlak i trajanje tretmana, dok su za hladnu plazmu bili frekvencija i trajanje tretmana. Dugoročni utjecaj ovih tehnika ispitan je pri odabranim optimalnim uvjetima na vinima sa standardnom koncentracijom SO_2, sa sniženom koncentracijom SO_2 uz dodatak glutationa i sa sniženom koncentracijom SO_2. Generalno, primjena različitih tretmana rezultirala je i različitim intenzitetom promjena u ispitivanim parametrima kvalitete vina. Obradom vina blažim procesnim uvjetima (niža amplituda, frekvencija, temperatura, tlak, te kraće trajanje tretmana) postignut je povoljniji učinak na cjelokupnu kvalitetu crnog i bijelog vina. Među primijenjenim tehnikama, ultrazvuk visokih snaga i visoki hidrostatski tlak pokazali su blaži i povoljniji utjecaj na oba vina u odnosu na hladnu plazmu. Promjene uočene tijekom 12 mjeseci starenja u bocama, prvenstveno u sastavu polifenolnih i aromatskih spojeva te kromatskih karakteristika bile su svojstvene dozrijevanju vina, što ukazuje na pogodnost ovih tehnika za ubrzavanje procesa starenja vina. Nadalje, viša koncentracija antioksidansa (SO_2 i glutation) rezultirala je

sporijim smanjenjem koncentracije većine analiziranih spojeva i senzorskih karakteristika. Posebice, HPU i HHP tretirani uzorci sa standardnom koncentracijom SO_2 i sniženom koncentracijom SO_2 i glutationom pokazali su sličan kemijski sastav, što implicira da bi ove tehnike u kombinaciji s glutationom i nižom koncentracijom SO_2 potencijalno mogle sačuvati vino od kvarenja.

Ključne riječi: *netoplinske tehnike, antioksidansi, sumporov dioksid, glutation, kvaliteta vina*

Information about the supervisor – PhD Karin Kovačević Ganić, Full Professor

KARIN KOVAČEVIĆ GANIĆ is a professor at the Faculty of Food Technology and Biotechnology of the University of Zagreb. Since 2015 she is at the position of Full Professor, and from 2011 to 2020 she was head of the Laboratory for Technology and Analysis of Wine. She obtained a PhD in 2005 with a thesis titled: *Aroma precursors of Malvasia istriana wine*. Her field of research is wine chemistry and technology related to the influence of technological parameters of production on the phenolic and aroma composition of wine, the use of non-thermal technologies such as high power ultrasound, high hydrostatic pressure and cold plasma as an alternative to sulfur dioxide in winemaking, sensory analysis of wine and the impact of wine on health. In academic year 2019/2020 and 2020/2021 she was the Vice-Dean for Science at the Faculty of Food Technology and Biotechnology. She was the head of the undergraduate study Food Technology at the same Faculty from 2014 to 2019. She participates in teaching in several subjects at the undergraduate, graduate, postgraduate doctoral study and at the postgraduate specialist study. She is the author of two peer-reviewed teaching materials, and under her mentorship 32 graduate and 21 final theses have been prepared, and she is the mentor of three doctoral dissertations and two master's theses. To date she has published over 100 significant scientific papers, which have been cited over 1300 times (source: ISI Web of Science). She has participated in many international and domestic scientific conferences. She was the principal investigator on a project funded by the Croatian Science Foundation "New enological tools for the reduction of sulfur dioxide and production of high-quality wine" (2015-2019). As an associate, she participated in five scientific projects funded by European funds. She is a member of the editorial board of the journal Food Technology and Biotechnology and a representative of the Republic of Croatia at the International Organization of Viticulture and Enology (OIV) in Paris. She is a member of the Section for Processing of Agricultural Products and Biotechnology within the Scientific Council for Agriculture and Forestry of the Croatian Academy of Sciences and Arts and numerous commissions, committees and working groups, both at the Faculty of Food Technology and Biotechnology and at the University of Zagreb. She is the winner of the National Science Award for significant scientific achievement in the field of Biotechnical Sciences in 2008.

CONTENTS

1. GENERAL INTRODUCTION

In the food industry, the usage of additives is regulated by legal regulations that are harmonized with the regulations of the European Union (EU). With the accession of Croatia to the EU, wine producers have become obliged to indicate "Contains sulfites" on the labels if the total concentration of sulfur dioxide (SO_2) is more than 10 mg/L. In enological terms, sulphites are residues that remain in wine after the application of SO_2 (E220), most often in the form of potassium metabisulphite, aqueous sulfuric acid, sulfur strips or gaseous SO_2. In fact, SO_2 is most commonly used additive in wine prodution due to its antioxidant and antimicrobial properties. However, its usage is today under greater consideration. The fact is that the eating habits of the population are changing and that competition in the world food market is increasing. In addition, a number of people are extremely sensitive to this additive, and it is therefore included in the list of allergens. Hence, the necessity of using SO_2 in wine production is even questioned, and wines with a lower concentration of SO_2 are becoming increasingly popular among consumers. Consequently, the scientific community is focused on finding alternative technological solutions with the aim of reducing the usage of SO_2 in wine production. One of the many potential solutions are the addition of natural antioxidants (i.e. glutathione) and the application of non-thermal techniques (high hydrostatic pressure, high power ultrasound, etc.).

1.1. Sulfur dioxide

Due to its numerous functions, SO_2 is still an almost irreplaceable additive in wine production. Its functions are as follows:

- antimicrobial activity (selective action in the must microflora and antimicrobial action during its aging – wine preservation)
- antioxidant action (dissolved oxygen inhibition, which enables protection of wine from chemical oxidation, i. e. oxidation of some phenolic and aroma compounds)
- inhibits the effect, and sometimes causes denaturation, of oxidative enzymes (polyphenol oxidase, PPO) in the must, resulting in protection of the must from oxidation before fermentation
- in higher doses in contact with the skin of grapes, promotes the diffusion of substances responsible for the color located in the vacuoles, which favors the release of anthocyanins

- improvement of the sensory characteristics of wine (binding to acetaldehyde or pyruvic acid, compounds responsible for smell or taste)
- clarifying agent for the acceleration of spontaneous precipitation, since it promotes the coagulation of colloidal substances (Giacosa et al., 2019).

Consequently, it is important to point out that SO_2, precisely with the aim of preventing oxidation and microbial spoilage, is used throughout the wine production process, from the processing of grapes to the bottling of wine. In general, SO_2 is a gas readily soluble in water and it is found in wine in free and bound form. The majority of free SO_2 is in the form of a bisulfite ion that prevents oxidation of wine, while only the form of molecular SO_2 has antimicrobial activity (Jackson, 2008). SO_2 is known to react with a number of wine ingredients, such as carbonyl and phenolic compounds, sugars and others. Namely, the free bisulfite ion reacts with the oxidation products of phenolic compounds, quinones and hydrogen peroxide, and thus prevents the formation of acetaldehyde and brown pigments that are otherwise a sign of oxidative changes (Waterhouse and Laurie, 2006). Furthermore, molecular SO_2 is the most active against wine spoilage because it limits the growth of a wide range of microorganisms, including yeasts and bacteria. SO_2 concentration is dependent on the pH value of wine, ethanol concentration, and temperature (Picariello, 2017).

Proper sulfuring or adding the optimal amount of SO_2 is very important. Molecular SO_2 concentration in a range from 0.6-0.8 mg/L is thought to be required to achieve adequate antimicrobial activity, while 20-40 mg/L in the form of free SO_2 provides an antioxidant effect (Waterhouse et al., 2016). At the very beginning of the technological process of wine production, the role of SO_2 is to prevent oxidative (enzymatic) browning of the must and to inactivate the natural microflora before the beginning of fermentation with the desired yeasts. In addition, wine storage comes after bottling of wine and it is the longest and uncontrolled part. If the usage of SO_2 is avoided during bottling, it can have negative effect on the microbial stability of the wine. Antioxidant activity issue presents a far more complex problem, particulary with young wines, which are characterized by fermentation and fruit aromas. In the absence of antioxidants, such as SO_2, sensory characteristics are difficult to protect (Giacosa et al., 2019). Contrary, overusage of SO_2 has negative consequences on the wine quality. This is primarily visible through a reduction in color intensity because SO_2 acts as a bleaching agent (Jackson, 2008; Garcia et al., 2016). Higher concentrations of SO_2 can negatively affect the

wine aroma but can also induce the formation of a reductive aroma (Jackson, 2000; Ribéreau-Gayon et al., 2006).

With all mentioned above, the use of SO_2 raises health community concerns about serious allergic reactions that occur in vulnerable populations, which has ultimately resulted in the certain restricitve measures to be accepted by World Health Organization (WHO) and the International Organization of Vine and Wine (OIV, fr. *Organization Internationale de la Vigne et du Vin*). Total SO_2 maximum permitted doses are 150 mg/L (red wine) and 200 mg/L (white wine), with some exception which depends on the sugar content (Commission Regulation (EC) No 607/2009). Currently, no wine-producing additive is available that can exert antimicrobial and antioxidant activity as SO_2. Consequently, various technological methods, from microbiological, physical to chemical, are being intensively investigated with the aim of reducing the use of SO_2 in wine production (Table 1).

Table 1. Review of researched microbial, physical and chemical methods for reduction of SO_2 in wine production.

Microbial methods	Physical methods	Chemical methods
Selection of different strains of *Saccharomyces* yeast (Suzzi et al., 1985; Eglinton and Henschke, 1996; Werner et al., 2009; Wells and Osborne, 2011; Miranda-Castilleja et al., 2015; Pezley, 2015)	High hydrostatic pressure (Delfini et al., 1995; Santos et al., 2019) High power ultrasound (García Martín and Sun, 2013; Gracin et al., 2016; van Wyk and Silva, 2019) Ultraviolet radiation (Falguera et al., 2013) Pulsed electric field (García Martín and Sun, 2013; van Wyk and Silva, 2019) High-pressure homogenization (van Wyk and Silva, 2019) High voltage electrical discharge (Delsart et al., 2015; Delsart et al., 2016) Cold plasma (Sainz-García et al., 2019)	Dimethyl dicarbonate (Costa et al., 2008) Lysozyme (Gao et al., 2002; Bartowsky et al., 2004; Sonni et al., 2011a) Phenolic compounds (García-Ruíz et al., 2011; González-Rompinelli et al., 2013) Chitosan (Ferreira et al., 2013; Valera et al., 2017) *β*-glucanase (Enrique et al., 2010) Bacteriocins (Rojo-Bezares et al., 2007) Silver nanoparticles (Izquierdo-Cañas et al., 2012) Short-/medium-chain fatty acids (Guilloux-Benatier et al., 1998) Toxin killer strains of yeast (Ciani and Fatichenti, 2001) Glutathione (Kritzinger et al., 2013a) Ascorbic acid (Bradshaw et al., 2011)

2. THEORETICAL BACKGROUND

2.1. Non-thermal techniques and their application in production of wine

The application of non-thermal techniques has become one of the main concepts in the food industry with the aim of maintaining and improving product quality (Knorr et al., 2011). Physical techniques (i.e. high hydrostatic pressure, high power ultrasound, pulsed electric field, cold plasma) in the last 20 years have shown a great potential, due to intensive research. First, their efficiency in microbial control of products is emphasized, which could replace or reduce the usage of existing additives. Namely, the goal is to produce a microbial stable product with minimal disruption of its sensory and nutritional characteristics. In general, the main advantages of these techniques are shorter processing times and lower processing temperatures compared to conventional methods, energy and time savings, less environmental pollution, and investment savings. This is especially important for a medium such as wine, as it is a food product that is very sensitive to processing involving the use of high temperatures. Moreover, conventional processing methods, possibly have a degrading effect on the color, aroma and taste of wine. All of these are sensitive sensory characteristics of the wine. Having in mind the above, the wine industry is very interested in the application of non-thermal techniques in the segment of production, aging and preservation of wine. However, it should be emphasized that despite the good efficacy of non-thermal techniques, none of them can completely replace the multiple action of SO_2 (antimicrobial and antioxidant). Therefore, a multidisciplinary approach, i.e., a combination of non-thermal techniques and the addition of antioxidants (e.g. reduced concentration of SO_2 and glutathione), is suggested as an alternative solution to reach this goal. This is especially evident with wine stability during aging. The non-thermal techniques used in this research are described in more detail below (high hydrostatic pressure, high power ultrasound, high voltage electrical discharge plasma – cold plasma).

2.1.1. High power ultrasound

In general, ultrasound is considered as one of the alternative techniques in the food industry for food processing in terms of preserving and extending the durability of the product. Ultrasound can be divided according to frequencies into diagnostic ultrasound (MHz) and high power ultrasound (kHz) (Mason et al., 2003). Namely, high power ultrasound (HPU) is a non-thermal technique with great potential in terms of controlling microbial stability and preserving wine. The mechanism of action of ultrasound on the liquid is that the emission of ultrasonic waves

leads to the formation of longitudinal waves that lead to the alternating formation of the compression and decompression phases. Due to pressure changes, a phenomenon known as cavitation occurs. The application of sound intensity or acoustic intensity of an ultrasonic wave between 10 and 1000 W/cm^2 with frequencies from 20 to 100 kHz causes the formation of cavitation bubbles which volume increases in the decompression phase up to the critical size. Implosion or collapse of cavitation bubbles generates an increase of temperature (5000°C) and pressure (50000 kPa), together with the formation of free radicals, shock waves and shear forces (van Wyk and Silva, 2019). High-frequency bubbles generate a uniform acoustic field of a smaller intensity, which has a higher cavitation collapse rate in the time of ultrasound treatment (Kentish and Feng, 2014).

Ultrasound generators are most often based on electroacoustic systems (Brnčić et al., 2009). Electroacoustic systems are characterized with a feature of converting electricity into high frequency alternating current, which is afterwards converted into mechanical vibrations (Silva and Sulaiman, 2017). The most used ultrasonic reactors are the directly immersed probe system and the ultrasonic bath (Brnčić et al., 2009). Ultrasonic bath is commonly used, since it has low running costs and easy maintenance. Parts of the ultrasonic bath are container with several piezoelectric transducers (40–130 kHz). Piezoelectric transducers are connected at the bottom or the side. Generated sound waves go through a liquid medium, where a food product is immersed (Figure 1a) (Astráin-Redín et al., 2019). The intensity of ultrasound within such a system is from 0.1 to 1.0 W/cm^2 and does not have an even distribution (Kentish, 2017). In the food industry, such systems have a purpose for surface cleaning, degassing, enzymatic and microbial inactivation, mass transfer improvement, etc. (Bermúdez-Aguirre et al., 2011). However, the ultrasound probe (direct system) is characterized by a higher intensity of ultrasound (> 5 W/cm^2), but also by a higher price compared to ultrasonic baths. It consists of three parts: a transducer, an ultrasonic signal amplifier, and a horn (probe) (Figure 1b) (Astráin-Redín et al., 2019). In such system, the sample is in direct contact with a probe that transmits ultrasonic energy, so this design is mainly used to process liquid products.

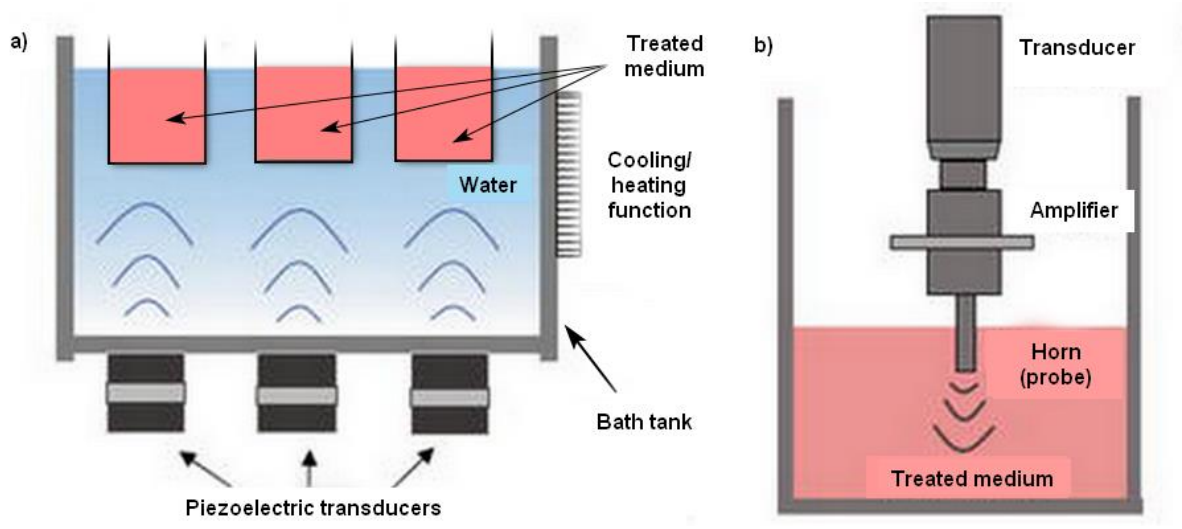

Figure 1. High power ultrasound generating systems: a) ultrasonic bath and b) ultrasonic probe [the picture has been adopted from Asträin-Redín et al. (2019) and modified].

Regardless of the system used for HPU treatment, for the treatment to induce cavitation it is necessary to know and select the appropriate frequency and intensity (process parameters). Ultrasound effectivity also depends on the properties of the fluid being treated such as viscosity, density, and surface tension (Brnčić et al., 2009). This ultrasound mechanism is responsible for the inactivation of microorganisms, so it is used primarily for this purpose (Figure 2). Current ultrasonic treatment systems have shown varying degrees of efficacy in yeast and bacterial inactivation. The ultrasonic inactivation is dependent on several characteristics of the microorganism being treated (shape, size and type) (Luo et al., 2012; Kentish and Feng, 2014; Gracin et al., 2016; Evelyn et al., 2017). It should be emphasized that in addition to achieving microbial stability of the wine, careful consideration should be taken to avoid damage to its overall quality throughout processing by HPU technique, i.e. that sensory and nutritional characteristics are preserved or even improved. In addition to managing wine microbiology, this technique has also been applied for the purpose of extracting phenolic compounds during maceration (Bautista-Ortín et al., 2017), extracting aroma compounds from must and wine (Cocito et al., 1995; Vila et al., 1999; Cabredo-Pinillos et al., 2006), substitution of preservative additives (Clodoveo et al., 2016) and valorization of by-products in wine production (Tao et al., 2014b; Roselló-Soto et al., 2015; Barba et al., 2016; Landeka Jurčević et al., 2017; Poveda et al., 2018; Romero-Díez et al., 2019). The effectiveness of this technique was officially recognized in 2019 by the OIV, which passed a special resolution on the application of ultrasound to grape crushing procedures so they can enhance phenolic compound extraction. Also, ultrasound is treated as the most prosperous technique for the wine aging, in

the terms of acceleration of this process (García Martín and Sun, 2013; Tao et al., 2014a). Particularly, effectiveness is considered to be important for red wine (changing chromatic characteristics and phenolic composition) (Masuzawa et al., 2000; Ferraretto and Celotti, 2016; Zhang et al., 2016a; Zhang and Wang, 2017).

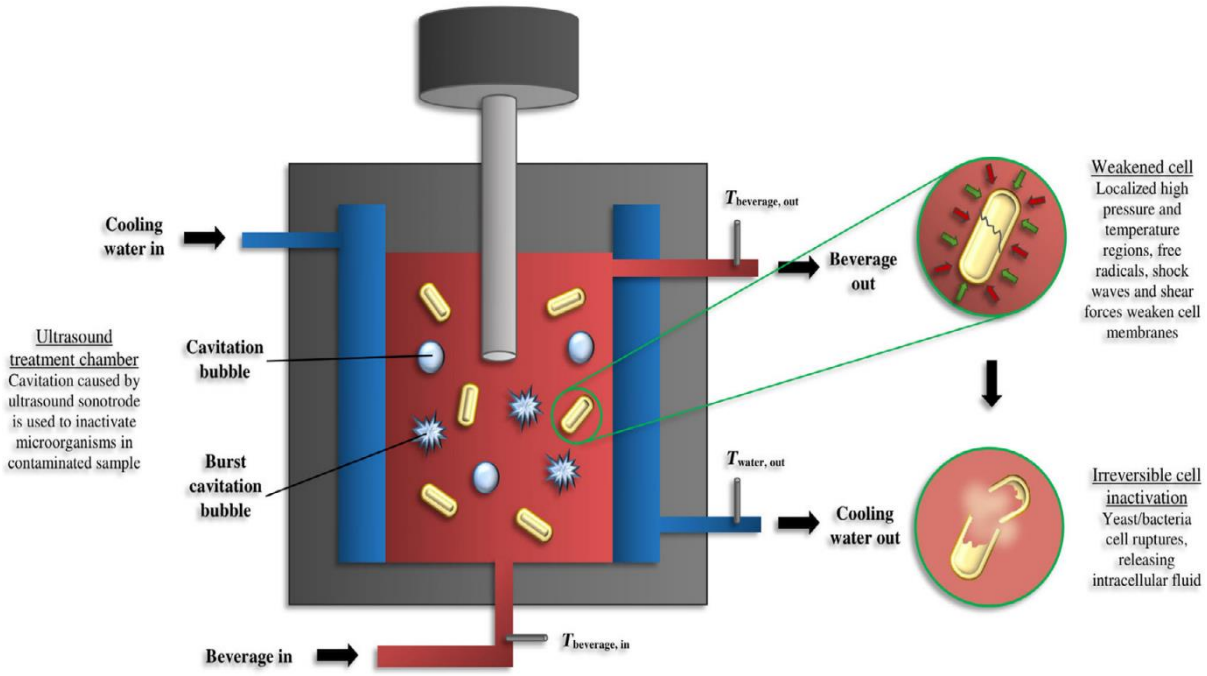

Figure 2. The mechanism of inactivation of undesirable microorganisms in wine using high power ultrasound (T refers to temperature) (van Wyk and Silva, 2019).

When HPU is applied to wine, it causes physical (micro-mechanical shocks caused by cavitation) and chemical (free radical formation) effects (Carbonell-Capella et al., 2017), which are expected to affect chemical composition and improve quality of wine during processing (García Martín and Sun, 2013). A review of the literature to date shows that the effects of HPU on wine quality are different, depending on whether red or white wine has been treated, and which ultrasonic systems and process conditions have been applied. Therefore, ultrasound treatment should be accurately modeled particularly in the case when the physical and chemical properties of the wine are the dependent variables, i.e. the ratio of different groups of phenolic compounds in the sample. Most research has been conducted on red wines, while there is still insufficient published data on the impact of HPU on the quality of white wines. Studies have shown that different ultrasonic treatment conditions result in changes of red wine color and total phenolics (Masuzawa et al., 2000; Ferraretto and Celotti, 2016; Zhang et al., 2016a; Zhang and Wang, 2017; Celotti et al., 2020). This happens due to stimulating polymerization reactions

that happen throughout the natural aging process of wine. However, it was shown that it does not affect the physicochemical parameters (pH, total and volatile acidity) (Ferraretto and Celotti, 2016; Zhang et al., 2016a; Zhang and Wang, 2017). Furthermore, some authors state that ultrasound affects the wine's electrical conductivity (Zhang et al., 2016b), initiates the formation of free radicals (Zhang et al., 2015), and causes changes in sensory characteristics and aroma composition (Singleton and Draper, 1963; Luo et al., 2012). Celotti et al. (2020) provided the study results in which ultrasound treatment can preserve phenolic compounds without causing their degradation in young red wine, emphasizing this as a fundamental aspect when the initial concentration of phenolic compounds is low, and the preservation of chromatic and sensory characteristics is of primary importance. HPU effect on the phenolic and aroma composition of white wine was reported in a small number of studies (Singleton and Draper, 1963; Vila et al., 1999), and its effectiveness as a stand-alone treatment and in combination with SO_2 in improving the quality and microbial stability of sweet white wine (Cui et al., 2012).

2.1.2. High hydrostatic pressure

High hydrostatic pressure (HHP) is one of the most researched non-thermal techniques, used in food preservation or modification in the last decade. HHP in a broad sense involves the usage of pressure (range from 100 to 600 MPa) on food, placed in a chamber, with or without packaging (van Wyk and Silva, 2019). The action of high pressure reduces the volume of the system to which the pressure is applied. According to Le Chatelier's principle, in equilibrium conditions, the application of high pressure to a closed system stimulates those reactions that lead to a decrease in a volume, while at the same time it slows down those reactions that lead to an increase in system volume (Krešić et al., 2011). Consequently, high pressure shows an impact only on non-covalent bonds (hydrogen, ionic and hydrophobic), while stronger covalent bonds in food remain intact (Yaldagard et al., 2008). The media used to transfer the pressure ensures that the pressure acts evenly on all sides of the food, so that the volume and shape of the food itself ultimately do not affect the outcome of the treatment itself. Furthermore, this technique is characterized by a small temperature increase during processing. Also, a small effect is visible on the low molecular weight compounds (Muntean et al., 2016). Namely, during the compression phase there is an increase in temperature within the treated sample, so-called adiabatic warming. For water and foods containing a high proportion of water, the temperature increase is about 3°C/100 MPa (Krešić et al., 2011). Processing at room temperature is the main advantage of this technique for application in wine technology, since

the treatment does not increase the temperature of the wine itself, which ensures the retention of physicochemical properties as well as the overall quality. One of the goals of HHP treatment is to achieve microbial stability or inactivation of microorganisms and enzymes (i.e. Figure 3). The sensory and nutritional characteristics of the treated product should be minimally affected. With that in mind, the major research focus was the usage of HHP in winemaking, with the focus on the microbial control of wine (González-Arenzana et al., 2016; van Wyk and Silva, 2017a; van Wyk and Silva, 2017b; van Wyk et al., 2018; Tomašević et al., 2020). Figure 3 shows the mechanism of inactivation of microorganisms in wine by HHP.

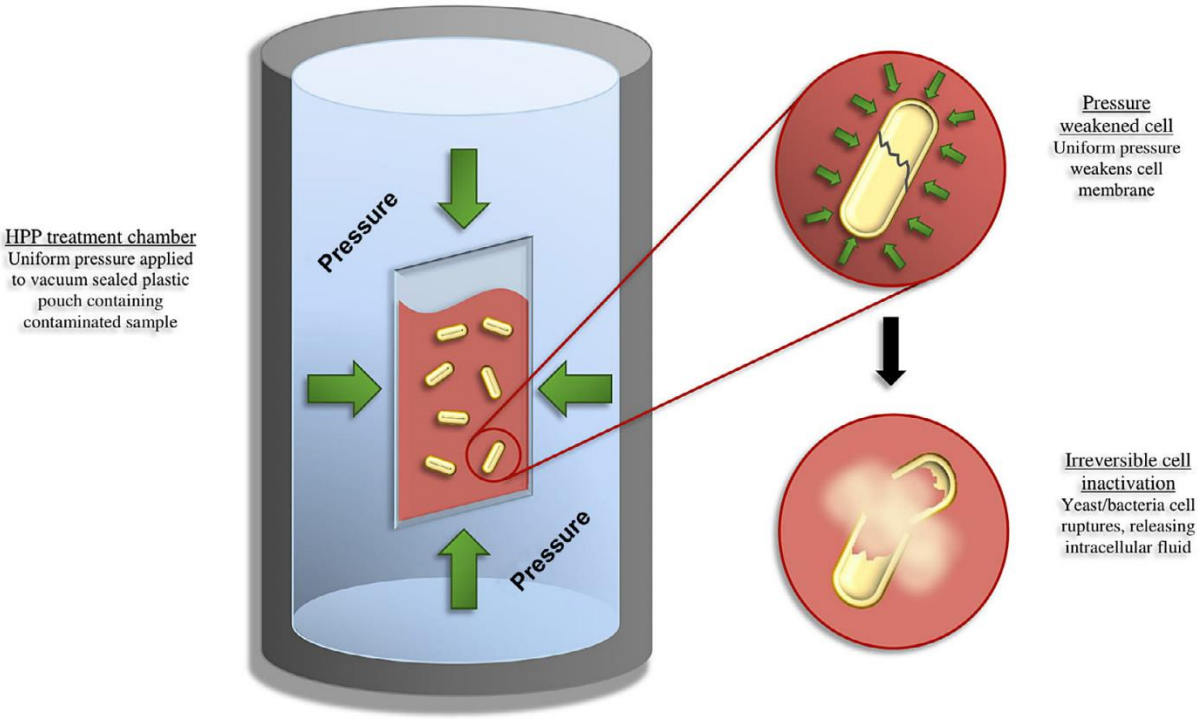

Figure 3. Usage of high hydrostatic pressure for the inactivation of microorganisms in wine (van Wyk and Silva, 2019).

With the before mentioned, it is important to point out what impact this technique has on the overall quality of wine. First, research has shown that the use of HHP can provide activation energy to trigger the chemical reactions in wine and thus accelerate the aging process (Tao et al., 2012). This leads to the enhancement of polymerization, esterification and oxidation reactions which can lead to an improvement in the color, aroma and taste of the wine (Buzrul, 2012; Sun et al., 2015). Furthermore, Li et al. (2005) reported that applying a pressure of 300 MPa for 2 h significantly contributes to better wine taste. Morata et al. (2012) subjected red

wine to a pressure of 100 MPa over a longer period of 24 h and concluded that there were no changes in the composition of anthocyanins and aroma compounds. Also, it has been shown that the application of HHP to wine in the short-term period has no impact on its quality, while in the long-term it improves or accelerates the aging process (Santos et al., 2012). Namely, some of the changes observed after 6 months of storage of red and white wines treated with HHP (425 and 500 MPa for 5 min) were more intense red-orange color, reduced concentration of total phenolics and antioxidant activity (Santos et al., 2015). White wine without SO_2 was subjected to pressures of 425 and 500 MPa for 5 min, and such treated wines showed a more pronounced brown hue (lower L* value and higher values a* and b*), lower value of antioxidant activity and total phenolics, compared to untreated wine containing 40 mg/L of SO_2 (Santos et al., 2013b). Also, the application of HHP has been shown to accelerate Maillard reactions thus producing wines with similar physicochemical and sensory characteristics as those that have been aged for an extended period (Santos et al., 2013b). Newest study by Santos et al. (2019) presents that after 5 months of aging, samples treated with HHP (500 MPa/5 min), had a lower concentration of anthocyanins, phenolic acids, and flavonols compared to wines treated by conventional methods and that those wines showed a similar degree of tannin polymerization and phenolic composition as a wine microoxygenated with the addition of oak chips. Notwithstanding the above, special emphasis should be placed on the adequate selection of process parameters. A study of Tao et al. (2012) has shown that extreme treatments with HHP (650 MPa for 1 to 2 h) change the physicochemical and sensory characteristics of the wine in a significant manner.

From the evidence provided above, HHP technique shows great potential in several areas (modification of the chemical composition of wine, processing of wine with low aging potential, and reducing the use of SO_2 during wine production). The rising start of the multidisciplinary approach appeared in last few years. The main goal was to produce high-quality wine with low or no SO_2 concentration with the combination of microbial, physical, and chemical processes (Ferrer-Gallego et al., 2017). Namely, most authors examined the influence of HHP on wines without SO_2 (Santos et al., 2013a; Santos et al., 2013b; Santos et al., 2015) or in wines with a single concentration of SO_2 (Santos et al., 2016; Sun et al., 2016; Briones-Labarca et al., 2017; van Wyk et al., 2018). Only recently Christofi et al. (2020) have studied HHP treatment in combination with different concentrations of SO_2 (0, 30, 60, and 100 mg/L) in wine, and the results showed that a combination of HHP treatment (350 MPa/10 min) and 60 mg/L SO_2 can suspend the rate of chemical reactions. Usually, in treated samples,

this takes place much faster. From the same research, after 12 months of aging, there was no difference in the chemical composition between these two groups, containing ≥ 60 mg/L SO_2. This suggests that HHP is a potential wine preservation technique, in the combination with an additional reduction of SO_2 concentration.

2.1.3. High voltage electrical discharge plasma – cold plasma

High voltage electrical discharge plasma, also called non-thermal plasma (NTP) or cold plasma (CP), is among the newer non-thermal techniques that have been intensively researched in the last decade. This technique shows great potential for application in the food industry in terms of inactivating microorganisms and improving food safety (Knorr et al., 2011; Mishra et al., 2016). Although most research to date has primarily focused on the inactivation of different types of microorganisms (Gurol et al., 2012; Fernandez et al., 2013; Misra et al., 2014; Vukušić et al., 2016) the focus shifted to the use of cold plasma for food properties modification (Segat et al., 2015; Zhu, 2017), inactivation of enzymes (Surowsky et al., 2013; Pankaj et al., 2013; Tappi et al., 2016), and extraction improvement of bioactive compounds (Elez Garofulić et al., 2015; Herceg et al., 2016; Bao et al., 2020).

Plasma can be described as the fourth state of matter. In specific, plasma is a fully or partially ionized gas with characteristic electrical, chemical, and physical properties (Petitpas et al., 2007). It is composed of excited atoms, molecules, ions, electrons, radicals, and photons. To generate plasma, it is necessary to provide an energy source that can ionize the gas. Namely, bringing energy above a certain limit in the gaseous phase causes ionization of molecules resulting in the formation of plasma (Thirumdas et al., 2014). Electric or electromagnetic fields have been shown to be most effective in gas ionization (Pankaj and Keener, 2018). In laboratory conditions, plasma is most often obtained by high-voltage electrical discharge or electrical discharges between two electrodes connected to an external energy source. Negatively charged electrons and positively charged ions in motion create electric and magnetic fields, which provide the energy needed for further ionization of the gas, i.e. the formation of plasma (Vukušić, 2016). There are different types of plasma, so regarding the working pressure of the gas at which the plasma is formed, we distinguish low-pressure and atmospheric plasmas, while according to the temperature at which they are applied, they are divided into hot plasma and cold plasma (Vukušić, 2016). Hot plasmas are in thermodynamic equilibrium, i.e. the temperature of electrons and other particles present in the plasma is the same (Fridman, 2008). On the other hand, cold plasma is characterized by electrons that have

a higher temperature than the weight ionic particles in the plasma, so this plasma is also called nonequilibrium plasma (Chen et al., 2004).

In recent years, the application of cold plasma or electrical discharge in liquids has been increasingly investigated, given that this technique shows great potential for processing liquid products that are sensitive to heat, such as fruit juices or wine. Namely, the advantage of cold plasma is that it does not cause a large increase in temperature after treatment, which thus ensures that the desired compounds remain preserved. The temperature of cold plasma is in the range of 30–60°C, and as such is desirable in the food industry because a small amount of energy is required to generate plasma (Santos Jr et al., 2018). The principle of plasma formation in liquids is as follows: plasma first develops in blowing bubbles of gas and then spreads through the liquid; by applying a very high voltage between the two electrodes, an electron is accelerated that has a sufficient amount of energy to induce physical and chemical changes in the liquids; the resulting electron avalanche (streamer discharge) travels to the opposite charged electrode and at the moment it reaches the electrode, a large number of free radicals are generated, and hydrodynamic cavitation, UV light and a strong electric field are developed. Figure 4 shows the application of high voltage electric discharge plasma in wine. Parameters that affect the electrical discharge plasma in liquids are the distance between the electrodes, electrode surfaces, impurities in the liquids, hydrostatic pressure, dissolved gases and electrical conductivity (Vukušić, 2016).

Prior to the implementation of this technique in food production, the possible impact of this technique on the nutritional and sensory characteristics of the product should be determined. The goal, as with all non-thermal techniques, is to produce a microbial stable product, but at the same time preserve its quality. During cold plasma treatment, reactive oxygen species (ROS) are induced in large quantities. This includes hydroxyl radical, atomic oxygen, hydrogen peroxide, singlet oxygen, and ozone. The resulting reactive oxygen species can destroy the bacterial structure by a variety of means. This applies to cell leakage, DNA damage, lipid peroxidation, protein denaturation, and interference in cell metabolism (Cheng et al., 2020). Although the exact mechanism of cold plasma inactivation of microorganisms has not been fully explained, the general belief is that ROS plays an important role in their inactivation (Pan et al., 2019). In general, the effectiveness of cold plasma in the control of undesirable microorganisms depends on many factors such as the type of microorganism, selected process parameters (voltage, frequency, power, polarity, reactor configuration) and physicochemical characteristics of the treated medium (pH, electrical conductivity, temperature, generated

radicals) (Misra et al., 2011; Vukušić et al., 2016; Liao et al., 2017; Stulić et al., 2019; Tomašević et al., 2019).

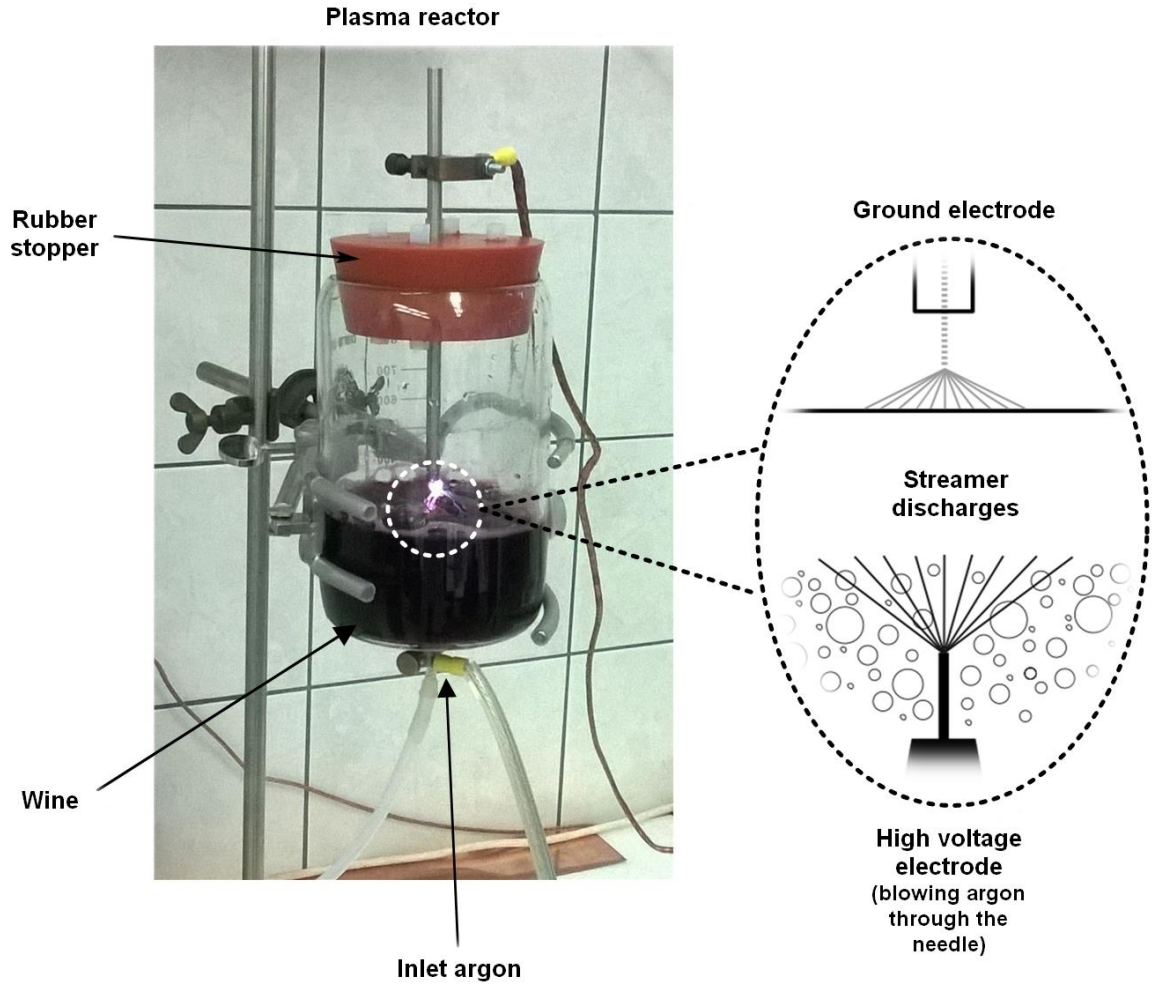

Figure 4. High voltage electrical discharge plasma – cold plasma treatment of wine.

In addition to microbial inactivation, recent studies have focused on the influence of cold plasma on the physicochemical and sensory characteristics of food products. A review of previous research on the effects of cold plasma on fruit juices was given by Pankaj and Keener (2018). Herceg et al. (2016) noted an increase in phenolic acids and tannins in pomegranate juice after gas plasma application. Bursać Kovačević et al. (2016) found an increase in anthocyanin concentration and a decrease in the L* value in pomegranate juice after treatment with atmospheric gas plasma. Elez Garofulić et al. (2015) also reported an increase in the concentration of anthocyanins and phenolic acids in cherry juice after plasma treatment. On

the other hand, Almeida et al. (2015) found a decrease in antioxidant capacity and an increase in the L* parameter in orange juice after plasma treatment. Pankaj et al. (2017) reported that total phenolics and antioxidant capacity decresed in grape juice after cold plasma treatment, while at the same time total flavonols increased. Contrary, the same authors found that cold plasma treatment did not affect pH and electrical conductivity. Given the previous research related to other food products and the positive results found in the control of the microbial population, the application of cold plasma in wine production could potentially result in a reduction of SO_2. Furthermore, recently Sainz-García et al. (2019) have compared the impact of serial and continuous cold atmospheric plasma treatments on quality characteristics of red wine. The same authors concluded that serial cold plasma treatment resulted in wine with higher intensity and lower color tonality, and increased concentration of total phenolics and anthocyanins. On the other hand, continuous cold plasma treatment did not lead to a significant improvement of young red wine (chromatic and phenolic composition) nor its deterioration (Sainz-García et al., 2019). However, there are still insufficient information on the impact of this technique on the overall quality of wine, as well as its long-term effect. Also, limiting factors are the lack of a standardized method for plasma treatment, and thus more difficult drawing a conclusion about its effectiveness, as well as energy efficiency that affects the cost-effectiveness of such technological process.

2.2. Addition of antioxidants – glutathione

Despite the mentioned innovative physical solutions, common methods of wine protection like the use of antioxidants such as SO_2 are still the first choice in wine production. In addition to SO_2 which is the most common antioxidant and preservative, glutathione is mentioned as a possible replacement. It is thought that glutathione may act in a similar way as SO_2 and may contribute to reducing the concentration of SO_2 used for the antioxidant protection of wine. The application and role of glutathione in wine has gained significant scientific and commercial value, and the primary reason for this is related to the control of oxidative spoilage of wine (Kritzinger et al., 2013a).

Glutathione (reduced form of glutathione, GSH) is a naturally occurring antioxidant in grapes as well as in yeasts, where it has similar physiological and biochemical roles (De Vero et al., 2017). GSH (tripeptide) is one of the most common non-protein thiols in living organisms. The composition of GSH consists of three amino acids cysteine, glutamic acid and glycine (Anderson, 1998). In recent years, this important sulfhydryl compound in grapes, must and

wine has become the subject of a growing number of researchers. It affects the quality of wine directly or indirectly, and its concentration in wine is very variable. There are numerous factors that affect it, from grape variety, *terroir* and harvest date (Cheynier et al., 1989), to the yeast strain and production technology (Kritzinger et al., 2013b). The values of GSH concentration usually range from few to 100 mg/L in must and up to 70 mg/L in wine (Kritzinger et al., 2013a).

The role of GSH in wine is primarily related to its redox properties. Namely, GSH is involved in the prevention of browning reactions, which can occur in must as a result of enzymatic or non-enzymatic reactions involving phenolic compounds (Oliveira et al., 2011). The antioxidant character of GSH has a protective role in wine: it stabilizes the color of wine by inhibiting the polymerization of phenolic compounds (Sonni et al., 2011b; Sonni et al., 2011c); with its sulfhydryl group (SH), as an electron-rich nucleophile, can reduce *o*-quinone compounds back to phenols (Comuzzo and Zironi, 2013). In addition, GSH can prevent the formation of sotolone and aminoacetophenone, compounds characteristic for atypical wine aging (Dubourdieu and Lavigne, 2004), and can protect aroma compounds or prevent the loss of varietal thiols, esters and terpenes (Roussis et al., 2009; Tirelli et al., 2010). Studies have shown a beneficial effect of glutathione addition for the production technology of white wine, especially for preservance of varietal aroma and color stability (Roussis et al., 2007; El Hosry et al., 2009; Ugliano et al., 2011). Moreover, glutathione (thiol) can potentially compete with varietal thiols to bind to *o*-quinones resulting in preservation of varietal flavor (Tirelli et al., 2010). In the wine bottling procedure, glutathione showed that it limits acetaldehyde accumulation. Also, it preserves the aroma and freshness of wine in a 12-months' storage period (Webber et al., 2017). Furthermore, a synergistic effect of glutathione and SO_2 in preventing the oxidation of phenolic and aroma compounds during the microoxygenation of red wine (Gambuti et al., 2015) and the aging of sparkling wine (Webber et al., 2017) was also found. The results of the research by Roussis et al. (2007) pointed out the following, when glutathione (20 mg/L) is added at a lower SO_2 concentration (35 mg/L) it has a more significant effect on the preservation of individual esters and linalool than a high concentration of free SO_2 (50 mg/L).

These are just some of the studies that are a good basis for future action in the segment of wine production, especially white wines. Also, the addition of GSH as a pure substance is allowed in the case of must and wine (max. concentration of 20 mg/L) according to current OIV directives (OIV, 2021). Thus, the use of GSH in wine production could partially replace SO_2 as an antioxidant.

2.3. Quality of wine

Wine is a very complex medium that contains over a thousand different chemical ingredients. Therefore, a problem in predicting wine quality is first of all related to its complexity. The OIV considers wine quality as a set of characteristics that distinguish one wine from another, and one of those characteristics is the consumer experience of taste (OIV, 2021). Despite different understandings of quality, the generally accepted and less subjective basic wine quality can be defined through its sensory and chemical characteristics. Namely, the quality and phenolic and aroma compounds are especially closely related in wine. It is known that phenolic compounds represent a vital role for the sensory characteristics of wine: color, astrigency and bitterness (Hornedo-Ortega et al., 2020). Also, aroma compounds are important for wine quality because these compounds produce an effect on the sensory senses of smell and taste (Vilanova et al., 2010). In addition to the above, analysis of physicochemical characteristics is crucial for assessing the quality of wine. The control of oxygen and SO_2 is of great importance in wine production since these parameters can drastically affect the composition of wine due to participation in numerous chemical reactions (Dimkou et al., 2013; Fracassetti et al., 2013).

2.3.1. Phenolic composition and chromatic characteristics

When we compare the complexity of chemical compounds in wine vs. grapes, it is higher because of the formation of numerous new compounds. During the process of wine production and aging, various chemical reactions occur, which lead to copigmentation, cycloaddition, polymerization and oxidation of phenolic compounds. These complex transformations lead consequently to changes in their concentration and composition, which is ultimately reflected in the intensity and color shade, and the bitterness of the final product.

The most important phenolic compounds in grapes and wines are the following: anthocyanins, flavan-3-ols, condensed tannins (proanthocyanidins) and phenolic acids. Anthocyanins are glycosides of anthocyanidins, mainly found in the skin of grapes. The most common anthocyanidins of red grapes and wines are cyanidin, peonidin, delphinidin, pelargonidin, petunidin and malvidin, the last of which is the most significant in *Vitis vinifera* L. (Gómez-Míguez et al., 2006). We also distinguish the 3-*O*-monoglucosides of these six anthocyanidins. Furthermore, some of the important flavan-3-ols are (+)-catechin and its enantiomer (-)-epicatechin, and some catechin derivatives such as gallocatechin, epigallocatechin, epicatechin gallate and epigallocatechin gallate (Mattivi et al., 2006). Oligomers and polymers of flavan-3-ols, (+)-catechin and (-)-epicatechin, are the main proanthocyanidins found in

grapes (Zhao et al., 2010). In young wines they occur as dimers and trimers, and during aging their concentration decreases because of oxidation and precipitation processes (Cheynier et al., 2006). The mentioned phenolic compounds are crucial for the quality of red wine due to their great contribution to its sensory characteristics. Anthocyanins are responsible for the color of red wines, while flavan-3-ols and condensed tannins are key compounds in the color stabilization, astrigency and bitterness. Namely, the chemical changes that occur are direct or indirect condensation reactions among anthocyanins and tannins, with formation of colored polymeric pigments and stabilization of wine color (Fulcrand et al., 2006; Cano-López et al., 2007; Chira et al., 2012). This ultimately affects the intensity and hue of the color of red wine, so in aged wines the appearance of brick red to brown tones could be observed (Cheynier et al., 2006).

In comparison between red and white wines, white wines have a significantly lower concentration of phenolic compounds. Phenolic acids, hydroxycinnamic and hydroxybenzoic, represent the primary group of phenolic compounds in white wines. Primarily, they are characterized by a higher concentration of caftaric acid. The hydroxycinnamic acids are represented with *p*- coumaric, caffeic, ferulic and sinapic acids. Their oxidation is associated with browning of wine (Niculescu et al., 2018). Browning is the result of a series of complex reactions that gives brown color, reduced brightness, increased color intensity and browning index (Kallithraka et al., 2009). Precisely, the *o*-quinones produced in oxidation of phenolic compounds (flavan-3-ol monomers of (+)-catechin and (-)-epicatechin, caffeic and other hydroxycinnamic acids) will go on with polymerization reactions and formation of brown pigments (Guyot et al., 1996). Among the most important hydroxybenzoic acids are *p*-hydroxybenzoic, protocatechuic, vanillic, gallic and syringic acids (Niculescu et al., 2018). For red wines, hydroxycinnamic acids and their tartrate esters represent a main group of non-flavonoid phenolic compounds. They participate in the formation of new more stable pigments (pyranoanthocyanins). Also, they are considered as stabilizers of color in young red wines (copigmentation with anthocyanins) (Heras-Roger et al., 2016). What is more relevant is their association with the sensory perception of astrigency and bitterness (Ferrer-Gallego et al., 2014).

2.3.2. Aroma composition

Important quality characteristic which implies the differences between wines is aroma. Aroma is a direct function of the chemical composition of wine. Essentially, aroma is created by interaction of numerous chemical compounds obtained from multiple sources, such as grapes, fermentation, and aging process (Vázquez-Pateiro et al., 2020). Although there are numerous chemical compounds located in grapes-wine, only a few actually contribute to the aroma (Zhu et al., 2016).

Significant compounds in the primary aroma of wine are terpenes, which can be free and glycosidically bound, and are indicators of the specificity of the wine variety and winegrowing region (Mele et al., 2020). At concentrations above the sensory threshold, these compounds form an active component of the aroma in many wines (Piñeiro et al., 2006). The most scented monoterpenes are monoterpene alcohols such as linalool, α-terpineol, nerol, geraniol, citronellol and hotrienol, which are carriers of floral aromas (Pereira et al., 2020). The importance of monoterpenes in wines ariseses from the fact that these compounds have a synergistic effect on other aroma compounds, and thus can affect the aroma composition of wine (Coetzee and du Toit, 2015).

Representatives of the secondary aroma, esters, higher alcohols and volatile fatty acids, are quantitatively the most important for wine aroma, and thus for the sensory characteristics and quality of wine (Stashenko et al., 1992). Esters are the largest and the most important compound group that have influence on wine aroma. Formation happens during alcoholic and malolactic fermentation, as well as aging. The origin of esters in wines can be different, and these are grapes, yeasts and bacteria. Yeasts synthesize the most valuable esters for wine aroma (Belda et al., 2017). Esters greatly influence wine aroma, when compared to higher alcohols, but they are present in small concentrations (mg/L), and further decrease during aging under chemical hydrolysis reactions (Garofolo and Piracci, 1994). They generally give off fruity or floral odor notes. In a case when they are present in higher concentrations, they mask the varietal aroma, and reduce the wine complexity (> 90 mg/L of ethyl acetate or 200 mg/L of total esters is considered to be a wine flaw) (Belda et al., 2017). In wine there can be found two main groups of esters: ethyl esters of fatty acids and acetate esters of higher alcohols. For wine aroma, ethyl esters are less important than acetate esters. The most important acetate esters are *i*-butyl acetate (fruit aroma), *i*-amyl acetate (banana) and 2-phenylethyl acetate (flower aroma) (Styger et al., 2011).

Furthermore, higher alcohols are formed by yeasts during alcoholic fermentation (decarboxylation) or from amino acids (deamination). Higher alcohols pass through the Ehrlich reaction (decomposition of amino acids) and directly affect the wine aroma. Often, they are precursors in ester formation (Belda et al., 2017). Quantitatively, *i*-butanol, phenylethyl alcohol, and *i*-amyl alcohol are primary higher alcohols in wine (Tao et al., 2008). Below 300 mg/L higher alcohols give the wine desired aroma complexity, while concentrations above this level negatively affect the wine aroma (Rapp and Versini, 1995).

Volatile fatty acids in wine can be divided into short-chain (acetic, propane and butanoic acid) and medium-chain saturated acids (hexane, octanoic, decadic and dodecadic). Short-chain acids are formed as by-products of alcoholic fermentation metabolism, while medium-chain acids are considered to be intermediates in the biosynthesis of long-chain fatty acids (Lambrechts and Pretorius, 2000). Relatively low concentrations (4-10 mg/L) of C6-C10 volatile fatty acids give the wine a mild and pleasant aroma, however at concentrations above 20 mg/L, their effect on wine becomes negative (Shinohara, 1985; Jiang and Zhang, 2010).

The tertiary aroma of wine includes all volatile compounds that are formed during aging of wine, giving the so-called "bouquet" of wine. During storage, through physicochemical and biological reactions, the transformation of aroma compounds produced in the previous stages occurs, which causes significant changes in the post-fermentative aroma of wine (Pereira et al., 2020). It is known that during wine aging they lose the floral aroma associated with monoterpenes. For example, there is a decrease in linalool concentration, while α-terpineol concentration initially increases (probably due to oxidation of other terpene alcohols) and then decreases at a later stage (Coetzee and du Toit, 2015). Furthermore, there is a loss of fruit character due to the reduction of the ester concentration due to chemical hydrolysis or oxidation by hydroxyl radicals or the interaction of the ester with *o*-quinones. In particular, the reduction in the concentration of acetate esters contributes to the loss of freshness and fruitiness in white wines during aging in bottles. Also, there may be a decrease in the concentration of higher alcohols due to their oxidation, but it can also remain unchanged (Coetzee and du Toit, 2015). On the other hand, it has been shown that hexanol concentration can increase during aging due to oxidation of linoleic and linolenic acids (Oliveira et al., 2006). Regarding volatile fatty acids, some compounds have been reported to increase (due to the hydrolysis of ethyl esters), and on the other hand, during aging others decrease or remain stable (Coetzee and du Toit, 2015).

2.3.3. Physicochemical characteristics

The introductory part of this thesis already describes the importance and role of SO_2 in wine production. Furthermore, monitoring the concentration of total and free SO_2 in wine is crucial during production and storage in order to ensure timely protection and to comply with legal restrictions on the maximum permitted levels of total SO_2 in wines. Apart from the fact that high concentrations of SO_2 can affect the final quality of wine, primarily sensory characteristics, they can also cause serious problems in people with allergic diseases or symptoms of food intolerance (Giménez-Gómez et al., 2017). Therefore, SO_2 is one of the most frequently analyzed components of wine and its concentrations are carefully controlled to ensure its effective action without negative effects on sensory characteristics. In addition to analytical monitoring of the concentration of SO_2, an important parameter for quality control of wine is the concentration of dissolved oxygen. Oxygen control is extremely important in the production of high quality wines. Namely, during production (pumping, flow or filtration) due to the contact of wine with air, oxygen dissolves in the wine. Depending on the degree of wine exposure to oxygen, various chemical reactions occur that can positively but also negatively affect wine sensory characteristics (color, aroma and taste) (Du Toit et al., 2006; Ribéreau-Gayon et al., 2006; Karbowiak et al. 2009). For example, it is known that exposure of wine to low oxygen concentrations can have a positive effect on the development of red and white wines. Additionaly, it can reduce the possibility of development of reductive odors (Ugliano et al., 2009). On the other hand, exposure of wine to uncontrolled, high oxygen concentrations can cause shortage of freshness and fruitiness. Also, oxidation and browning in wine appear, as defects (Ribéreau-Gayon et al., 2006; Lopes et al., 2009). Therefore, a better understanding of the impact of oxygen on wine quality and the control of wine exposure to oxygen during production is extremely important in order to reduce and optimize the use of SO_2.

2.3.4. Sensory characteristics

In addition to determining the chemical composition, an indispensable part for determination of wine quality is its sensory characteristics assessment. The sensory characteristics of wine are crucial for defining its acceptability. The relationship between sensory evaluation and the chemical composition of wine is a major subject of research in enology (Jones et al., 2008; Chira et al., 2011; Villamor, 2012). The results of chemical and sensory analysis of wine complement each other, which gives a complete picture of the characteristics and quality of wine. Sensory analysis of wine means a detailed analysis of the impressions that wine leaves

on the senses of sight, smell and taste (Ivandija, 2011). In general, it involves evaluating characteristics such as appearance, color, clarity, aroma, and taste of the wine. These sensory characteristics chemically belong to phenolic and aroma compounds, which are described in more detail in the previous subsections. In short, wine is a chemically dynamic medium and contains high concentrations of oxidizable compounds, especially phenols, which promotes changes primarily in wine color. Furthermore, it contains various volatile compounds that are interconnected, forming complexes, and finally wine aroma, while the main components of wine such as alcohol, extract, sugar, acids and tannins are responsible for complex impression of taste (Ivandija, 2011). Therefore, it should be noted that the chemical composition and sensory characteristics of wine are very closely related and that the assessment of the overall quality of wine can not be based solely on one of these two properties, nor it is necessary to consider all parameters.

2.4. Hypothesis, goals and expected scientific contribution of the research

Based on previous knowledge and literature review, the hypothesis was defined that non-thermal techniques (high power ultrasound, high hydrostatic pressure and high voltage electrical discharge plasma – cold plasma) are potential methods for use in production of wine with reduced SO_2 concentration due to efficiency in inactivation of microorganisms, acceleration of oxidation-reduction reactions and preservation and improvement of product quality.

Therefore, the aim of this doctoral thesis is to determine the short-term impact of non-thermal techniques (high power ultrasound, high hydrostatic pressure and high voltage electrical discharge plasma - cold plasma) on the overall quality of red and white wines. After optimizing each technique in order to preserve and improve the quality of wine, the impact of each technique along with antioxidants addition (SO_2 and glutathione) will be investigated during 12 months of aging in bottles. The findings of this research will show the effectiveness of these non-thermal techniques along with antioxidants in the production of high quality wines with reduced SO_2 concentration.

Thus, in this doctoral thesis, the short-term and long-term impact of high power ultrasound (Lukić et al., 2019a (PAPER 1); Lukić et al., 2020a (PAPER 2); Lukić et al., 2019b (PAPER 3); APPENDIX 1), high hydrostatic pressure (Lukić et al., 2019b (PAPER 3); Tomašević et al., 2017 (PAPER 4); Lukić et al., 2020b (PAPER 5); APPENDIX 2) and high voltage electrical discharge plasma - cold plasma (Lukić et al., 2019c (PAPER 6); Lukić et al., 2017 (PAPER 7); APPENDIX 3) on the phenolic, chromatic, aroma, physicochemical and sensory characteristics of red and white wines were examined. An overview of complete scientific papers is given below. Also, the unpublished results of this scientific research necessary for a complete analysis of the selected topic are given in *Chapter 8. Sensory and analytical data supplement* in the form of APPENDIX 1-3.

3. SCIENTIFIC PAPERS

3.1. List of scientific papers

1. **Lukić, K.**, Brnčić, M., Ćurko, N., Tomašević, M., Valinger, D., Denoya, G. I., Barba, F. J., Kovačević Ganić, K. (2019a) Effects of high power ultrasound treatments on the phenolic, chromatic and aroma composition of young and aged red wine. *Ultrason. Sonochem.* **59**, 104725. doi: 10.1016/j.ultsonch.2019.104725

2. **Lukić, K.**, Brnčić, M., Ćurko, N., Tomašević, M., Tušek, A. J., Kovačević Ganić, K. (2020a) Quality characteristics of white wine: The short-and long-term impact of high power ultrasound processing. *Ultrason. Sonochem.* **68**, 105194. doi: 10.1016/j.ultsonch.2020.105194

3. **Lukić, K.**, Tomašević, M., Ćurko, N., Sivrić, A., Ružman, E., Kovačević Ganić, K. (2019b) Influence of non-thermal processing techniques on sulfur dioxide and oxygen concentrations in young and aged wines. *Croatian Journal of Food Technology, Biotechnology and Nutrition* **14**(3-4), 65-75. doi: 10.31895/hcptbn.14.3-4.7

4. Tomašević, M., **Lukić, K.**, Bosiljkov, T., Kelšin, K., Ćurko, N., Kovačević Ganić, K. (2017) Effect of high hydrostatic pressure on the volatile compounds in wine. *Works of the Faculty of Agriculture and Food Science* **62**(67 (2)), 505-516.

5. **Lukić, K.**, Ćurko, N., Tomašević, M., Kovačević Ganić, K. (2020b) Phenolic and Aroma Changes of Red and White Wines during Aging Induced by High Hydrostatic Pressure. *Foods* **9**, 1034. doi: 10.3390/foods9081034

6. **Lukić, K.**, Vukušić, T., Tomašević, M., Ćurko, N., Gracin, L., Kovačević Ganić, K. (2019c) The impact of high voltage electrical discharge plasma on the chromatic characteristics and phenolic composition of red and white wines. *Innov. Food Sci. Emerg. Technol.* **53**, 70-77. doi: 10.1016/j.ifset.2017.11.004

7. **Lukić, K.**, Tomašević, M., Vukušić, T., Kelšin, K., Gracin, L., Kovačević Ganić, K. (2017) Influence of high voltage electrical discharge plasma treatment on the physicochemical characteristics of wine. *Works of the Faculty of Agriculture and Food Science* **62**(67 (2)), 517-524.

Paper 1

Lukić, K., Brnčić, M., Ćurko, N., Tomašević, M., Valinger, D., Denoya, G. I., Barba, F. J., Kovačević Ganić, K. (2019a) Effects of high power ultrasound treatments on the phenolic, chromatic and aroma composition of young and aged red wine. *Ultrason. Sonochem.* **59**, 104725. doi: 10.1016/j.ultsonch.2019.104725

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Effects of high power ultrasound treatments on the phenolic, chromatic and aroma composition of young and aged red wine

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ABSTRACT

In this study, the effects of both ultrasonic bath and probe treatments on the phenolic, chromatic and aroma composition of young red wine Cabernet Sauvignon were studied and modeled by artificial neural networks (ANNs). Moreover, the effect of high power ultrasound (HPU) along with antioxidants addition (sulfur dioxide and glutathione) was investigated during 6 months of aging in bottles. Lower amplitude and temperature, shorter treatment duration and particularly lower frequency showed a more favorable and milder effect on the chemical composition of wine. In the case of the ultrasonic probe treatment, similar effect was achieved primarily by a larger probe diameter as well as lower amplitude and treatment duration. Selected ANN models showed the best predictions for the chromatic characteristics followed by total phenolics and anthocyanins. The changes induced by HPU treatment after 6 months of aging were mainly detected in the composition of phenolic compounds (both total and individual), where higher concentration of antioxidants (sulfur dioxide and glutathione) slowed down the decrease rate of these compounds during aging. However, HPU treatment did not influence most of the chromatic characteristics and aroma compounds, except lightness and fatty acids. The obtained results indicated that suitable ultrasound treatment might accelerate some aging reactions and shorten the period of wine aging.

1. Introduction

High power ultrasound (HPU) is an innovative processing technology that could be used on wines for many applications. For instance, over the last years, many studies have been carried out on the use of ultrasound for wine microbial stabilization [1–5] and for the acceleration of wine aging process [6–9].

Despite the mentioned studies, most of the conducted researches regarding the application of HPU in wine production are related to the effect of the technology on the extraction of different bioactive compounds (phenolics, flavonoids, tannins and others) responsible for wine color, flavor and taste [10–16].

Generally, when it is applied to a wine, HPU causes both physical and chemical effects, which are expected to modify the physicochemical properties and enhance the quality of the product during processing. But first of all, the application of HPU should ensure the preservation of sensory properties of wines and the antimicrobial effect at the same time. The replacement of the antioxidant and antimicrobial effect of sulfur dioxide (SO_2) is still hard to accomplish. However, the combination of HPU together with antioxidants addition (lower SO_2 and glutathione) could be a suitable practice to achieve this purpose, especially regarding the wine stability during aging. In other words, it was reported that the combination of SO_2 and glutathione implicates a respectable protective effect in wines [17]. Additionally, reduced glutathione has been proposed as an alternative method due to its specific antioxidant effects in preserving aroma compounds and preventing oxidation [18].

Recently, García Martín et al. [19] reviewed the effect of ultrasound on the quality properties of red wines. Additionally, other authors reported that different conditions of ultrasound treatment influence the color characteristics and significantly modify the content of total phenolics through stimulation of polymerization reactions that take place

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during natural aging of wine, without major changes in basic physicochemical parameters such as pH, total and volatile acidity [9,20,21]. Moreover, some studies showed that ultrasound influences the electrical conductivity of red wine [22], triggers the generation of free radicals into the wine [23] and causes changes in the wine aroma composition and sensory properties (formation of oxidized aromas) [4,24]. However, the results obtained in these studies are still not sufficient to conclude how the use of different ultrasound systems such as ultrasonic baths or ultrasonic immersion probes could lead to different effects on quality properties of wine as well as its characteristics during aging.

Hence, further investigation about the effect of different ultrasound systems and process conditions (i.e., frequency, intensity, treatment duration and temperature) on a wider range of wine quality properties is necessary.

Aside from the aforementioned facts, the stochastic nature of ultrasound process and its dependence on numerous interdependent parameters make it difficult and almost impossible to develop one general and precise mathematical model suitable for investigating the process and product parameters [15]. Therefore, the artificial intelligence based techniques for prediction have attracted increasing attention in recent years, particularly for process modeling. Artificial neural networks (ANNs) are one of the important artificial intelligence methods that can be used to solve the problems that are not suitable for standard statistical methods [25].

Given the above, the aim of this study was (i) to evaluate the effect of HPU treatment applied by an ultrasonic bath and by an ultrasonic probe on the phenolic composition, chromatic characteristics and aroma composition of a young red wine Cabernet Sauvignon; (ii) to evaluate the ability of ANNs to predict aforementioned quality properties of ultrasonic bath and ultrasonic probe treated red wine, and (iii) to study the effect of HPU along antioxidants addition (SO_2 and glutathione) on phenolic, chromatic and aroma composition of red wine during 6 months of storage.

2. Material and methods

2.1. Chemicals

The chemicals used in this work were: Folin-Ciocalteu reagent (Kemika, Zagreb, Croatia), sodium bisulfite (Acros Organics, Geel, Belgium), hydrochloric acid (37%, Carlos Erba, Val del Reuil, Spain), sodium chloride (pro analysis, Carlo Erba, Val del Reuil, Spain), ethanol (96%, Gram-Mol, Zagreb, Croatia), Sodium carbonate anhydrous (99%, T.T.T. Sveta Nedjelja, Croatia), formic acid (98–100%, T.T.T., Sveta Nedjelja, Croatia), acetonitrile (HPLC grade, J.T. Baker, Deventer, Netherlands), ethanol (HPLC grade, J.T. Baker, Deventer, Netherlands). Malvidin-3-*O*-glucoside chloride, (+)-catechin, (−)-epicatechin, B1 [(−)-epicatechin-(4ß-8)-(+)-catechin] and B2 [(−)-epicatechin-(4ß-8)-(−)-epicatechin], as well as the aroma reference standards, and L-glutathione reduced (≥98%) were purchased from Sigma Aldrich (St. Louis, USA). The aqueous solution of potassium bisulfite (Bisulfite 15) was purchased from Laffort (Bordeaux, France).

2.2. Wine samples

The work was done with young wine Cabernet Sauvignon (*Vitis vinifera* L.), vintage 2017, from winery Erdutski, Erdut, Croatia. The wine had the following physicochemical characteristics: alcohol 12.8 vol%, total acidity (as tartaric acid) 5.6 g/L, volatile acidity (as acetic acid) 0.4 g/L, pH = 3.5, reducing sugars 5.0 g/L, free SO_2 10 mg/L, and total SO_2 20 mg/L.

2.3. High power ultrasound (HPU) treatments

The ultrasound studies were carried out using two different techniques: ultrasonic bath (experiment 1) and ultrasonic probe (experiment 2). The HPU experiment 1 was carried out using an ultrasonic bath system (Elmasonic P, Elma Schmidbauer GmbH, Singen, Germany), with dimensions of 505 × 300 × 200 mm and maximum capacity of 28 L. The wine (200 mL) was placed in a round-bottom glass vessel (400 mL), which served as a treatment chamber, and then immersed in the ultrasonic bath. The samples were then treated by ultrasound running at different combinations of process parameters, namely ultrasound frequency (37 and 80 kHz), ultrasound amplitude (40, 60 and 100%), bath temperature (20, 40 and 60 °C) and treatment duration (20, 50, 65 and 90 min), selected based on literature data [7,21,23] and preliminary experiments (data not shown). The sonicator generated the power of 380 W. The ultrasonic energy was delivered from the bottom to the water in the bath with an automatic control of frequency. The control of water temperature inside the bath during the HPU treatments was achieved by cold water cooling of the treatment chamber.

On the other hand, the HPU experiment 2 was carried out using an ultrasonic processor system (Q700, Qsonica Sonicators, Newton, CT, USA) with dimensions of 400 × 400 × 800 mm, which was set at a nominal power of 700 W and a constant frequency of 20 kHz. The HPU probe was centered and dipped 2 cm inside a 400 mL glass vessel containing 300 mL of the sample. To study the effects of the ultrasound treatment, the experimental design considered different process parameters, namely the diameter size of ultrasound probe (12.7, 19.1 and 25.4 mm), ultrasound amplitude (25, 50, 75 and 100%) and treatment duration (3, 6 and 9 min), selected based on literature data [4,20,26] and preliminary experiment (data not shown). The samples were kept at room temperature (25 °C) by cooling the reactor during the treatment. Each HPU treatment in both experimental sets 1 and 2 was conducted in duplicate [144 (72 × 2) and 72 (36 × 2) trials in total]. Finally, after ultrasound exposures, the wine samples were subjected to different analyses in order to evaluate the effects of the treatments on the main wine quality properties. Wine that was not subjected to any HPU treatment was used as control sample in both HPU experiments.

2.4. Storage stability and changes in the chemical composition of red wines processed by HPU

According to the results of both HPU experiments, a second experiment (ultrasonic probe) was chosen for small scale performing at following process conditions: probe diameter of 25.4 mm, ultrasound amplitude of 25% and treatment duration of 6 min. The aim of this was to study the effect of HPU along antioxidants addition (SO_2 and glutathione) on phenolic, chromatic and aroma composition of red wine during 6 months of storage in bottles. Before HPU treatment, three experimental wines were prepared: (i) wine with standard SO_2 concentration (25 mg/L of free SO_2), (ii) wine with low SO_2 concentration and addition of glutathione (10 mg/L of free SO_2 with 20 mg/L of glutathione), and (iii) wine with low SO_2 concentration (10 mg/L of free SO_2). Control wine was untreated wine with standard concentration of SO_2 (25 mg/L of free SO_2). After HPU treatments, the wines were stored for 6 months in 750 mL bottles, sealed with natural cork stoppers and stored in a dark place at 12 °C. HPU treatments were carried out in triplicate and chemical analyses were conducted after 0, 3 and 6 months of aging.

2.5. Analysis of chromatic characteristics

The chromatic characteristics of the wine samples were measured with a Specord 50 Plus spectrophotometer (AnalytikJena, Jena, Germany) using the CIELab space [27]. The values of L* (lightness), a* (redness/greenness), b* (yellowness/blueness), C* (chroma) and H* (hue angle) were determined. All measurements were performed in triplicate. The total color difference value (ΔE*) between the control and treated wine samples was calculated by the following Eq. (1):

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (1)$$

2.6. Spectrophotometric analysis of phenolic compounds

Determination of total phenolics (TP) content was done by the Folin-Ciocalteu method as described in detail by Singleton and Rossi [28]. The results were expressed as mg/L of gallic acid equivalents (mg GAE/L). The total anthocyanins (TA) content was measured by the bisulfite bleaching method as previously described by Ribéreau-Gayon and Stonestreet [29]. The results were expressed as mg/L. Measurement of total tannins (TT) content was carried out according to Ribéreau-Gayon and Stonestreet [30] and the results were expressed as g/L. All these spectrophotometric analyses were carried out in triplicate by a Specord 50 Plus spectrophotometer.

2.7. HPLC analysis of phenolic compounds

The HPLC analyses were performed on an Agilent 1100 Series LC-MSD system (Agilent Technologies, Waldbronn, Germany) with autosampler, binary pump, thermostated column compartment, DAD detector, FLD detector, and single quadrupole mass detector equipped with electrospray ionization interface, coupled to an Agilent Chemstation data analysis software. Wine samples were filtered through a 0.45 μm pore size cellulose acetate syringe filters (Nantong FilterBio Membrane, Nantong City, Jiangsu P.R China) prior to injection.

Free anthocyanins separation in the red wine samples was carried out according to the method previously described by Lorrain et al. [31] by using a Phenomenex Nucleosil C18 (4.6 mm × 250 mm, 5 μm) column. The mobile phase consisted of two solvents, water/formic acid (95:5, v/v) (solvent A) and acetonitrile/formic acid (95:5, v/v) (solvent B) and it was applied at a flow rate of 1 mL/min as follows: 0–25 min, 10–35% B linear; 25–26 min, 35–100% B linear; 26–28 min, 100% B isocratic; 28–29 min, 100–10% B linear. The column was re-equilibrated between runs for 29–35 min under initial gradient conditions. Free anthocyanins were eluted under following conditions: injection volume 20 μL, column temperature 40 °C and detection at 520 nm. The identification and peak assignment of anthocyanins were based on the comparison of their retention times, UV–Vis and mass spectral data with those of standards [32,33]. The following nine major free anthocyanins were determined: delphinidin-3-*O*-glucoside, cyanidin-3-*O*-glucoside, petunidin-3-*O*-glucoside, peonidin-3-*O*-glucoside, malvidin-3-*O*-glucoside, peonidin-3-*O*-acetylglucoside, malvidin-3-*O*-acetylglucoside, peonidin-3-(6-*O*-*p*-coumaroyl) glucoside and malvidin-3-(6-*O*-*p*-coumaroyl) glucoside. The quantification was performed by using an external standard calibration curve of malvidin-3-*O*-glucoside chloride. All analyses were conducted in triplicate and the results were expressed as the sum of the free individual anthocyanins quantified.

The analysis of flavan-3-ols was performed by using a Lichrospher 100-RP18 (4.6 mm × 250 mm, 5 μm) column, according to the method of Ćurko et al. [34] with a slight modification of the solvent gradient conditions. The mobile phase consisted of two solvents, water/formic acid (99:1, v/v) (solvent A) and acetonitrile/formic acid (99:1, v/v) (solvent B) and it was applied at a flow rate of 1 mL/min as follows: 0–11 min, 3–8% B linear; 11–16 min, 8% B isocratic; 16–20 min, 8–10% B linear; 20–27 min, 10% B isocratic; 27–32 min, 10–12% B linear; 32–34 min, 12–14% B linear; 34–45 min, 14–25% B linear; 45–46 min, 25–100% B linear; 46–50 min, 100% B isocratic, 50–51 min, 100–3% B linear. The column was re-equilibrated between runs for 51–55 min under initial gradient conditions. The injection volume was 20 μL and the column temperature was 25 °C. The detection was conducted at 280 nm excitation wavelength and 320 nm emission wavelength with low fluorescence intensity. The identification and peak assignment of flavan-3-ols were based on the comparison of their retention times and mass spectral data with those of standards [35,36]. The following flavan-3-ols were determined: (+)-catechin (C), (−)-epicatechin (EC), dimers B1, B2, B3, B4 and trimer C1. The quantification was performed by using an external standard calibration curve in the case of C, EC, B1, B2. On the other hand, the dimers B3, B4 and trimer C1 were quantified as dimer B1 equivalents. All analyses were conducted in triplicate and the results were expressed as the sum of the free individual flavan-3-ols.

2.8. GC/MS analysis of aroma compounds

Aroma compounds were extracted from the wine by solid-phase microextraction (SPME) and analyzed by gas chromatography coupled with mass spectrometry (GC/MS) using an Agilent Gas Chromatography 6890 series equipped with an Agilent 5973 Inert mass selective detector (Agilent Technologies, Santa Clara, USA) according to the method described by Tomašević et al. [17]. The identification of wine aroma compounds was done with the help of GC/MS using the Enhanced Chemstation software (Agilent Technologies, Santa Clara, CA, USA), and the peak retention times of the total compounds in wine were compared with those of standards as well as their mass spectra were matched with the Nist08 mass library (Wiley & Sons, Hoboken, NJ, USA). The quantification of aroma compounds was carried out by preparing and analyzing calibration curves for each compound using GC/MS at the same extraction and chromatographic parameters as for the wine samples. The identified aroma compounds included esters (ethyl butyrate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, diethyl succinate, ethyl acetate, *i*-butyl acetate, *i*-amyl acetate, hexyl acetate and 2-phenylethyl acetate), higher alcohols (amyl alcohol, phenylethyl alcohol, 1-hexanol and *cis*-3-hexenol), fatty acids (hexanoic acid, octanoic acid and decanoic acid) and terpenes (linalool and α-terpineol). All the analyses were conducted in triplicate and the results were expressed as the sum of determined individual aroma compounds, sorted by main aroma groups.

2.9. Data analysis

Overall differences in both HPU experiments were examined using multivariate analysis of variance (MANOVA) testing for the effects of process (input) variables, followed by univariate ANOVAs performed on each dependent variable, as listed in Table 1. The statistical data analysis was performed using Statistica v.10.0 software (StatSoft, Tulsa, USA). To predict total phenolics, total anthocyanins, total tannins, total free anthocyanins, total flavan-3-ols, chromatic characteristics, total esters, total higher alcohols, total fatty acids and total terpenes in both HPU experiments, artificial neural network (ANN) modeling was applied. The ANN trainings were performed with random separation of data into training, test and validation sets at different ratios. Multiple layer perceptron (MLP) networks trained by Broyden–Fletcher–Goldfarb–Shanno (BFGS) algorithm were selected to develop the prediction models. The performances of the developed models were statistically measured by the root mean squared error (RMSE) and correlation coefficient (R^2). Overall differences in bottled wines were examined using one-way ANOVA. In order to compare variable means and to examine which wines were different, Tukey's HSD test was used as a comparison test when samples were significantly different after ANOVA ($p < 0.05$). All multivariate analyses of experimental data and ANN calculations were carried out using Statistica v.10.0 software (StatSoft, Tulsa, USA).

3. Results and discussion

3.1. Influence of high power ultrasound (HPU) process parameters on the quality properties of red wine

The effects of different HPU process variables (inputs) on the quality properties of red wine (outputs) were studied (Table 1). The results obtained for the phenolic composition, chromatic characteristics and

Table 1
Experimental design used in the two High Power Ultrasound experiments.

	Independent variables (inputs)				
	Amplitude (%)	Frequency (kHz)	Bath temperature (°C)	Treatment duration (min)	Dependent variables (outputs)
Experiment 1	40	37	20	20	Total phenolics
Ultrasonic bath	60	80	40	50	Total anthocyanins
	100		60	65	Total tannins
				90	Total free anthocyanins*
	Probe diameter (mm)	Amplitude (%)	Treatment duration (min)		Total flavan-3-ols*
					Chromatic characteristics
Experiment 2	12.7	25	3		Total esters*
Ultrasonic probe	19.1	50	6		Total higher alcohols*
	25.4	75	9		Total fatty acids*
		100			Total terpenes*

* Sum of individual compounds: total free anthocyanins [delphinidin-3-*O*-glucoside, cyanidin-3-*O*-glucoside, petunidin-3-*O*-glucoside, peonidin-3-*O*-glucoside, malvidin-3-*O*-glucoside, peonidin-3-*O*-acetylglucoside, malvidin-3-*O*-acetylglucoside, peonidin-3-(6-*O*-*p*-coumaroyl)glucoside and malvidin-3-(6-*O*-*p*-coumaroyl) glucoside)], total flavan-3-ols [(+)-catechin, (−)-epicatechin, dimers B1, B2, B3, B4 and trimer C1], total esters (ethyl butyrate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, diethyl succinate, ethyl acetate, *i*-butyl acetate, *i*-amyl acetate, hexyl acetate and 2-phenylethyl acetate), total higher alcohols (amyl alcohol, phenylethyl alcohol, 1-hexanol and *cis*-3-hexenol), total fatty acids (hexanoic acid, octanoic acid and decanoic acid), total terpenes (linalool and α-terpineol).

aroma composition of the red wine treated by ultrasonic bath and ultrasonic probe in different conditions were listed (as a supplementary material) in Tables S1–S3, respectively. The summarized results of the analysis of variance are given in Table 2. The ANOVA revealed that all process variables (inputs) and their interactions showed statistically significant effect on analyzed variables (outputs) in both HPU experiments ($p < 0.0001$, $p < 0.001$, $p < 0.01$, Wilk's lambda).

3.1.1. Influence of HPU process parameters on phenolic composition

In ultrasonic bath experiment (Table S1 and Table 2-Experiment 1), ultrasound frequency was the most important variable influencing TP, TA, TT and total free anthocyanins, while the ultrasound amplitude had a greater effect on total flavan-3-ols (higher *F* values). Besides, the largest part of the variation due to an interaction between variables in the phenolic composition of the treated wine was due to frequency × bath temperature (X_2X_3) (Table 3). Generally, a higher value of frequency resulted in a lower content of TP, TA, TT and total free anthocyanins, independently from the other process variables. Similarly, a higher ultrasound frequency along with higher bath temperature also resulted in a lower content of phenolic compounds (Table S1). It was already reported that the ultrasound degradation of phenolic compounds was frequency-dependent and that a low-frequency ultrasound (20 kHz) did not affect the stability of phenolics [37]. Furthermore, it is known that phenol degradation is greater at higher frequencies [38].Specifically, the highest content of TP, TA and total free anthocyanins was observed at the conditions of 40% amplitude, 37 kHz frequency and 60 °C after 50–65 min of sonication, and was the closest to that of the untreated wine (Table S1). On the other hand, the highest content of total flavan-3-ols was achieved under 100% amplitude, 80 kHz frequency and 40 °C after 90 min of sonication (Table S1). Singleton and Draper [24] reported similar results at their work, in which the ultrasound treatments were used to accelerate aging of wine. A similar behavior was also observed by Zhang et al. [21] for the ultrasonic bath treatment of red wine Cabernet Sauvignon. These authors reported that the lowest content of TP was obtained at the highest process conditions (300 W, 100 kHz, 60 °C, 100 min), with the greatest influence of ultrasound frequency and exposure time. Zhang et al. [21] suggested that the high volatility of the wine (due to the ethanol content) promotes the formation of free radicals by cavitation phenomenon which in turn causes oxidative damage, primarily of phenolic compounds. For example, the aforementioned phenomenon could probably induce the degradation of anthocyanins resulting in the opening of the benzene ring and the formation of a chalcone. During HPU treatment, various physical (cavitation, mechanical effects and micro-mechanical shocks) and chemical effects (formation of free radicals and ions) occur simultaneously or separately, and affect the quality of the treated medium [39]. Also, it is important to highlight that the increase of ultrasound intensity, which is directly correlated to the ultrasound amplitude, results in an increase of sonochemical effects (more violent bubble collapse) [40,41]. Moreover, the transient cavitation bubbles are less numerous at low frequencies, which favor the physical effects instead of the chemical ones [42,43]. On the other hand, higher temperatures induce an increase of vapor pressure, which causes more solvent vapors to enter the bubble cavity and consequently, the sonication effects due to less violent bubble collapse are reduced [44]. The effect of HPU on wine is mainly attributed to acoustic cavitation that creates localized high temperatures and pressures, and consequently induces chemical reactions that naturally occur during wine aging [7,9,45,46]. Masuzawa et al. [47] confirmed an effect of polymerization of phenolic compounds in red wine promoted by ultrasound treatment at low sound pressures. However, some researches indicate a lower degree of chemical decomposition of phenolic compounds when ultrasound is used as extraction method at low frequencies of 20–40 kHz in comparison to conventional processing technologies [15].

Regarding the ultrasonic probe experiment (Table S2 and Table 2-Experiment 2), ANOVA showed that the ultrasound amplitude was the most important variable influencing TP and TT, while the probe diameter and the treatment duration had significantly higher effect on TA and total free anthocyanins (higher *F* values) respectively. Also, the treatment duration showed to be the most important variable affecting total flavan-3-ols. Besides, a decrease in the content of TP, TA, TT, total free anthocyanins and total flavan-3-ols was observed when the probe diameter was reduced. On the other hand, an increase of the ultrasound amplitude or the treatment duration resulted in lower concentrations of phenolic compounds. Moreover, among the interaction effects, the interaction between the probe diameter and the treatment duration (X_1X_3) was the one that affected in greater extend the phenolic composition of the wine (higher *F* values), with the exception of total flavan-3-ols. As can be seen in Table S2, the experiments performed with 25.4 mm probe and 25% amplitude during 6 min of sonication resulted in a higher content of TP and TA. In addition, HPU treatment with 19.1 mm probe also resulted in a higher content of total free anthocyanins at identical amplitude and treatment duration (Table S2). On the other hand, the highest content of total flavan-3-ols was obtained with the 19.1 mm probe, but at higher amplitude (75%) after only 3 min of sonication (Table S2). All together, these results demonstrated that there is no clear trend in the overall phenolic composition at different amplitudes and treatment durations of sonication, which could be due to enhanced polymerization/depolymerization, co-pigmentation, isomerization and decomposition reactions during the

Table 2
Analysis of variance (*F* values) for High Power Ultrasound experiments 1 and 2.

Experiment 1
Ultrasonic bath

Source	TP	TA	TT	Total free anthocyanins	Total flavan-3-ols	L*	a*	b*	C*	H*	Total esters	Total higher alcohols	Total fatty acids	Total terpenes
Amplitude (X_1)	11466.33[a]	6997.72[a]	8985.55[a]	4163.65[a]	**689.32**[a]	295.11[a]	164.24[a]	197.36[a]	177.74[a]	**60.29**[a]	30.34[a]	**831.52**[a]	4.58[d]	**14.99**[a]
Frequency (X_2)	**30376.99**[a]	**17444.76**[a]	**19404.84**[a]	**18894.49**[a]	29.31[a]	251.74[a]	250.29[a]	167.99[a]	238.86[a]	0.48	0.59	97.01[a]	5.80[d]	0.30
Bath temperature (X_3)	9477.43[a]	5334.66[a]	3929.93[a]	6334.38[a]	106.56[a]	**485.22**[a]	**352.06**[a]	**242.64**[a]	**337.31**[a]	9.00[b]	**603.55**[a]	530.76[a]	**139.84**[a]	3.00
Treatment duration (X_4)	170.95[a]	273.25[a]	220.38[a]	289.33[a]	12.11[a]	138.84[a]	114.82[a]	74.80[a]	94.78[a]	3.65[d]	179.46[a]	497.95[a]	21.42[a]	1.86
X_1X_2	369.91[a]	676.76[a]	492.10[a]	1137.94[a]	53.58[a]	37.64[a]	17.38[a]	24.75[a]	16.21[a]	**22.00**[a]	32.08[a]	46.65[a]	10.75[b]	3.99[d]
X_1X_3	1580.41[a]	972.85[a]	1724.44[a]	1686.47[a]	82.36[a]	**37.97**[a]	**61.44**[a]	**56.72**[a]	**66.79**[a]	8.99[a]	15.22[a]	71.22[a]	7.67[a]	12.74[a]
X_2X_3	**10412.22**[a]	**6952.71**[a]	**5790.01**[a]	**6946.66**[a]	**143.58**[a]	6.49[c]	6.28[c]	7.71[b]	3.17[d]	17.57[a]	41.86[a]	110.05[a]	**47.54**[a]	10.67[b]
X_1X_3	1132.48[a]	988.60[a]	1401.35[a]	1085.46[a]	18.08[a]	6.56[a]	5.69[b]	8.01[a]	5.14[b]	1.44	12.63[a]	136.07[a]	13.32[a]	4.02[c]
X_2X_4	2867.60[a]	1426.89[a]	2125.43[a]	1164.82[a]	9.62[a]	1.44	1.60	1.67	1.44	0.36	**67.03**[a]	94.27[a]	13.92[a]	11.44[a]
X_3X_4	684.81[a]	543.01[a]	861.30[a]	642.37[a]	12.45[a]	28.39[a]	24.76[a]	24.08[a]	27.77[a]	3.16[c]	27.47[a]	**383.58**[a]	27.86[a]	5.08[b]
$X_1X_2X_3$	1917.46[a]	1177.55[a]	1045.64[a]	1640.55[a]	70.54[a]	24.17[a]	31.81[a]	18.82[a]	26.33[a]	12.34[a]	19.61[a]	305.29[a]	6.20[b]	**13.82**[a]
$X_1X_2X_4$	596.44[a]	415.44[a]	270.40[a]	211.76[a]	10.40[a]	3.94[c]	3.61[c]	3.10[c]	2.56[d]	0.43	7.64[a]	313.40[a]	9.99[a]	7.42[a]
$X_1X_3X_4$	1112.54[a]	1059.93[a]	1096.39[a]	878.14[a]	17.61[a]	1.84	1.66	1.97[d]	1.42	0.95	6.44[a]	201.85[a]	6.92[a]	12.23[a]
$X_2X_3X_4$	1070.77[a]	712.54[a]	1168.78[a]	448.43[a]	5.72[b]	3.16[c]	2.43[d]	2.27[d]	1.51	0.28	4.00[c]	222.80[a]	11.20[a]	1.44
$X_1X_2X_3X_4$	841.10[a]	519.76[a]	599.79[a]	506.28[a]	6.94[a]	1.40	1.31	1.26	1.04	0.21	31.70[a]	225.02[a]	6.84[a]	8.43[a]

Experiment 2
Ultrasonic probe

Source	TP	TA	TT	Total free anthocyanins	Total flavan-3-ols	L*	a*	b*	C*	H*	Total esters	Total higher alcohols	Total fatty acids	Total terpenes
Probe diameter (X_1)	1272.89[a]	**3150.84**[a]	51.19[a]	349.09[a]	3.41[d]	**1228.68**[a]	**403.61**[a]	**697.85**[a]	**432.09**[a]	**816.01**[a]	**15.96**[a]	**56.44**[a]	10.54[b]	**12.67**[b]
Amplitude (X_2)	**1409.70**[a]	215.05[a]	**98.52**[a]	46.45[a]	8.01[b]	8.58[b]	4.20[d]	11.78[a]	11.42[a]	8.50[b]	5.29[c]	49.99[a]	0.56	0.14
Treatment duration (X_3)	325.68[a]	561.04[a]	75.54[a]	**472.84**[a]	**330.18**[a]	255.85[a]	293.39[a]	170.03[a]	79.55[a]	91.92[a]	6.04[c]	32.97[a]	**11.42**[b]	1.98
X_1X_2	26.10[a]	11.05[a]	1.82	21.75[a]	12.14[a]	9.10[a]	1.65	4.63[c]	15.86[a]	14.71[a]	6.59[b]	15.94[a]	1.10	1.89
X_1X_3	**167.95**[a]	**276.18**[a]	**21.43**[a]	**191.18**[a]	13.78[a]	**314.89**[a]	**219.70**[a]	**255.94**[a]	**135.10**[a]	**314.52**[a]	4.93[c]	6.36[b]	2.89[d]	**9.22**[a]
X_2X_3	35.97[a]	5.14[b]	0.43	57.29[a]	**58.09**[a]	12.13[a]	4.39[c]	4.75[c]	15.58[a]	8.05[a]	**9.19**[a]	**31.67**[a]	**3.64**[c]	3.21[d]
$X_1X_2X_3$	35.20[a]	11.46[a]	1.17	45.58[a]	5.45[a]	5.47[a]	1.49	2.65[d]	11.03[a]	7.50[a]	3.47[c]	22.52[a]	2.75[c]	6.10[a]

[a]$p < 0.0001$, [b]$p < 0.001$, [c]$p < 0.01$, [d]$p < 0.05$. The most significant effect (higher *F* values) of process (input) variables and their interactions on each output variable are shown in bold. Error terms for experiments 1 and 2 are df = 143 and df = 71. Abbreviations: TP, total phenolics; TA, total anthocyanins; TT, total tannins.

Table 3
Performance parameters of Artificial Neural Network (ANN) models of High Power Ultrasound experiments 1 and 2.

	Experiment 1 Ultrasonic bath					Experiment 2 Ultrasonic probe				
Network number	**1**	2	3	4	5	**1**	2	3	4	5
Network name[a]	**MLP 4/10/14**[b]	MLP 4/9/14	MLP 4/9/14	MLP 4/8/14	MLP 4/10/14	**MLP 3/8/14**[b]	MLP 3/9/14	MLP 3/6/14	MLP 3/10/14	MLP 3/6/14
Training performance	**0.8402**	0.8148	0.8010	0.7981	0.8042	**0.7878**	0.7715	0.7680	0.7662	0.7183
Training error	**0.0907**	0.1062	0.1147	0.1133	0.1271	**0.1354**	0.1439	0.1391	0.1578	0.1596
Test performance	**0.7919**	0.8137	0.7693	0.7800	0.7902	**0.7607**	0.7302	0.7367	0.7475	0.7330
Test error	**0.1282**	0.1201	0.1402	0.1298	0.1790	**0.2346**	0.2502	0.2410	0.2449	0.2307
Validation performance	**0.7921**	0.7452	0.7997	0.7916	0.7945	**0.7771**	0.7601	0.7343	0.6988	0.7482
Validation error	**0.1243**	0.1525	0.1310	0.1404	0.1782	**0.1126**	0.1417	0.1586	0.1793	0.1511
Hidden activation	**Tanh**	Logistic	Logistic	Tanh	Logistic	**Logistic**	Tanh	Tanh	Tanh	Tanh
Output activation	**Logistic**	Logistic	Tanh	Logistic	Logistic	**Identity**	Identity	Tanh	Exponential	Logistic

Abbreviations: MLP, multilayer perceptron.
[a] Number of input variables/number of neurons in hidden layer/number of output variables.
[b] The most suitable ANN is marked bold.

ultrasound treatment. So, changes in the phenolic composition are probably related to the already mentioned cavitation phenomenon, which triggers oxidation reactions in wines (phenols are oxidized to quinones, while oxygen is reduced to hydrogen peroxide). Cavitation also produced a variety of chemical reactions by the free radicals generated and that is considered the main cause of the degradation of phenolic compounds. In the same way that in our work, Tiwari et al. [26] observed that the anthocyanins content of red grape juice decreased during prolonged sonication with a 19 mm probe at higher amplitudes. On the other hand, Ferraretto and Celotti [20] found that free anthocyanins in red wines were not modified by ultrasound treatment (200 W output, 20 kHz, 13 mm probe, 30–90%, 1–5 min), whereas the higher process conditions (higher amplitudes and longer exposures) resulted in an increase of flavan-3-ols, namely the monomeric catechins. The possible explanation for the increment of catechin content is that the ultrasound treatment promotes the depolymerization and recombination reactions of phenolic compounds [20]. In addition, the results in the current study also suggest that application of ultrasound with an appropriate process conditions might accelerate the wine aging reactions.

Similarly to the results of the present work, many studies have confirmed that the HPU treatment using an ultrasonic probe has a higher and localized intensity of ultrasound in comparison to the treatment using an ultrasonic bath, which is characterized by a lower ultrasound or cavitation intensity and an uneven distribution of ultrasound [48]. Generally, higher amplitudes can lead to higher ultrasound intensities, which can promote some undesirable effects (compound degradation). But also, higher amplitudes can cause erosion of the ultrasonic probe, leading to agitation instead of cavitation and a weak distribution of ultrasound through the treated medium [44]. According to the results obtained in both HPU experiments, it is necessary to avoid extreme process conditions (i.e., frequency, amplitude, treatment duration) in order to maintain the phenolic composition of wine.

3.1.2. Influence of HPU process parameters on chromatic characteristics

The effect of the different independent variables of the HPU using an ultrasonic bath on the chromatic characteristics would be ranked in the following order: bath temperature > amplitude > frequency > treatment duration (higher F values) according to the results of the ANOVA analysis (Table 2. Experiment 1). Among interaction effects, amplitude × bath temperature (X_1X_3) was the most significant variable in affecting L*, a*, b* and C* values (Table 2. Experiment 1). The sonicated samples presented slightly different values of the CIELab parameters, when compared with the unsonicated wine (Table S1). Moreover, the values of chromatic characteristics (L*, a*, b* and C*) varied according to the applied ultrasound conditions, where higher bath temperatures, ultrasound amplitudes as well as treatment durations resulted in slightly lower values of these parameters (Table S1). Contrary, an increase of ultrasound frequency resulted in slightly higher values of L*, a*, b* and C*, while H* in general remained constant. For example, the lowest values of chromatic characteristics were obtained at 100% amplitude and 60 °C after 90 min of sonication (Table S1). In order to determine the total color difference of the wine samples against the control, the parameter ΔE* was calculated (Table S3). For the assessment, it was considered that when the value of ΔE* between two samples is in a range from 2 to 10, the difference in color is clearly perceptible, while in the case of values higher than 10 the colors are more opposite than similar [49]. Also, according to Ramirez-Navas and Rodriguez de Stouvenel [50], all the color differences with ΔE* values higher than 6 are considerable. The values of the total color difference (ΔE*) between treated and control samples were mostly in the range of 2–6, which means there were perceptible differences between these wine colors (Table S3). Furthermore, only the values of ΔE* between the samples sonicated at 100% amplitude and 60 °C during 90 min, as well as at 37 and 80 kHz frequency and 20 °C during 20 min compared to the control sample were higher than 6, being clearly perceptible by the human eye. It is important to consider that the wine color is mainly influenced by the presence of various anthocyanins, the applied wine-making technique and the numerous reactions that take place during natural aging [51]. For anthocyanins is well-known that they are highly unstable and very susceptible to degradation. It is interesting to highlight that, comparing the obtained results, the chromatic characteristics and the anthocyanins were both influenced by the same investigated variables during HPU, namely ultrasound amplitude, frequency and bath temperature. Probably, the localized high temperatures and pressures generated from the acoustic cavitation in the ultrasound treatment initiate some chemical reactions related to the color changes in red wine [9]. Additionally, these extreme physical conditions can also lead to accelerated isomerization of color pigments [52].

When the wine is treated by an ultrasonic probe (Table 2- Experiment 2), it was shown that the probe diameter as well as the interaction probe diameter × treatment duration (X_1X_3) on wine chromatic characteristics were the most significant (higher F values) compared to the rest of the experimental variables and their interactions. As can be seen from Table S2, the sonicated samples showed slightly different values of the CIELab parameters when compared to the unsonicated wine. Particularly, the lowest values of chromatic characteristics were obtained with 19.1 mm probe at all amplitudes and treatment durations (Table S2). Moreover, as we can observe in Table S3, the total color differences ΔE* between most of the sonicated samples and the control sample were in the range of 0.5–3, all being slightly perceptible. The samples treated with a smaller probe diameter (12.7 mm) for 3 min showed the

values of ΔE* around 4–5, which means there were perceptible differences between these samples and control. Nevertheless, there were no considerable color differences between sonicated samples and control (untreated) sample, since obtained ΔE* values were not higher than 6. As previously suggested, these changes in the chromatic characteristics can be related to the changes in the content of anthocyanins, which are known to be responsible for the red color of the wine and to react with catechins during natural aging of wine [7].Then, higher ultrasound powers may cause the breakdown of the existing colored polymeric pigments in the red wine and consequently lead to a decrease in color characteristics [9]. On the contrary, a weaker ultrasound irradiation may initiate and accelerate chemical reactions involving anthocyanins due to ultrasound-generated free radicals and this way positively modify the wine color [9].

3.1.3. Influence of HPU process parameters on aroma composition

Interestingly, from the statistical analysis of aroma composition of ultrasonic bath treated wine (Table 2-Experiment 1), it can be seen that the bath temperature was the most important variable influencing total esters and total fatty acids, whereas ultrasound amplitude had the greatest effect on total higher alcohols and total terpenes (higher *F* values). Additionally, among the interaction effects, the one between bath temperature and treatment duration (X_3X_4), and the one between bath temperature or treatment duration and frequency (X_2X_3, X_2X_4) showed to play the most significant role in affecting the aroma composition of treated wine (higher *F* values). Besides, the lowest content of total esters and total higher alcohols was observed at the highest bath temperatures (40–60 °C) and treatment durations (65–90 min) (Table S1), probably due to the heating effect of ultrasound energy which could accelerate the evaporation of aroma compounds. Furthermore, an increase in ultrasound amplitude resulted also in a lower content of total esters, total higher alcohols, total fatty acids and total terpenes in sonicated wines, when compared to the untreated wine.

As can be seen in Table S1, the content of total esters and total higher alcohols decreased in the range of 60–100% amplitude and a bath temperature of 40–60 °C at 37 kHz frequency after 90 min of HPU. However, the results of total fatty acids and especially of total terpenes showed no particular trend at all combinations of applied process parameters (Table S1). In an alcoholic beverage such as wine, ultrasound can cause an acceleration of oxidation, polymerization and condensation of alcohols, aldehydes, esters and others compounds [53]. Then, the changes observed in the aroma composition of ultrasound treated wine are probably due to oxidation reactions (occurring as a result of various interactions with free radicals) generated during the HPU [23,54]. Singleton and Draper [24] found that ultrasound bath treatment decreases volatile esters in wines at higher process conditions, relating this to a possible degassing effect of ultrasound. Moreover, Chemat et al. [55] reported a relation between the increase of ultrasound power (higher amplitudes and temperatures) and the degradation of wine phenolics, which could prevent the oxidative degradation of aroma compounds. Due to the complexity of the wine aroma, some wine components were divided into a group of esters, higher alcohols, fatty acids and terpenes. It is known, that the majority of the aroma compounds in wine are fermentation compounds, primarily higher alcohols and esters [56]. Also, the volatile fatty acids and terpenes can contribute significantly to the overall flavor and aroma of wine [57].

Additionally, from Table 2 it can be seen that among the three process variables in ultrasonic probe experiment, the probe diameter was the most significant variable that influenced the content of total esters, total higher alcohols and total terpenes (higher *F* values). Secondly, the treatment duration showed to be the most important variable influencing total fatty acids (higher *F* value). Interestingly, the content of total esters, total higher alcohols and total fatty acids showed first an increase by increasing the probe diameter, achieving highest values using 19.1 mm probe, while afterwards slightly decreased (Table S2). On the other hand, an increase in the probe diameter resulted in a lower content of total terpenes. As can be seen from Table S2, treatments performed with a 12.7 mm probe and 25% amplitude during 6 min provoked lower content of total esters in the wine, while HPU conditions of 100% amplitude and 3 min with the same probe diameter resulted in lower content of total higher alcohols compared to the unsonicated wine. Furthermore, lower content of total fatty acids was observed at 75% amplitude after 9 min of HPU treatment with a 12.7 mm probe. Contrary, the lowest content of total terpenes was achieved at conditions of 25% amplitude after 9 min of HPU treatment with a 25.4 mm probe (Table S2). From these results, it could be suggested that, in general, a smaller probe diameter along with higher amplitudes or longer ultrasound exposures caused a major degradation of the compounds responsible for the wine aroma. These changes could be related to the various mechanisms that can act simultaneously or separately when applying ultrasound, such as the thermal effects of the implosion of cavitation bubbles and consequently the formation of free radicals, mechanical effects of the microstreaming, implosion and shock waves [58,59]. The extreme physical conditions (high temperatures and pressures) that occur inside the bubbles during cavitation collapse at the micro-level [60] are responsible for the observed degradation of aroma compounds. Furthermore, the sonolysis of water as a consequence of cavitation, induces the formation of hydroxyl radicals that can be involved in the degradation, esterification and ring opening and formation of chalcones [61]. Also, the formation of hydroxyl ions (OH^-) increases linearly with the increase of ultrasound amplitude [62].

Finally, the obtained results demonstrated that the choice of proper ultrasound conditions in both HPU experiments is crucial, in order to avoid the occurrence of excessive oxidation and degradation of phenolic compounds and the compounds responsible for wine aroma, and to maintain the overall wine quality and color.

3.2. ANN modeling of HPU processes

In the present study, ANN models were developed in order to test whether it is possible to predict the content of TP, TA, TT, total free anthocyanins, total flavan-3-ols, total esters, total higher alcohols, total fatty acids, total terpenes, and chromatic characteristics (L*, a*, b*, C* and H*) based on HPU process parameters of experiment 1 (ultrasonic bath) and experiment 2 (ultrasonic probe). The data generated from the experimental designs of HPU experiments (Tables S1 and S2) were used to figure out the optimal ANNs. Firstly, the total experimental set of each HPU experiment was randomly divided into seven sets for the training, validation and testing of the neural networks. Based on the results of the training process, the separation of data into training, test and validation set as 60:20:20 ratios showed to be the most suitable for both HPU experiments. Among the various structures, models of good performance were developed for both experiments 1 and 2 and their performance parameters are presented in Table 3.

Regarding HPU experiment 1 (ultrasonic bath), nearly all of the selected networks had higher linear correlation coefficient (R^2) for training, test and validation with lower Root mean square error (RMSE) values (Table 3). As can be seen, there are three different ANN regarding the number of neurons in hidden layer (8, 9 and 10) since all of them have 4 neurons in input layer and 14 neurons in output layer. Moreover, the hidden activation and the output activation of the ANNs with the same numbers of neurons in hidden layer were different. When observing the correlation coefficient for training, for all the five networks, the highest values was observed for ANN 1 (R^2 = 0.8402) with the lowest training error (RMSE = 0.0907). The ANN 2 had the highest value for test performance (R^2 = 0.8137) with the lowest training error (RMSE = 0.1201). For the validation performance ANN 3 showed the highest performance (R^2 = 0.7997) which was slightly higher than ANN 1 (R^2 = 0.7921) but in term of validation error ANN 3 showed higher values (RMSE = 0.1310) than ANN 1 (RMSE = 0.1243). Based

on these results, ANN 1 was selected as the optimal one for HPU experiment 1 (Table 3).

The results of HPU experiment 2 (ultrasonic probe) demonstrated that almost all of the developed networks had lower linear correlation coefficient (R^2) for training, test and validation with higher RMSE values (Table 3). As indicated in the table, there are four different ANN considering the number of neurons in hidden layer (6, 8, 9 and 10) since all of them have 3 neurons in input layer and 14 neurons in output layer. The hidden activation and the output activation of the ANNs with the same number of neurons in hidden layer were different. Furthermore, the highest value of correlation coefficient for training was observed for ANN 1 ($R^2 = 0.7878$), which also had the lowest training error (RMSE = 0.1354). Also for training performance, ANN 1 had the highest training performance ($R^2 = 0.7607$) as well as the highest validation performance ($R^2 = 0.7771$) with the lowest training and validation errors (RMSE = 0.2346 and RMSE = 0.1126, respectively). Based on these results, ANN 1 was selected as the optimal one for HPU experiment 2 (Table 3).

The performance of the final selected ANN models (4/10/14 and 3/8/14) to predict each of the output variables (TP, TA, TT, total free anthocyanins, total flavan-3-ols, chromatic characteristics, total esters, total higher alcohols, total fatty acids and total terpenes) in experiments 1 and 2 is presented in Table 4. Also, in order to get a clearer picture for each of the tested parameter in terms of ANN predictions, the results of both HPU experiments are presented as correlation of experimental and model predicted data in Figs. 1 and 2.

Based on the results presented in Table 4, the best correlations between experimental data and the ANN predictions in experiment 1 (ultrasonic bath) for training, test and validation were obtained for chromatic characteristic L* ($R^2 = 0.9725$, $R^2 = 0.9333$, $R^2 = 0.9852$), followed by C* ($R^2 = 0.9702$, $R^2 = 0.9143$, $R^2 = 0.9870$), and a* and b* which had negligible differences in values. Such good correlations are visible in Fig. 1f–i. Moreover, it is observed that the correlation coefficients for validation between the measured and predicted data for TP, TA, TT, total free anthocyanins, H* and total esters were also satisfactory ($0.7773 \leq R^2 \leq 0.8565$) (Table 4). Meanwhile, the least acceptable results of the ANN performance belonged to total flavan-3-ols, total higher alcohols, total fatty acids and total terpenes (Fig. 1e, l, m and n).

Further, regarding HPU experiment 2 (ultrasonic probe), the best correlations between experimental data and the ANN predictions for training, test and validation were again obtained for chromatic characteristic L* with R^2 values of 0.9564, 0.9663 and 0.9882 for training, test and validation (Table 4). The second highest value for validation was observed for chromatic characteristic a*, followed by b* and C* values. Moreover, the values of correlation coefficients for validation for TP ($R^2 = 0.9263$) and TA ($R^2 = 0.9580$) were much higher than in the first experiment. Also, the correlation coefficients for validation between the measured and predicted data for TT, total flavan-3-ols, total free anthocyanins and H* value were satisfactory ($0.8526 \leq R^2 \leq 0.8770$) (Table 4). On the other hand, the least acceptable results (the highest data dispersion) of the ANN performance belonged to total esters, total fatty acids and total terpenes with total higher alcohols at the last place (Fig. 2k, m, n and l).

In general, a good-fitting model should have the R^2 values above 0.90, while the values between 0.70 and 0.90 show that the models can be considered moderately precise. On the other hand, the R^2 values below 0.70 imply that the model can be used for qualitative differentiation without the ability to be used in quantitative prediction [63,64]. As a result, for the ultrasonic bath experiment, the selected ANN 1 model showed the best prediction for monitoring chromatic characteristics (except H*) and also very good prediction for certain parameters such as TP, TA, TT, total free anthocyanins and total esters, while total higher alcohols, total fatty acids and total terpenes did not give satisfactory predictions. For the ultrasonic probe experiment, the ANN 1 showed that chromatic characteristics, TP and TA could be easily predicted but, in the same way than in the first experiment, total higher alcohols, total fatty acids, and total terpenes with addition of total esters had the least acceptable results.

3.3. Effect of HPU treatment along with SO_2 and GSH additions on the phenolic, chromatic and aroma composition of red wine during storage

The effect of HPU treatment along with antioxidants addition (SO_2 and GSH) on the phenolic, chromatic and aroma composition of red wine during 6 months of storage in the bottles is shown in Table 5. Although the analyzed parameters were influenced by the content and type of antioxidants used, a general trend for all wines can be observed. As it can be seen, there is a decreasing trend in the content of TP, TA, total free anthocyanins and total flavan-3-ols with time. After 3 and 6 months of aging, significant differences ($p < 0.05$) were observed among the different treatments indicating that HPU treatment affected both total and individual phenolic compounds, except TT content which remained constant during observed period of storage. Specifically, after 6 months of storage the sonicated samples showed significantly lower content of phenolic compounds when compared with the untreated wine. It is already known that the content of phenolic compounds decrease during storage due to their potential chemical oxidation,

Table 4
Performance of the final selected Artificial Neural Network (ANN) model to predict each of the dependent variables (outputs) of High Power Ultrasound experiments 1 and 2.

	Experiment 1 Ultrasonic bath Correlation coefficient (R^2)			Experiment 2 Ultrasonic probe Correlation coefficient (R^2)		
Output variables	Training	Testing	Validation	Training	Testing	Validation
Total phenolics	0.8860	0.8496	0.8565	0.9123	0.8093	0.9263
Total anthocyanins	0.8765	0.8279	0.8525	0.9375	0.9035	0.9580
Total tannins	0.8897	0.8773	0.8387	0.9262	0.8603	0.8576
Total free anthocyanins	0.8608	0.8754	0.8295	0.5959	0.7364	0.8770
Total flavan-3-ols	0.8905	0.8193	0.6899	0.8180	0.7464	0.8526
L*	0.9725	0.9333	0.9852	0.9564	0.9663	0.9882
a*	0.9608	0.9093	0.9879	0.9094	0.9529	0.9881
b*	0.9656	0.9069	0.9885	0.9353	0.9607	0.9554
C*	0.9702	0.9143	0.9870	0.9258	0.7975	0.9496
H*	0.8755	0.8215	0.7773	0.9379	0.9717	0.8553
Total esters	0.8482	0.8519	0.8090	0.5359	0.3888	0.3234
Total higher alcohols	0.6297	0.5395	0.4765	0.5792	0.3480	0.0188
Total fatty acids	0.7559	0.6816	0.6253	0.6205	0.6550	0.6579
Total terpenes	0.3807	0.2785	0.3854	0.4394	0.5513	0.6716

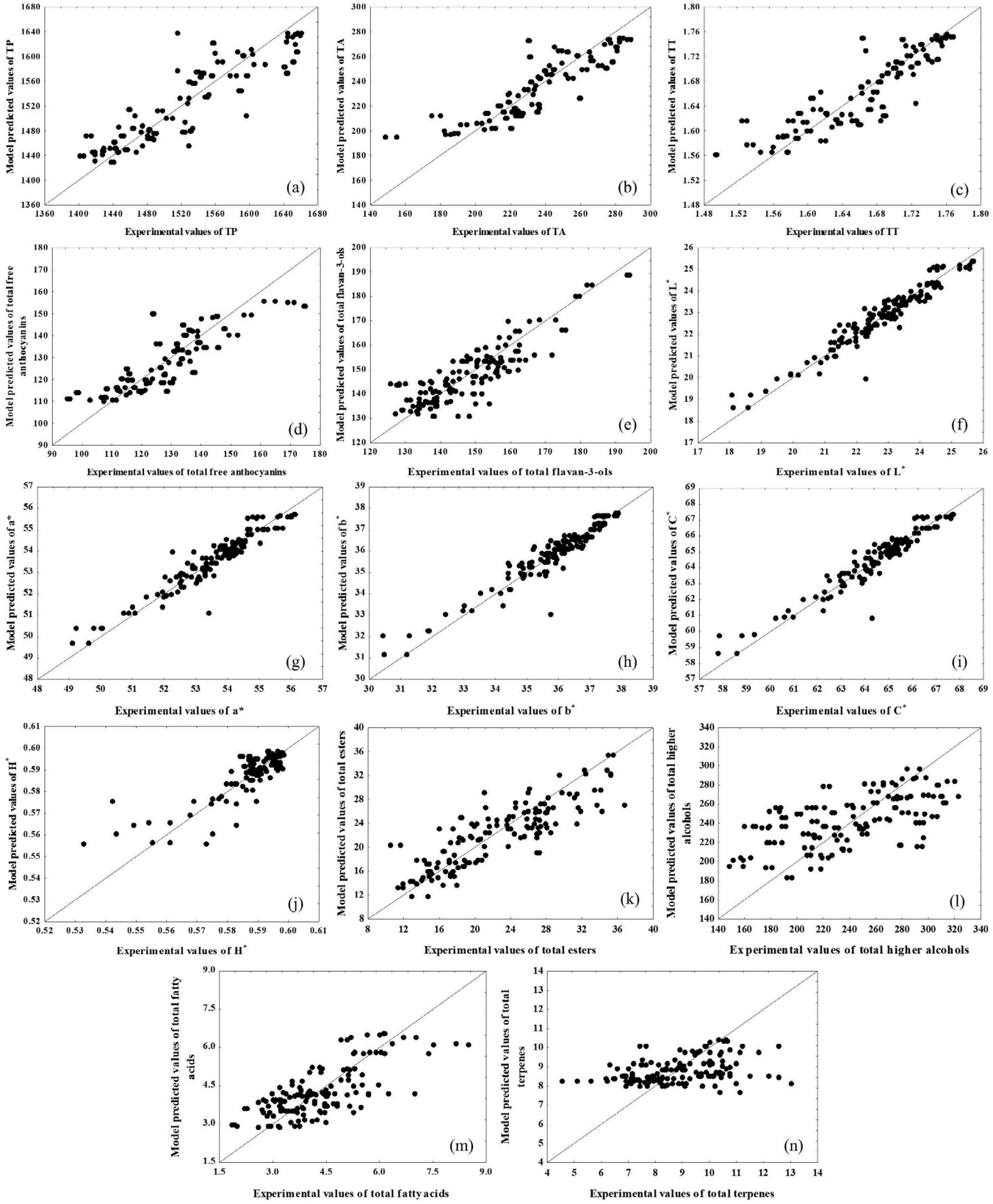

Fig. 1. Comparison between experimental data and Artificial Neural Network models predicted data for High Power Ultrasound experiment 1 (ultrasonic bath) for (a) total phenolics – TP, (b) total anthocyanins – TA, (c) total tannins – (TT), (d) total free anthocyanins, (e) total flavan-3-ols, (f) L*, (g) a*, (h) b*, (i) C*, (j) H*, (k) total esters, (l) total higher alcohols, (m) total fatty acids, and (n) total terpenes.

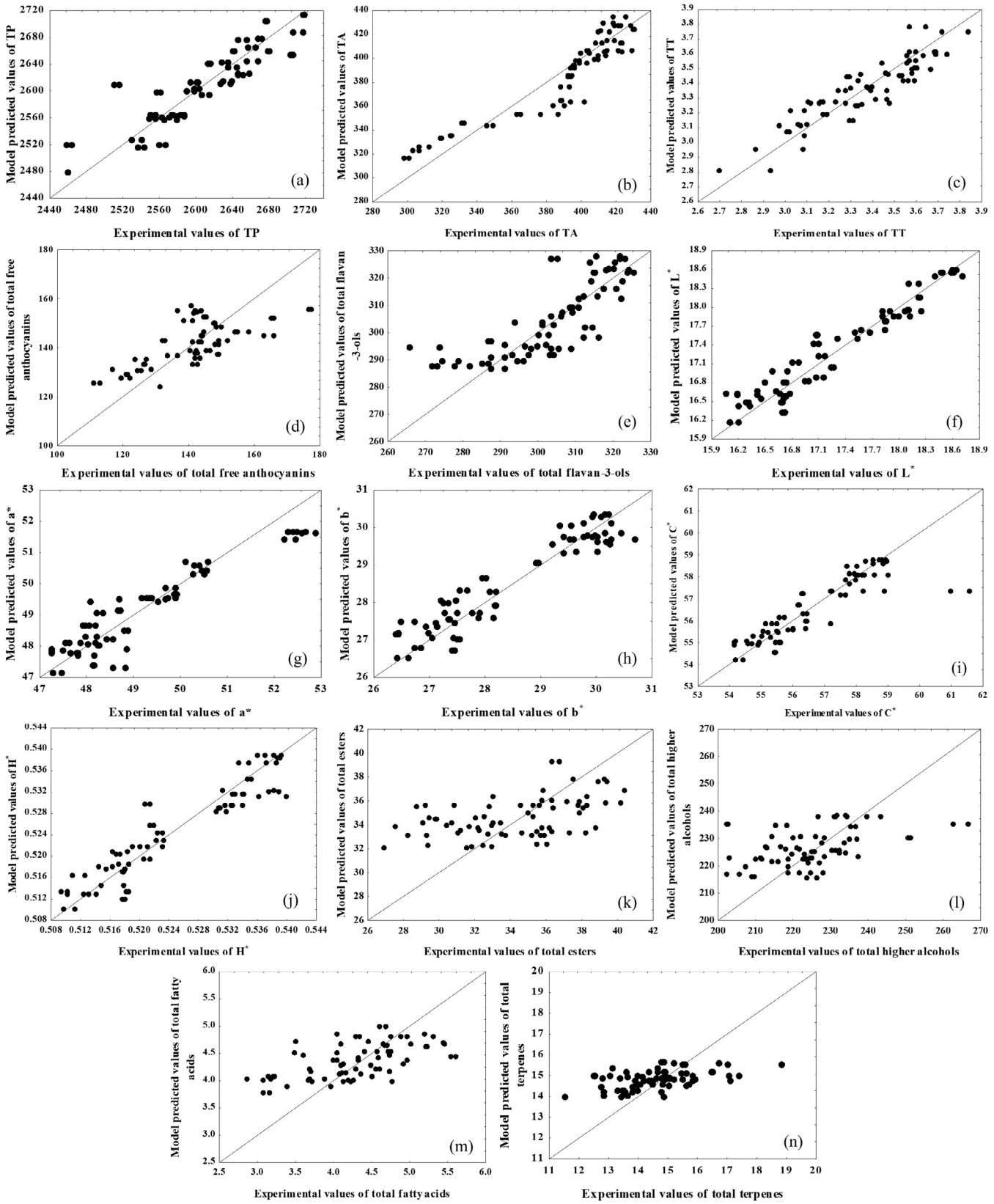

Fig. 2. Comparison between experimental data and Artificial Neural Network models predicted data for High Power Ultrasound experiment 2 (ultrasonic probe) for (a) total phenolics – TP, (b) total anthocyanins – TA, (c) total tannins – (TT), (d) total free anthocyanins, (e) total flavan-3-ols, (f) L*, (g) a*, (h) b*, (i) C*, (j) H*, (k) total esters, (l) total higher alcohols, (m) total fatty acids, and (n) total terpenes.

Table 5
Effect of High Power Ultrasound treatment (ultrasonic probe) along with sulfur dioxide (SO_2) and glutathione (GSH) additions on the phenolic, chromatic and aroma composition of red wine during 6 months of bottle aging.

Months		Wine			
		Control (untreated)	Standard SO_2	Low SO_2 and GSH	Low SO_2
TP (mg/L)	0	2960.42 ± 5.30^{a}	2940.00 ± 8.25^{a}	2790.49 ± 1.87^{b}	2688.75 ± 8.84^{c}
	3	2891.82 ± 9.00^{a}	2841.36 ± 7.07^{b}	2732.73 ± 12.86^{c}	2634.09 ± 14.78^{d}
	6	2820.00 ± 7.71^{a}	2719.55 ± 3.21^{b}	2662.27 ± 9.64^{c}	2544.55 ± 10.29^{d}
TA (mg/L)	0	261.80 ± 4.70^{a}	253.18 ± 2.04^{ab}	243.21 ± 1.55^{b}	217.31 ± 1.42^{c}
	3	259.09 ± 1.11^{a}	245.74 ± 1.61^{b}	237.04 ± 0.37^{b}	184.98 ± 4.21^{c}
	6	243.56 ± 1.20^{a}	233.78 ± 3.02^{b}	186.34 ± 0.11^{c}	153.74 ± 2.97^{d}
TT (g/L)	0	4.49 ± 0.04^{a}	4.46 ± 0.04^{a}	4.40 ± 0.03^{a}	4.33 ± 0.09^{a}
	3	4.45 ± 0.13^{a}	4.35 ± 0.06^{a}	4.24 ± 0.01^{a}	4.25 ± 0.06^{a}
	6	3.75 ± 0.02^{a}	3.69 ± 0.12^{a}	3.65 ± 0.09^{a}	3.57 ± 0.04^{a}
Total free anthocyanins (mg/L)	0	156.55 ± 1.59^{a}	151.89 ± 0.95^{a}	139.28 ± 0.03^{b}	125.14 ± 2.87^{c}
	3	140.88 ± 2.72^{a}	129.71 ± 0.44^{b}	118.63 ± 0.82^{c}	103.93 ± 0.65^{d}
	6	132.61 ± 1.86^{a}	112.83 ± 1.24^{b}	102.64 ± 0.73^{c}	92.53 ± 0.93^{d}
Total flavan-3-ols (mg/L)	0	441.46 ± 3.25^{a}	427.75 ± 0.91^{b}	408.00 ± 3.60^{c}	400.08 ± 0.56^{c}
	3	439.19 ± 1.17^{a}	417.88 ± 2.86^{b}	393.44 ± 2.08^{c}	379.20 ± 1.66^{d}
	6	427.83 ± 3.05^{a}	404.97 ± 0.31^{b}	378.34 ± 3.78^{c}	362.66 ± 3.23^{d}
L*	0	22.28 ± 0.03^{a}	20.93 ± 0.03^{b}	18.84 ± 0.05^{c}	17.30 ± 0.22^{d}
	3	25.56 ± 0.13^{a}	23.81 ± 0.07^{b}	20.99 ± 0.07^{c}	17.45 ± 0.06^{d}
	6	26.90 ± 0.03^{a}	25.48 ± 0.03^{b}	23.95 ± 0.00^{c}	20.39 ± 0.14^{d}
a*	0	52.13 ± 0.06^{a}	51.09 ± 0.07^{b}	46.23 ± 0.08^{c}	47.58 ± 0.30^{d}
	3	54.68 ± 0.16^{a}	53.59 ± 0.10^{b}	50.91 ± 0.10^{c}	48.27 ± 0.07^{d}
	6	54.12 ± 0.03^{a}	53.35 ± 0.06^{a}	53.88 ± 0.03^{a}	50.43 ± 0.39^{b}
b*	0	36.55 ± 0.06^{a}	34.86 ± 0.07^{b}	31.35 ± 0.09^{c}	29.44 ± 0.37^{d}
	3	41.03 ± 0.18^{a}	39.02 ± 0.12^{b}	34.44 ± 0.16^{c}	29.74 ± 0.10^{d}
	6	40.67 ± 0.03^{a}	39.53 ± 0.11^{b}	39.38 ± 0.02^{b}	30.53 ± 0.18^{c}
C*	0	63.67 ± 0.08^{a}	61.85 ± 0.09^{b}	55.86 ± 0.12^{c}	55.95 ± 0.45^{c}
	3	68.36 ± 0.23^{a}	66.29 ± 0.15^{b}	61.47 ± 0.17^{c}	56.69 ± 0.11^{d}
	6	67.70 ± 0.04^{a}	66.40 ± 0.11^{b}	66.73 ± 0.04^{b}	57.46 ± 0.36^{c}
H*	0	0.61 ± 0.00^{a}	0.60 ± 0.00^{a}	0.54 ± 0.00^{b}	0.55 ± 0.00^{b}
	3	0.64 ± 0.00^{a}	0.63 ± 0.00^{a}	0.59 ± 0.00^{b}	0.55 ± 0.00^{c}
	6	0.64 ± 0.00^{a}	0.64 ± 0.00^{a}	0.63 ± 0.00^{a}	0.54 ± 0.00^{b}
ΔE*	0	–	2.41 ± 0.18^{b}	8.59 ± 0.22^{a}	9.80 ± 0.43^{a}
	3	–	2.87 ± 0.43^{c}	8.86 ± 0.14^{b}	15.30 ± 0.13^{a}
	6	–	1.98 ± 0.05^{c}	3.23 ± 0.03^{b}	12.61 ± 0.06^{a}
Total esters (mg/L)	0	61.31 ± 8.19^{a}	56.39 ± 7.34^{a}	51.42 ± 1.35^{a}	44.31 ± 0.21^{a}
	3	45.31 ± 1.22^{a}	42.96 ± 0.55^{ab}	41.78 ± 0.16^{b}	36.88 ± 0.27^{c}
	6	34.81 ± 2.53^{a}	31.73 ± 1.80^{a}	31.61 ± 1.11^{a}	30.08 ± 0.55^{a}
Total higher alcohols (mg/L)	0	95.57 ± 1.82^{a}	93.61 ± 2.07^{ab}	88.67 ± 0.85^{ab}	88.20 ± 1.89^{b}
	3	103.96 ± 1.30^{a}	103.69 ± 5.15^{a}	98.83 ± 1.21^{a}	93.59 ± 1.50^{a}
	6	105.50 ± 6.30^{a}	104.10 ± 0.48^{a}	102.63 ± 1.24^{a}	100.38 ± 1.40^{a}
Total fatty acids (mg/L)	0	2.57 ± 0.05^{a}	2.45 ± 0.01^{a}	2.25 ± 0.01^{b}	2.17 ± 0.02^{b}
	3	2.36 ± 0.04^{a}	2.03 ± 0.03^{b}	1.93 ± 0.04^{b}	1.77 ± 0.03^{c}
	6	1.92 ± 0.02^{a}	1.59 ± 0.00^{b}	1.50 ± 0.01^{c}	1.40 ± 0.03^{d}
Total terpenes (μg/L)	0	17.66 ± 0.26^{a}	16.02 ± 0.57^{b}	14.81 ± 0.06^{bc}	13.87 ± 0.03^{c}
	3	13.30 ± 0.12^{a}	13.23 ± 0.35^{a}	12.45 ± 0.76^{a}	11.01 ± 0.91^{a}
	6	9.89 ± 1.26^{a}	8.40 ± 0.17^{a}	7.93 ± 0.18^{a}	7.38 ± 0.03^{a}

Data presented as average value of six analytical repetitions with standard deviation. ANOVA to compare data; different letters indicate statistical differences between wine samples of all treatments at the same time (Tukey's test, $p < 0.05$). Abbreviations: TP, total phenolics; TA, total anthocyanins; TT, total tannins.

polymerization, condensation and/or precipitation [65]. This tendency was significantly enhanced when ultrasound is applied to wines probably due to specific chemical reactions among phenolic compounds that take place during sonication. Moreover, the lowest concentrations of analyzed phenolic compounds were found in wine with lower content of SO_2. As already well-known the addition of SO_2 in winemaking is essential, in the first place, for preventing microbial spoilage, but also for the management of oxidative aging of wine. This antioxidant removes hydrogen peroxide formed by the oxidation of phenolic compounds and reacts with quinones, reducing them to the catechols, thereby increasing the oxygen consumption rate in wine [66]. Additionally, GSH also influenced, though modest, chemical composition of wine, resulting in slightly higher content of most phenolic compounds (except TT) at the beginning of storage as well as after 6 months compared to wine with lower content of SO_2 aged without GSH (Table 5). This is probably due to the fact that the reduced glutathione has the ability to protect the easily oxidized compounds such as phenolics by reducing oxygen consumption rate [18].

Regarding chromatic characteristics, there is an increasing trend in parameters L*, a*, b*, C* and H* of the presented wine samples along storage, changing into more orange and clear color, respectively. At the beginning of storage and after 3 months, significant differences can be observed in parameters L*, a*, b* and C* among the different treatments of the wine samples. However, after 6 months of storage there were no significant differences in a*, b*, C* and H* values, except in lightness. Furthermore, sonicated samples were characterized by slightly lower values of chromatic characteristics compared to control wine, indicating that HPU treatment did not affect significantly most of the chromatic characteristics, except lightness. On the other hand, the wines with higher content of antioxidants (sulfur dioxide and glutathione) showed higher values of chromatic characteristics. This could be probably due to the fact that the content of sulfur dioxide is able to strongly affect the color of red wine by its bleaching effect on the free anthocyanins [67]. Earlier studies showed that the addition of glutathione appeared to have an improving effect on the wine aroma [17], as well as the impact on wine color by increasing chromatic

characteristics during aging [68]. After the calculation of the total color difference (ΔE*) between treated and control samples, it can be observed that ΔE* values for the sample with standard SO_2 as well as for the sample with lower content of SO_2 and GSH after 6 months of storage were in the range of 1–4, which means that the color differences in these cases were slightly perceptible. Only treated sample with lower content of SO_2 showed ΔE* value higher than 10, which means there was remarkable color difference compared to the control sample. These observations showed that the total color differences between treated and control samples during aging were primarily influenced by the content of antioxidants (SO_2 and GSH) in wine (Table 5). Regarding aroma composition, a slight decrease in total esters, total fatty acids and total terpenes was observed for all the wines along storage, whereas the content of total higher alcohols slightly increased, independently of treatment applied. In general, during wine aging, the decrease of most aroma compounds can be observed due to various chemical and biochemical reactions such as hydrolysis or oxidation. A well-known is loss of fresh and fruity character of a wine during aging as a consequence of decrease of esters [69]. Furthermore, higher alcohols were generally stable during aging, however their increase could be a result of hydrolysis of esters [70] or oxidation of fatty acids [71]. However, uniform trend was not observed in content of volatile fatty acids during aging, as some compounds can increase while others can decrease or remain stable [72].

There is still lack of information in the scientific literature about the effect of ultrasound on the aroma composition of wine, especially on important aroma groups such as higher alcohols, fatty acids and terpenes. As it can be seen from Table 5, no significant differences among the different treatments of the wine samples were not observed, indicating that HPU treatment did not affect total esters, total higher alcohols and total terpenes of the wines immediately after the HPU treatment as well as through the whole period of storage. However, after 6 months of storage the sonicated samples presented lower concentrations of total fatty acids when compared with untreated wine, indicating that HPU treatment influenced this group of aroma compounds. Aside that, the effect of antioxidants addition (SO_2 and GSH) was not noticeable on the content of total esters, total higher alcohols and total terpenes, while higher concentration of antioxidants (SO_2 and GSH) resulted in wines with higher content of total fatty acids. In addition, it was reported that GSH in the combination of lower content of SO_2 could slow down oxidation rate of aroma compounds such as volatile thiols, monoterpenes and esters [18,73,74].

4. Conclusions

The ultrasonic bath and probe treatments influenced the chemical composition of young red wine Cabernet Sauvignon. In both cases, the mild ultrasound conditions (lower frequency, amplitude, temperature, treatment duration, and proper probe diameter size) showed in general a more favorable and lighter impact on the phenolic, color and aroma composition of the treated red wine, while on the contrary, higher process conditions resulted in a decrease of aforementioned tested parameters. Respectively, among the four different parameters of ultrasonic bath experiment, the frequency (37–80 kHz) proved to be the most important one influencing chemical composition of red wine, followed by bath temperature (20–60 °C) and amplitude (40–100%). Regarding ultrasonic probe experiment, statistical analysis suggested that the selection of the probe diameter (12.7–25.4 mm) was the most significant parameter affecting red wine chemical composition, followed by treatment duration (3–9 min) and amplitude (25–100%). Moreover, their interaction effects also contributed significantly to a large part of the total variation in the whole data set. When considering ANN prediction for all the 14 parameters in both HPU experiments, the chromatic characteristics had the highest correlation of experimental and predicted data. For the second HPU experiment (ultrasonic probe) TP and TA showed very good correlation, while in both cases total higher alcohols, total fatty acids, total terpenes and total esters did not have good prediction. HPU treatment influenced the phenolic composition of wine after 6 months of storage in the bottles. Particularly, the lower content of total phenolics, total anthocyanins, total free anthocyanins and total flavan-3-ols was observed in sonicated samples. However, HPU treatment did not affect the content of total tannins. Also, the addition of higher concentration of antioxidants (SO_2 and glutathione) delayed the loss of aforementioned phenolic compounds during aging. Moreover, identical trends noticed for phenolics were observed in lightness (L*) as well as the content of total fatty acids. On the other hand, HPU treatment after 6 months of aging did not influence the chromatic parameters a*, b*, C* and H*, as well as the content of total esters, total higher alcohols and total terpenes regardless of the antioxidants addition in wine, since no significant differences among sonicated samples were observed. This shows that HPU can be applied with lower content of SO_2 without causing changes in the aforementioned chromatic and aroma characteristics. Our results indicated that proper HPU treatment might slightly accelerate chemical reactions which naturally occur during aging of red wine.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ultsonch.2019.104725.

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Supplementary Table 1. Effects of different High Power Ultrasound process variables (ultrasonic bath) on the phenolic, chromatic and aroma composition of red wine (experiment 1)

	Experiment 1 Ultrasonic bath																
	Independent variables*				Response												
Run	X_1	X_2	X_3	X_4	TP (mg GAE/L)	TA (mg/L)	TT (g/L)	Total free anthocyanins (mg/L)	Total flavan-3-ols (mg/L)	L*	a*	b*	C*	H*	Total esters (mg/L)	Total higher alcohols (mg/L)	Total fatty acids (mg/L)	Total terpenes (μg/L)
0	0	0	0	0	1668.82±1.67	292.73±1.79	1.78±0.01	179.94±1.18	195.06±3.94	24.74±0.18	55.59±0.24	37.65±0.28	66.72±0.24	0.55±0.00	38.21±0.93	325.64±2.59	8.37±0.08	12.68±0.13
1	100	80	20	20	1580.50±4.95	251.30±0.12	1.72±0.00	135.64±0.50	139.22±3.14	24.57±0.12	55.67±0.10	37.06±0.03	66.63±0.03	0.56±0.03	27.88±1.70	212.63±1.05	4.84±0.45	8.94±0.11
2	100	80	20	50	1603.00±0.00	265.56±0.00	1.73±0.00	138.88±0.07	154.63±2.41	23.51±0.06	54.43±0.15	36.51±0.29	65.64±0.18	0.57±0.02	27.12±0.14	190.38±1.97	3.77±0.13	8.35±0.16
3	100	80	20	65	1602.00±0.00	264.91±0.19	1.73±0.00	138.75±0.05	142.55±3.87	22.57±0.23	53.91±0.05	35.53±0.49	65.24±0.06	0.56±0.02	18.63±1.15	290.61±0.03	2.97±0.37	8.81±0.28
4	100	80	20	90	1557.50±0.71	245.00±0.00	1.71±0.00	133.89±0.28	163.53±1.21	21.46±0.24	53.12±0.29	34.60±0.24	64.48±0.07	0.55±0.03	17.47±0.28	190.10±2.38	2.26±0.05	6.59±0.49
5	100	80	40	20	1451.50±2.12	215.95±0.74	1.60±0.00	114.39±0.20	175.07±0.85	23.07±0.09	54.12±0.10	36.08±0.08	65.04±0.12	0.59±0.00	26.62±0.90	213.10±2.99	3.12±0.14	6.92±0.83
6	100	80	40	50	1463.50±0.71	218.97±0.06	1.61±0.00	115.46±0.05	179.16±0.86	22.39±0.01	53.48±0.01	35.59±0.03	64.24±0.03	0.59±0.00	23.00±0.82	248.41±5.80	3.91±0.16	9.92±0.66
7	100	80	40	65	1525.00±0.00	232.62±0.06	1.67±0.00	127.60±0.11	182.41±0.93	22.48±0.17	53.57±0.12	35.81±0.17	64.44±0.19	0.59±0.00	23.86±0.25	221.30±1.21	4.96±0.43	9.51±0.22
8	100	80	40	90	1552.86±0.19	243.47±0.43	1.71±0.00	133.36±0.29	193.67±0.41	21.86±0.15	52.96±0.16	35.25±0.20	63.62±0.24	0.59±0.00	16.51±0.16	230.91±2.93	5.32±0.26	9.85±0.03
9	100	80	60	20	1474.00±0.00	222.47±0.43	1.62±0.00	117.65±0.32	151.94±2.68	22.37±0.12	53.39±0.08	35.58±0.06	64.16±0.10	0.59±0.00	26.59±1.15	227.41±1.58	3.91±0.28	10.77±0.53
10	100	80	60	50	1528.00±0.00	233.58±0.31	1.68±0.00	127.76±0.02	140.41±4.37	20.87±0.30	52.02±0.31	34.17±0.38	62.24±0.46	0.58±0.00	19.46±2.52	234.31±5.83	4.03±0.06	7.29±0.47
11	100	80	60	65	1529.95±1.35	235.77±0.19	1.69±0.00	129.05±0.13	154.07±4.98	20.88±1.98	52.07±1.92	34.09±2.34	62.24±2.88	0.58±0.01	16.10±1.73	155.27±5.53	4.37±0.01	10.75±1.05
12	100	80	60	90	1651.36±0.64	278.29±0.19	1.75±0.00	145.68±0.13	162.39±4.15	18.36±0.41	49.50±0.40	30.86±0.58	58.33±0.64	0.56±0.00	11.68±0.40	194.67±2.77	6.85±0.27	9.28±0.01
13	100	37	20	20	1565.09±4.37	249.33±0.19	1.72±0.00	134.88±0.16	152.80±2.99	23.57±0.12	54.48±0.09	36.53±0.10	65.60±0.13	0.59±0.00	35.26±2.13	296.22±0.37	4.87±0.68	11.49±1.03
14	100	37	20	50	1539.14±2.64	239.75±0.12	1.69±0.00	131.94±0.19	160.49±0.47	23.33±0.02	54.23±0.01	36.42±0.02	65.33±0.01	0.59±0.00	22.90±2.35	185.57±3.53	3.18±0.26	9.95±0.33
15	100	37	20	65	1542.73±1.29	240.76±0.43	1.70±0.00	132.19±0.07	148.43±0.34	22.95±0.05	53.88±0.08	36.18±0.08	64.90±0.11	0.59±0.00	27.49±0.99	278.67±0.23	4.66±0.12	9.82±0.23
16	100	37	20	90	1534.50±2.12	236.78±0.25	1.69±0.00	131.04±0.14	155.69±1.94	22.87±0.02	53.78±0.04	36.20±0.02	64.83±0.05	0.59±0.00	26.68±1.68	234.41±0.52	4.11±0.13	7.52±0.49
17	100	37	40	20	1641.45±0.77	271.12±1.79	1.74±0.00	139.44±0.01	159.07±4.37	23.25±0.19	54.11±0.25	36.27±0.23	65.14±0.34	0.59±0.01	18.50±0.11	163.18±4.80	2.91±0.43	6.53±1.28
18	100	37	40	50	1644.27±0.39	274.75±0.74	1.74±0.00	141.16±0.58	155.32±2.78	22.60±0.06	53.54±0.06	35.87±0.05	64.45±0.08	0.59±0.00	27.64±0.71	230.14±2.27	4.50±0.45	7.97±0.18
19	100	37	40	65	1597.27±1.29	261.96±2.04	1.73±0.00	138.24±0.07	150.30±4.19	22.23±0.05	53.24±0.04	35.64±0.05	64.07±0.06	0.59±0.00	25.67±1.86	249.01±1.64	4.58±0.16	6.34±0.25
20	100	37	40	90	1555.91±0.64	244.56±0.37	1.71±0.00	133.60±0.04	154.63±2.41	21.73±0.41	52.77±0.37	35.25±0.46	63.46±0.56	0.59±0.00	11.10±0.79	206.33±2.79	3.50±0.98	6.90±0.04
21	100	37	60	20	1560.00±0.00	248.15±1.36	1.71±0.00	134.37±0.26	160.55±3.01	21.33±0.01	52.07±0.00	34.48±0.00	62.45±0.00	0.58±0.00	20.59±0.28	259.40±2.21	3.39±0.21	7.13±0.06
22	100	37	60	50	1655.50±0.71	280.74±0.06	1.76±0.00	150.72±1.93	158.18±2.11	20.05±0.16	50.97±0.15	33.11±0.20	60.78±0.23	0.58±0.00	15.05±0.23	265.71±4.08	3.30±0.06	8.38±0.91
23	100	37	60	65	1653.82±0.26	280.35±0.37	1.75±0.00	147.97±0.18	162.01±0.94	19.12±0.00	50.02±0.02	31.89±0.01	59.32±0.02	0.57±0.00	17.15±1.09	215.12±2.74	5.74±0.32	9.86±0.35
24	100	37	60	90	1644.77±0.32	276.02±0.80	1.75±0.00	143.79±0.15	170.46±3.17	18.35±0.31	49.37±0.35	30.83±0.49	58.20±0.55	0.56±0.00	13.83±1.33	178.23±3.17	5.52±0.28	9.95±0.62
25	60	80	20	20	1506.50±4.95	229.86±0.25	1.66±0.00	123.76±0.04	146.58±2.19	24.25±0.04	54.70±0.08	37.22±0.10	66.16±0.12	0.60±0.00	33.56±1.82	317.29±4.45	3.87±0.01	7.68±0.77
26	60	80	20	50	1482.00±0.00	224.26±0.00	1.64±0.00	121.06±0.11	135.51±2.14	23.91±0.13	54.39±0.18	36.91±0.13	65.75±0.24	0.60±0.00	16.77±1.11	201.29±2.54	1.89±0.04	7.58±0.54
27	60	80	20	65	1412.05±4.18	177.54±3.09	1.53±0.00	98.43±0.72	138.65±0.25	23.81±0.14	54.31±0.18	36.96±0.11	65.70±0.20	0.60±0.00	21.40±0.35	242.44±0.02	3.68±0.11	7.42±0.48
28	60	80	20	90	1480.00±0.00	223.43±0.06	1.63±0.00	120.02±0.07	130.74±0.74	23.48±0.06	53.83±0.09	36.59±0.10	65.09±0.13	0.60±0.00	22.50±4.20	304.88±2.48	2.44±0.64	7.38±0.78
29	60	80	40	20	1428.50±0.71	193.16±2.29	1.57±0.00	107.86±0.29	129.17±0.12	23.30±0.17	54.17±0.16	36.08±0.12	65.09±0.20	0.59±0.00	19.94±0.38	284.45±5.85	3.27±0.01	11.52±1.49
30	60	80	40	50	1531.50±0.71	236.21±0.19	1.69±0.00	129.91±0.56	135.63±4.75	24.00±0.42	54.60±0.61	36.81±0.03	65.85±0.53	0.59±0.00	26.04±1.21	255.04±4.69	4.04±0.47	7.24±0.17
31	60	80	40	65	1466.00±0.00	219.28±0.37	1.61±0.00	115.75±0.21	135.76±0.14	23.41±0.10	54.23±0.10	36.26±0.11	65.24±0.14	0.59±0.00	22.38±1.94	259.10±3.67	4.87±0.57	7.70±0.56
32	60	80	40	90	1549.09±2.57	242.24±1.05	1.70±0.00	132.70±0.02	126.92±1.86	23.24±0.09	54.07±0.11	36.25±0.10	65.10±0.15	0.59±0.00	16.91±1.64	250.76±4.04	3.14±0.09	7.63±0.66
33	60	80	60	20	1467.00±0.00	220.15±0.31	1.62±0.00	116.09±0.06	136.79±2.74	24.53±0.09	55.21±0.09	37.32±0.06	66.64±0.11	0.59±0.00	26.30±0.62	306.94±3.77	6.63±0.52	10.03±0.46
34	60	80	60	50	1447.00±0.00	213.81±0.25	1.59±0.00	113.29±0.40	135.81±2.62	23.10±0.19	54.05±0.24	36.57±0.23	65.26±0.32	0.59±0.00	18.00±1.16	251.44±2.65	6.47±1.29	9.07±0.04
35	60	80	60	65	1596.00±0.00	259.74±2.10	1.72±0.00	137.62±0.66	140.88±2.81	22.16±0.29	53.14±0.34	35.69±0.38	64.02±0.49	0.59±0.00	14.85±0.21	208.28±3.88	4.99±0.13	6.80±0.28
36	60	80	60	90	1459.05±0.06	218.58±0.37	1.60±0.00	115.10±0.13	136.23±2.97	21.48±0.01	52.57±0.07	35.10±0.06	63.21±0.09	0.59±0.00	14.83±0.02	208.88±1.71	6.14±0.01	9.81±1.32
37	60	37	20	20	1589.00±1.41	254.19±0.25	1.72±0.00	136.24±0.06	138.13±0.27	23.49±0.11	54.31±0.12	36.22±0.11	65.27±0.16	0.59±0.00	25.16±0.75	222.40±3.97	3.65±0.48	4.85±0.40
38	60	37	20	50	1481.41±0.58	223.83±1.36	1.64±0.00	120.38±0.00	130.47±4.44	23.36±0.06	54.17±0.08	36.15±0.08	65.13±0.12	0.59±0.00	30.99±0.58	316.66±8.86	5.07±0.86	9.50±1.58
39	60	37	20	65	1416.73±1.80	182.00±0.49	1.53±0.00	102.64±0.24	132.32±1.28	23.07±0.26	53.82±0.27	35.85±0.24	64.67±0.36	0.59±0.00	33.80±0.51	312.34±1.17	4.51±0.02	10.39±0.85
40	60	37	20	90	1423.14±5.85	188.91±1.18	1.56±0.00	107.29±0.20	136.07±3.39	23.11±0.18	53.89±0.21	36.04±0.19	64.83±0.28	0.59±0.00	21.04±0.08	182.65±5.23	2.91±0.23	10.25±0.34
41	60	37	40	20	1586.00±0.00	252.18±0.00	1.72±0.00	136.07±0.07	127.98±0.43	23.02±0.19	53.74±0.24	35.94±0.27	64.65±0.35	0.59±0.00	27.45±0.83	281.64±3.74	3.04±0.22	8.12±1.44
42	60	37	40	50	1612.00±9.90	266.66±0.31	1.74±0.00	139.02±0.03	143.11±0.62	22.42±0.23	52.61±0.46	35.44±0.31	63.85±0.38	0.59±0.01	24.08±0.37	276.25±0.44	4.66±1.17	12.02±1.44
43	60	37	40	65	1545.27±1.03	241.94±0.12	1.70±0.00	132.43±0.08	139.27±4.84	22.50±0.05	53.27±0.06	35.67±0.06	64.11±0.08	0.59±0.00	27.24±0.03	286.15±1.09	4.64±0.25	7.97±0.32
44	60	37	40	90	1519.09±0.00	232.01±0.06	1.67±0.00	126.40±0.50	136.93±3.77	22.31±0.03	53.09±0.05	35.60±0.06	63.92±0.07	0.59±0.00	27.85±0.92	274.81±0.13	3.72±0.36	6.85±0.11
45	60	37	60	20	1642.86±0.19	273.88±0.43	1.74±0.00	139.99±0.10	155.94±3.29	22.97±0.20	53.92±0.18	36.05±0.15	64.86±0.24	0.59±0.00	19.51±1.19	269.72±0.30	3.42±0.05	8.02±1.53
46	60	37	60	50	1647.95±4.18	276.89±0.12	1.75±0.00	145.27±0.32	149.59±0.55	21.35±0.25	52.36±0.24	34.58±0.28	62.75±0.35	0.58±0.00	14.75±0.20	249.12±1.59	3.48±0.40	7.90±0.66
47	60	37	60	65	1515.23±0.32	230.52±0.00	1.66±0.00	123.96±0.22	153.10±1.91	20.65±0.32	51.71±0.41	33.85±0.46	61.81±0.60	0.58±0.00	15.02±1.18	237.64±3.22	5.21±0.16	7.97±0.39

48	60	37	60	90	1656.18±0.26	281.93±0.31	1.76±0.00	155.58±1.63	150.47±1.69	20.37±0.66	51.49±0.67	33.64±0.87	61.51±1.03	0.58±0.01	12.75±1.07	154.05±7.34	5.71±0.62	9.98±0.33
49	40	80	20	20	1439.50±2.12	205.01±0.31	1.58±0.00	110.70±0.98	136.24±3.24	25.68±0.04	56.12±0.02	37.85±0.05	67.69±0.04	0.59±0.00	35.28±0.42	269.42±4.79	2.82±0.11	9.99±0.64
50	40	80	20	50	1442.50±0.71	208.03±0.99	1.59±0.00	112.04±0.48	141.37±4.15	25.41±0.22	55.85±0.25	37.68±0.17	67.37±0.31	0.59±0.00	33.79±2.02	210.73±4.73	3.99±0.69	9.76±1.02
51	40	80	20	65	1485.27±1.03	225.53±1.98	1.65±0.00	121.68±0.06	140.01±4.07	25.47±0.05	55.92±0.06	37.79±0.04	67.49±0.07	0.59±0.00	27.58±0.29	264.97±7.98	3.05±0.16	10.07±0.26
52	40	80	20	90	1473.00±0.00	221.38±1.24	1.62±0.00	116.86±0.12	132.48±3.70	25.40±0.23	55.79±0.30	37.69±0.26	67.33±0.39	0.59±0.00	22.61±1.66	294.97±2.31	2.90±0.42	10.62±0.21
53	40	80	40	20	1418.50±0.71	184.45±0.06	1.55±0.00	106.83±0.57	136.87±1.56	24.64±0.14	55.04±0.12	37.36±0.09	66.52±0.15	0.60±0.00	32.35±4.09	270.13±6.87	3.95±0.50	8.92±1.49
54	40	80	40	50	1445.50±0.71	210.35±0.19	1.59±0.00	112.78±0.00	155.89±0.40	24.57±0.19	54.93±0.22	37.34±0.17	66.42±0.28	0.60±0.00	33.05±1.71	293.95±4.83	4.08±0.23	9.50±0.83
55	40	80	40	65	1487.50±0.71	226.41±1.92	1.66±0.00	122.17±0.14	153.41±4.37	24.42±0.16	54.78±0.20	37.28±0.21	66.27±0.28	0.60±0.00	23.03±0.88	173.51±7.25	2.93±0.11	7.80±0.45
56	40	80	40	90	1529.00±0.00	234.46±0.06	1.68±0.00	127.90±0.11	169.25±3.67	24.39±0.21	54.80±0.22	37.27±0.03	66.27±0.20	0.60±0.00	16.46±0.86	161.19±5.47	3.72±0.04	7.83±0.86
57	40	80	60	20	1432.50±4.95	202.43±0.37	1.57±0.00	108.31±0.30	139.07±3.75	24.22±0.31	54.90±0.28	37.31±0.23	66.38±0.36	0.60±0.00	20.80±1.13	215.91±6.81	3.69±0.21	8.38±0.03
58	40	80	60	50	1532.86±0.19	236.47±4.83	1.69±0.00	130.76±0.18	138.85±0.07	23.14±0.10	53.94±0.10	36.68±0.09	65.23±0.13	0.60±0.00	19.65±0.70	293.14±2.83	7.26±1.27	8.85±1.65
59	40	80	60	65	1489.59±1.99	226.98±0.43	1.66±0.00	122.89±0.15	149.45±0.09	22.19±0.34	53.03±0.41	35.79±0.45	63.98±0.59	0.59±0.00	18.78±0.09	221.60±3.16	5.20±0.01	8.13±0.83
60	40	80	60	90	1403.00±2.38	151.64±1.11	1.49±0.00	95.48±0.42	135.94±2.36	22.19±0.22	53.09±0.18	36.03±0.23	64.16±0.28	0.60±0.01	13.89±0.61	214.14±5.44	5.83±0.25	9.85±1.29
61	40	37	20	20	1474.50±0.71	223.08±0.12	1.63±0.00	119.07±0.32	153.06±3.88	24.07±0.11	54.33±0.16	36.69±0.14	65.55±0.21	0.59±0.00	26.15±0.01	274.99±1.97	4.19±0.23	10.49±0.22
62	40	37	20	50	1441.41±0.58	206.59±0.31	1.58±0.00	111.61±0.09	144.71±1.83	24.09±0.20	54.33±0.25	36.71±0.23	65.57±0.34	0.59±0.00	27.81±2.68	239.63±2.77	5.01±0.15	10.04±0.01
63	40	37	20	65	1522.91±1.54	232.40±0.56	1.67±0.00	127.01±0.32	151.33±5.37	24.13±0.13	54.46±0.05	36.88±0.01	65.77±0.05	0.60±0.00	29.65±2.07	185.50±2.36	3.56±0.00	7.54±0.15
64	40	37	20	90	1494.91±4.37	227.89±0.06	1.66±0.00	123.20±0.13	149.42±4.35	23.86±0.23	54.16±0.30	36.69±0.28	65.42±0.40	0.60±0.00	30.14±1.98	282.11±3.15	5.39±0.44	11.48±0.47
65	40	37	40	20	1515.45±0.00	231.22±0.43	1.67±0.00	125.42±0.89	148.99±3.97	22.64±1.03	53.03±0.74	35.51±0.90	63.82±1.12	0.59±0.01	17.71±0.64	288.91±6.71	3.72±0.14	8.43±0.21
66	40	37	40	50	1483.00±1.41	224.39±0.06	1.65±0.00	121.39±0.16	145.53±2.68	22.41±0.65	52.99±0.42	35.48±0.59	63.77±0.67	0.59±0.00	26.16±0.29	294.19±5.31	4.29±0.21	9.55±0.65
67	40	37	40	65	1529.00±0.00	235.24±0.43	1.68±0.00	128.44±0.48	155.54±2.06	21.58±0.19	52.21±0.26	34.63±0.33	62.65±0.39	0.59±0.00	29.71±2.50	306.89±0.78	4.15±0.50	11.89±0.96
68	40	37	40	90	1455.32±2.38	217.74±0.43	1.60±0.00	114.91±0.12	161.89±0.57	21.50±0.28	52.18±0.35	34.64±0.35	62.63±0.48	0.59±0.00	19.61±1.46	246.87±5.49	5.06±0.13	10.62±0.10
69	40	37	60	20	1659.32±0.32	282.23±0.06	1.76±0.00	163.01±2.75	150.52±0.27	24.46±0.11	55.53±0.07	37.44±0.04	66.98±0.08	0.59±0.00	18.92±0.99	298.20±3.12	3.50±0.63	7.57±1.59
70	40	37	60	50	1660.95±0.06	283.46±1.42	1.76±0.00	170.01±1.48	159.53±0.11	22.66±0.21	53.89±0.27	36.19±0.26	64.92±0.37	0.59±0.00	17.16±0.64	175.59±2.97	4.21±0.15	8.88±0.57
71	40	37	60	65	1656.00±2.83	287.57±1.30	1.77±0.00	174.73±0.15	143.53±2.48	21.77±0.30	52.92±0.54	35.38±0.31	63.83±0.38	0.59±0.00	14.59±0.80	177.80±1.11	5.99±0.11	7.98±0.38
72	40	37	60	90	1593.00±1.41	258.34±0.56	1.72±0.00	136.98±0.08	141.73±0.54	21.21±0.06	52.57±0.05	34.87±0.05	63.08±0.07	0.59±0.00	12.89±0.16	222.33±5.84	8.01±0.69	8.90±0.18

*X_1 = amplitude (%), X_2 = frequency (kHz), X_3 = bath temperature (°C), X_4 = treatment duration (min). Experimental results are presented as means ± S.D. Abbreviations: TP, total phenolics; TA, total anthocyanins; TT, total tannins.

Supplementary Table 2. Effects of different High Power Ultrasound process variables (ultrasonic probe) on the phenolic, chromatic and aroma composition of red wine (experiment 2)

	Experiment 2 Ultrasonic probe															
	Independent variables*			Response												
Run	X_1	X_2	X_3	TP (mg GAE/L)	TA (mg/L)	TT (g/L)	Total free anthocyanins (mg/L)	Total flavan-3-ols (mg/L)	L*	a*	b*	C*	H*	Total esters (mg/L)	Total higher alcohols (mg/L)	Total fatty acids (mg/L)	Total terpenes (µg/L)
0	0	0	0	2757.92±1.77	434.18±0.49	3.74±0.04	174.01±1.63	343.13±0.71	17.22±0.29	48.62±0.24	27.83±0.18	56.35±0.75	0.52±0.00	40.65±0.88	257.19±0.69	5.82±0.11	18.67±0.35
1	12.7	25	3	2653.33±8.25	397.86±5.57	3.68±0.01	125.45±2.36	319.66±0.81	18.07±0.09	52.58±0.13	30.11±0.12	58.64±0.54	0.53±0.00	36.43±2.04	236.05±0.97	5.24±0.31	14.86±0.05
2	12.7	50	3	2560.00±3.54	388.50±0.62	3.53±0.01	126.48±0.40	322.53±0.93	17.86±0.06	52.37±0.07	29.92±0.08	57.86±0.11	0.52±0.00	31.29±3.07	216.88±2.19	4.38±0.40	15.55±0.06
3	12.7	75	3	2553.83±3.54	387.14±4.89	3.44±0.04	124.98±0.76	318.90±4.40	18.16±0.13	52.74±0.20	30.31±0.20	58.38±0.28	0.53±0.00	37.47±1.81	264.68±2.83	5.45±0.01	17.96±1.28
4	12.7	100	3	2535.83±8.25	381.81±6.99	3.30±0.01	121.32±0.26	304.55±1.28	17.89±0.10	52.35±0.16	29.90±0.17	57.83±0.23	0.53±0.00	31.70±0.74	202.55±0.12	3.98±0.68	16.00±1.08
5	12.7	25	6	2640.83±7.07	363.96±1.79	3.52±0.08	158.29±0.05	313.68±1.35	16.73±0.02	48.03±0.06	27.32±0.06	56.25±1.33	0.52±0.00	28.77±0.78	232.81±6.40	3.93±0.37	14.87±0.33
6	12.7	50	6	2594.58±6.48	347.51±2.29	3.26±0.02	164.41±2.00	314.20±2.87	16.72±0.08	48.10±0.17	27.40±0.16	55.36±0.23	0.52±0.00	33.11±0.57	207.19±5.97	3.22±0.01	13.93±0.35
7	12.7	75	6	2553.33±4.71	319.11±0.74	3.06±0.05	150.42±1.90	296.81±7.07	16.70±0.01	48.04±0.00	27.36±0.01	55.28±0.01	0.52±0.00	37.79±0.64	221.06±3.56	4.22±0.19	12.55±0.04
8	12.7	100	6	2540.42±5.30	304.76±2.72	3.01±0.01	148.53±0.65	269.99±5.67	16.27±0.08	47.40±0.17	26.62±0.17	54.36±0.23	0.51±0.00	32.85±2.52	210.76±1.16	4.22±0.74	16.20±1.76
9	12.7	25	9	2597.92±5.30	331.67±0.93	3.14±0.05	122.83±8.36	276.98±7.03	17.43±0.16	48.81±1.01	29.84±0.26	57.58±0.13	0.54±0.00	35.77±0.38	224.99±1.71	3.68±0.40	13.54±0.64
10	12.7	50	9	2578.75±6.48	325.06±0.49	3.02±0.07	120.89±1.83	295.46±1.00	17.08±0.01	49.19±0.70	29.74±0.73	56.30±0.00	0.53±0.00	30.94±2.18	209.42±0.47	4.25±0.74	15.55±0.12
11	12.7	75	9	2564.17±4.71	309.40±4.33	2.97±0.15	112.01±1.57	303.79±0.65	17.59±0.12	49.47±0.41	30.48±0.30	61.29±0.41	0.54±0.00	32.39±0.75	204.07±2.31	3.02±0.22	14.64±0.35
12	12.7	100	9	2460.42±0.59	299.38±1.92	2.82±0.16	131.33±0.11	307.34±2.45	17.71±0.19	49.65±0.36	29.68±0.37	58.55±0.49	0.53±0.00	29.23±3.28	226.31±2.30	3.82±0.96	15.05±2.04
13	19.1	25	3	2662.08±8.84	418.43±6.93	3.60±0.05	139.20±3.62	314.13±2.44	16.31±0.02	47.60±0.07	26.80±0.07	54.36±0.09	0.51±0.00	36.52±0.28	232.92±1.69	4.65±0.06	14.80±0.20
14	19.1	50	3	2649.58±2.95	414.84±0.25	3.58±0.02	142.71±1.38	318.47±5.75	16.73±0.02	48.27±0.02	27.53±0.03	55.57±0.03	0.52±0.00	38.39±1.25	235.55±5.84	4.63±0.81	13.12±0.42
15	19.1	75	3	2610.83±5.89	401.98±3.59	3.29±0.01	141.55±0.08	324.76±1.08	16.13±0.09	47.27±0.01	26.42±0.03	54.15±0.01	0.51±0.00	38.13±3.23	238.92±6.31	4.59±0.41	14.07±0.23
16	19.1	100	3	2572.92±8.84	397.91±1.18	3.20±0.05	165.47±0.45	321.38±4.30	16.31±0.16	47.53±0.37	26.72±0.38	54.52±0.51	0.51±0.00	35.65±3.71	228.89±3.12	4.83±0.69	14.43±0.08
17	19.1	25	6	2651.67±5.89	416.98±2.66	3.72±0.04	176.92±0.47	308.06±1.93	16.62±0.16	48.04±0.21	27.30±0.24	55.26±0.30	0.52±0.00	30.77±2.87	218.13±5.38	4.52±0.56	14.45±0.57
18	19.1	50	6	2632.50±4.71	403.68±0.43	3.62±0.06	142.62±0.28	302.81±1.97	16.59±0.18	48.00±0.25	27.25±0.28	55.20±0.36	0.52±0.00	30.04±1.11	224.30±5.90	4.19±0.20	14.91±1.16
19	19.1	75	6	2601.25±4.12	400.93±3.22	3.39±0.02	144.92±0.57	301.65±1.86	16.71±0.02	48.17±0.02	27.44±0.02	55.44±0.03	0.52±0.00	39.76±0.60	251.06±0.36	5.57±0.04	14.49±0.04
20	19.1	100	6	2567.50±5.89	393.36±1.18	3.33±0.01	139.87±2.01	287.39±0.63	16.16±0.07	47.38±0.13	26.54±0.14	54.31±0.18	0.51±0.00	36.04±0.40	224.10±2.83	4.59±0.25	15.74±0.30
21	19.1	25	9	2642.08±1.77	411.91±3.53	3.60±0.01	149.17±0.88	275.65±3.47	16.53±0.15	47.74±0.12	27.05±0.15	54.87±0.18	0.52±0.00	37.23±1.22	233.22±1.29	4.46±0.22	16.49±0.88
22	19.1	50	9	2598.75±5.30	395.54±0.31	3.43±0.05	142.12±1.12	276.81±3.25	16.98±0.03	48.51±0.09	27.84±0.09	55.93±0.13	0.52±0.00	34.92±0.52	216.97±5.60	4.48±0.69	16.47±0.86
23	19.1	75	9	2581.67±9.43	393.31±1.48	3.18±0.02	148.82±0.26	295.58±3.13	16.67±0.11	48.06±0.18	27.35±0.19	55.30±0.25	0.52±0.00	36.71±1.54	230.65±4.23	4.65±0.11	15.74±1.12
24	19.1	100	9	2462.08±4.12	390.95±3.59	3.09±0.00	142.03±1.03	298.18±2.35	16.84±0.05	48.29±0.09	27.63±0.09	55.64±0.12	0.52±0.00	36.78±2.15	236.02±1.60	4.69±0.03	13.91±1.07
25	25.4	25	3	2670.83±2.36	430.33±0.74	3.78±0.09	142.70±0.90	309.76±1.73	18.57±0.21	50.36±0.34	30.03±0.35	58.63±0.48	0.54±0.00	39.18±0.34	231.18±1.44	4.86±0.14	15.07±0.22
26	25.4	50	3	2661.67±5.89	425.60±4.33	3.59±0.05	142.25±0.32	319.27±2.34	18.55±0.10	50.35±0.08	30.02±0.10	58.62±0.12	0.54±0.00	37.51±0.53	230.81±0.90	5.23±0.01	15.37±0.43
27	25.4	75	3	2615.00±2.36	420.00±1.61	3.57±0.04	146.05±0.77	315.17±0.09	18.63±0.04	50.52±0.06	30.21±0.05	58.86±0.08	0.54±0.00	30.76±1.86	219.85±2.66	3.77±0.39	13.52±0.04
28	25.4	100	3	2513.75±4.12	410.94±0.19	3.35±0.00	141.24±1.35	317.44±4.72	18.55±0.07	50.40±0.16	30.06±0.16	58.69±0.21	0.54±0.00	29.62±2.96	214.76±2.84	4.18±0.80	13.22±0.51
29	25.4	25	6	2718.75±0.59	422.01±4.95	3.50±0.08	154.47±0.69	316.76±8.02	18.17±0.08	49.80±0.15	29.46±0.15	57.85±0.20	0.53±0.00	35.77±0.15	225.47±2.21	4.18±0.08	14.90±0.01
30	25.4	50	6	2712.08±7.66	415.58±4.02	3.41±0.09	144.41±0.03	308.98±0.61	18.24±0.01	49.91±0.03	29.57±0.05	58.02±0.05	0.53±0.00	34.45±1.01	215.24±1.11	3.78±0.14	13.27±0.57
31	25.4	75	6	2705.42±1.77	413.26±4.58	3.34±0.06	143.91±0.18	305.07±1.86	18.11±0.00	49.74±0.01	29.42±0.00	57.79±0.01	0.53±0.00	35.88±0.61	224.94±0.30	4.14±0.04	14.32±0.67
32	25.4	100	6	2635.00±7.07	409.94±2.47	3.22±0.09	142.54±1.89	297.62±5.05	17.85±0.01	49.32±0.03	28.95±0.03	57.20±0.04	0.53±0.00	33.97±2.51	221.36±2.20	4.30±0.28	13.30±0.75
33	25.4	25	9	2677.50±1.18	423.19±0.68	3.58±0.02	140.78±0.24	289.50±2.39	17.12±0.06	48.71±0.18	28.02±0.20	56.20±0.26	0.52±0.00	31.69±1.78	220.22±2.29	3.12±0.06	12.51±1.34
34	25.4	50	9	2680.42±1.77	425.08±5.82	3.45±0.17	147.75±0.22	286.26±1.22	17.27±0.02	48.87±0.01	28.19±0.01	56.42±0.01	0.52±0.00	31.85±1.64	212.92±8.05	3.67±0.84	13.67±0.47
35	25.4	75	9	2633.33±8.25	408.45±1.36	3.29±0.26	132.25±0.49	289.45±2.45	17.14±0.05	48.85±0.05	28.15±0.07	56.38±0.08	0.52±0.00	29.85±0.05	222.97±0.36	4.13±0.62	14.24±0.42
36	25.4	100	9	2583.75±6.48	395.11±1.79	3.08±0.03	135.00±1.99	300.98±3.87	17.08±0.04	48.71±0.04	28.00±0.04	56.18±0.05	0.52±0.00	33.89±1.97	225.29±0.33	4.45±0.18	14.93±0.16

*X_1 = probe diameter (mm), X_2 = amplitude (%), X_3 = treatment duration (min). Experimental results are presented as means ± S.D. Abbreviations: TP, total phenolics; TA, total anthocyanins; TT, total tannins.

Supplementary Table 3. The total color difference value (ΔE*) between the control and treated wine samples obtained in both High Power Ultrasound experiments

Experiment 1 Ultrasonic bath treatments					
Run	ΔE*	Run	ΔE*	Run	ΔE*
1	0.68 ± 0.19	25	8.34 ± 0.71	49	6.33 ± 1.07
2	1.85 ± 0.12	26	0.97 ± 0.11	50	0.82 ± 0.20
3	2.50 ± 0.25	27	0.93 ± 0.04	51	0.79 ± 0.07
4	3.55 ± 0.26	28	1.40 ± 0.36	52	0.72 ± 0.03
5	2.47 ± 0.07	29	1.58 ± 0.01	53	0.83 ± 0.23
6	2.49 ± 0.13	30	1.18 ± 0.04	54	0.42 ± 0.26
7	2.27 ± 0.04	31	1.53 ± 0.17	55	0.53 ± 0.14
8	3.00 ± 0.02	32	1.52 ± 0.29	56	0.38 ± 0.02
9	2.45 ± 0.21	33	1.59 ± 0.08	57	0.72 ± 0.27
10	4.35 ± 0.61	34	2.15 ± 0.42	58	1.99 ± 0.10
11	4.64 ± 1.51	35	2.91 ± 0.45	59	2.88 ± 0.47
12	7.74 ± 1.05	36	3.39 ± 0.31	60	2.65 ± 0.32
13	7.64 ± 0.54	37	2.42 ± 0.09	61	1.56 ± 0.46
14	1.44 ± 0.19	38	1.41 ± 0.09	62	0.78 ± 0.31
15	1.84 ± 0.26	39	1.75 ± 0.14	63	0.66 ± 0.38
16	1.87 ± 0.20	40	1.71 ± 0.28	64	0.96 ± 0.51
17	1.55 ± 0.31	41	1.79 ± 0.07	65	2.67 ± 1.42
18	2.26 ± 0.16	42	2.67 ± 0.38	66	2.69 ± 0.44
19	2.55 ± 0.11	43	2.39 ± 0.05	67	3.37 ± 0.10
20	3.10 ± 0.70	44	2.44 ± 0.15	68	3.25 ± 0.10
21	3.60 ± 0.01	45	2.03 ± 0.19	69	4.38 ± 0.55
22	5.01 ± 0.07	46	4.02 ± 0.37	70	2.93 ± 0.49
23	5.83 ± 0.23	47	4.25 ± 0.71	71	3.23 ± 0.54
24	6.52 ± 0.24	48	4.39 ± 0.87	72	3.61 ± 0.15
Experiment 2 Ultrasonic probe treatments					
Run	ΔE*	Run	ΔE*	Run	ΔE*
1	4.65 ± 0.16	13	1.72 ± 0.31	25	3.11 ± 0.15
2	4.34 ± 0.22	14	0.67 ± 0.37	26	3.09 ± 0.54
3	4.90 ± 0.05	15	2.24 ± 0.45	27	3.35 ± 0.47
4	4.32 ± 0.10	16	1.81 ± 0.93	28	3.15 ± 0.15
5	0.94 ± 0.33	17	1.00 ± 0.06	29	2.22 ± 0.60
6	0.84 ± 0.17	18	1.06 ± 0.00	30	2.40 ± 0.33
7	0.92 ± 0.42	19	0.78 ± 0.37	31	2.14 ± 0.37
8	1.97 ± 0.64	20	2.08 ± 0.20	32	1.47 ± 0.34
9	2.21 ± 0.52	21	1.37 ± 0.16	33	0.28 ± 0.08
10	2.01 ± 0.67	22	0.28 ± 0.27	34	0.48 ± 0.29
11	2.83 ± 0.45	23	0.92 ± 0.13	35	0.45 ± 0.13
12	2.22 ± 0.14	24	0.54 ± 0.53	36	0.41 ± 0.03

*The number of runs in both high power ultrasound experiments refers to different applied process conditions. The listed runs are the same one that are presented in Supplementary Table 1 (Experiment 1 - ultrasonic bath) and 2 (Experiment 2 - ultrasonic probe).

Paper 2

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Quality characteristics of white wine: The short- and long-term impact of high power ultrasound processing

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ABSTRACT

This research aimed to analyze the effects of ultrasound on the quality characteristics of white wine when processed by two different systems, i.e., ultrasonic bath and ultrasonic probe. In this regard, the multivariate statistical analysis and artificial neural network (ANN) techniques were used. Additionally, the efficiency of high power ultrasound (HPU) combined with sulfite and glutathione (GSH) treatments was explored during 18 months of bottle storage. Regarding ultrasonic bath experiment, the higher bath temperature caused the degradation of volatile compounds, precisely esters and higher alcohols, while the ultrasound effect on phenolic composition was much less pronounced. Interestingly, a combination of larger probe diameter and higher ultrasound amplitude showed a milder effect on phenolic and volatile composition in ultrasonic probe experiment. Both, ultrasonic bath and probe experiments did not cause great changes in the color properties. Moreover, implemented ANN models for flavan-3-ols, higher alcohols and esters resulted in the highest prediction values. HPU processing after 18 months of storage did not affect wine color. However, it modified phenolic and volatile composition, with greater effect in wines with lower concentration of antioxidants. In addition, there was no significant difference in the phenolic and volatile composition among sonicated low-sulfite-GSH wine and the one with standard-sulfite content. Therefore, a combined HPU and low-sulfite-GSH treatment might be a promising method for production of low-sulfite wines.

1. Introduction

Non-thermal techniques like high hydrostatic pressure, pulsed electric fields and ultrasound showed to be very useful tool for controlling wine microbial activity and quality [1]. These techniques can reduce the use of chemical preservatives, such as sulfur dioxide, while maintaining or improving the quality characteristics of the produced wine [1,2]. Despite their good efficiency, none of them cannot fully cover the actions of SO_2 (antimicrobial and antioxidant). Therefore, these techniques in combination with glutathione and lower doses of sulfites should be considered as a possible solution. The addition of compounds like glutathione, ascorbic acid, lysozyme or chitosan is the most common studied among the alternative practices for complementing the activity of SO_2 [3–9]. Particularly, the use of reduced glutathione (GSH) was highlighted to have beneficial impact in white wine production, especially for the preservation of important volatile compounds and color stability [10–12]. Some studies also demonstrated that the combination of GSH and sulfur dioxide gives respectable protection to wines [11,13], but it is still insufficiently explained.

Among mentioned non-thermal techniques, the application of high power ultrasound (HPU) in wine technology already showed great success in microorganism and enzyme inactivation, acceleration of wine aging process, extraction of bioactive phenolic and volatile compounds, as well as improvement of fermentation and barrel sanitation [14–21]. Namely, HPU possesses physical (micro-mechanical shocks caused by cavitation effect) and chemical (formation of free radicals) effects [22], that modifies chemical composition and improves the quality of the wine during processing [14]. Depending on the purpose, HPU treatment of liquids can be performed, generally, using a direct (ultrasonic probe) immersion or indirect (ultrasonic bath) contacting system [23–25]. Regarding HPU effect on wine quality, extensive

Abbreviations: ANN, artificial neural network; CONW, control wine; GSH, glutathione; HPU, high power ultrasound; MLP, multilayered perceptron neural network; SLGW, sonicated low-sulfite-GSH wine; SLW, sonicated low-sulfite wine; SSW, sonicated standard-sulfite wine; TE, total esters; TF, total flavan-3-ols; TFA, total fatty acids; THA, total higher alcohols; TP, total phenolics; TPA, total phenolic acids; TT, total terpenes

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research has been carried out on the ultrasound processing of red wine [18,20,26–30], but there is still lack of published data regarding its influence on quality characteristics of white wine. Only few studies reported about HPU effect on phenolic and volatile composition of white wine [26,31], and its efficiency as single treatment as well as combined with SO_2 treatment in improving the quality of low-alcohol sweet white wine and its microbiological stability [32]. Also, it should be noted that previous studies were mainly focused on the effect of ultrasonic probe treatment, while reports about the influence of ultrasonic bath treatment on wine quality are lacking. Therefore, due to different applied ultrasound systems and process parameters as well as differences between chemical composition of red and white wines, primarily in phenolics, it is important to investigate more detailed the effect of HPU on overall quality of white wine. In addition, it is important to take into account the modeling of HPU experimental conditions in order to avoid negative effect on sensory quality of wines, since some wines treated with ultrasound showed oxidative characteristics [26]. Due to ultrasound dependence upon various parameters and its specific characteristics, artificial neural network (ANN) seems to be reliable method for modeling the HPU process with very good predictive and calculation abilities [33].

Hence, the primary aim of this study was to investigate the influence of bath and probe ultrasound processing on the chemical composition of a young white wine Graševina. Moreover, the prediction capabilities of HPU treated white wine quality characteristics by ANNs were investigated. Additionally, another aim was to examine the impact of HPU combined with sulfite and GSH treatments on the white wine chemical composition through long-term storage period (18 months).

2. Material and methods

2.1. Chemicals

Hydrochloric acid was purchased from Carlo Erba (Val del Reuil, France), ethanol from Gram-Mol (Zagreb, Croatia), methanol from J. T. Baker (Deventer, Netherlands), sodium bisulfite from Acros Organics (Geel, Belgium), and Folin-Ciocalteu reagent from Kemika (Zagreb, Croatia). Formic acid and sodium carbonate anhydrous were obtained from T.T.T. (Sveta Nedjelja, Croatia). The aqueous bisulfite solution was obtained from Laffort (Bordeaux, France). All standards were acquired from Sigma-Aldrich (St. Louis, USA), including phenolic standards, hydroxybenzoic acids (gallic acid, protocatechuic acid, vanillic acid and syringic acid), hydroxycinnamic acids (caftaric acid, chlorogenic acid, caffeic acid, *p*-coumaric acid and ferulic acid), and flavan-3-ols [(+)-catechin, (-)-epicatechin, procyanidins B1 and B2], as well as standards of individual volatile compounds and reduced L-glutathione.

2.2. White wine HPU processing

The studied wine was young white wine Graševina (*Vitis vinifera* L.), produced in 2017 in the winery Erdutski vinogradi from Erdut, Croatia. The physicochemical properties of wine were alcohol 11.4%, volatile acidity 0.31 g/L as acetic acid, total acidity 5.1 g/L as tartaric acid, pH 3.4, reducing sugars 2.8 g/L and free SO_2 25 mg/L. Two separate HPU experiments were performed during this study. In each experiment, the effect of HPU on wine chemical composition was investigated with different combinations of process parameters. In the first experiment a bath sonicator (Elmasonic P, Elma Schmidbauer GmbH, Singen, Germany) was used for sonication of wine, operating at 37 kHz and 80 kHz frequencies. The total rated output power was 380 W. For ultrasound treatments, 200 mL of white wine was put into a 400 mL glass beaker and sonicated at amplitude levels of 40, 60 and 80%, and 20, 40 and 60 °C temperature during 20, 50, 65 and 90 min. The water bath temperature during the processes was controlled by adding cold water. The control sample was unsonicated wine. Each HPU treatment was done in duplicate [144 (72 × 2) trials in total]. For ultrasound treatments in the second experiment, 300 mL of white wine was placed in a 400 mL glass reactor and treated separately at amplitude levels of 25, 50, 75 and 100% using a 20 kHz probe sonicator (Q700, Qsonica Sonicators, Newton, CT, USA). The samples were sonicated with three ultrasonic probes with a diameter of 12.7, 19.1 and 25.4 mm during 3, 6 and 9 min, and the probe immerse depth was 2 cm. During HPU treatments, the wine sample temperature was maintained at 25 °C by ice-water cooling of the reactor. Each HPU treatment was conducted in duplicate [72 (36 × 2) trials in total]. The control sample was wine not subjected to HPU treatment. The process parameters for both bath and probe ultrasonic experiments were chosen based on the literature review [14,18,20,29,34,35] and preliminary investigations (data not shown).

2.3. HPU processing and bottle aging of white wine

According to obtained results of two different HPU techniques used in this study, ultrasonic bath system was selected for further investigation of HPU effect along with sulfite and GSH additions on white wine quality during storage period of 18 months. The effect of HPU processing was assessed with 80 kHz frequency and 100% amplitude at 27 °C for 30 min. The wine samples were prepared without and with addition of antioxidants (sulfite and GSH). The first sonicated standard-sulfite wine (SSW) contained 45 mg/L of free SO_2, the second one low-sulfite-GSH wine (SLGW) 25 mg/L plus 20 mg/L of GSH and the third sonicated low-sulfite wine (SLW) had 25 mg/L of free SO_2. The unsonicated standard-sulfite wine was used as a control wine (CONW). Afterwards, all wine samples (control and HPU treated) were bottled and stored at 12 °C for 18 months. Previously described wine variations were treated in triplicate and after that all measurements were performed after 0, 3, 6, 12 and 18 months of storage.

2.4. Spectrophotometric analyses

The content of total phenolics (TP) was determined with Folin-Ciocalteu's reagent [36]. The measurements of color properties and total color difference (ΔE*) were carried out using the CIELab [37].

2.5. Phenolic analysis by HPLC-DAD/MS

The phenolic acids and flavan-3-ols were analyzed by high-performance liquid chromatography-diode array detection/mass spectrometry (HPLC–DAD/MS) with direct injection of the 20 μL of sample, based on the method described by Monagas et al. [38]. The separation and detection of the compounds was carried out in a liquid chromatograph Agilent Technologies 1100 Series equipped with an automatic injector, diode array detector (DAD) and mass spectrometer fitted with an electrospray ionization source. A column Phenomenex Gemini C18 (4.6 mm × 250 mm, 5 μm) was used. The wine samples were filtered by 0.45 μm cellulose acetate syringe filters before injection into the equipment. A total of 13 compounds {9 phenolic acids (gallic acid, protocatechuic acid, vanillic acid, syringic acid, caftaric acid, chlorogenic acid, caffeic acid, *p*-coumaric acid and ferulic acid) and 4 flavan-3-ols [(+)-catechin, (-)-epicatechin, procyanidins B1 and B2]} were identified according to their retention times and the spectral properties (UV/Vis spectra and detected ions), respectively. Chromatographic and calibration parameters for determined phenolic compounds are presented (as a supplementary material) in Table S1. Total phenolic acids (TPA) and total flavan-3-ols (TF) contents were calculated as a sum of the contributions of individual compounds.

2.6. Volatile analysis by GC–MS

The volatile composition was determined by gas chromatography-mass spectrometry (GC–MS) following the protocol of Tomašević et al. [13]. The headspace solid-phase microextraction (HS-SPME) was used

for the extraction of volatiles. The analysis was performed on an Agilent 6890 GC fitted with an Agilent 5973 Inert MS. A total of 18 compounds [9 esters (ethyl acetate, *i*-butyl acetate, *i*-amyl acetate, ethyl hexanoate, hexyl acetate, ethyl octanoate, ethyl decanoate, diethyl succinate and 2-phenylethyl acetate), 4 higher alcohols (1-hexanol, *cis*-3-hexenol, amyl alcohol and phenylethyl alcohol), 2 terpenes (linalool and α-terpineol) and 3 volatile fatty acids (hexanoic acid, octanoic acid and decanoic acid)] were identified by comparing both the retention times and the mass spectrum with those of authentic standards, and the concentration of each compound was determined using their calibration curves. Chromatographic and calibration parameters for analyzed volatile compounds are presented (as a supplementary material) in Table S2. Total esters (TE), total higher alcohols (THA), total terpenes (TT) and total fatty acids (TFA) contents were calculated as a sum of individual compounds.

2.7. Statistical analysis

Data were evaluated with multivariate statistical analysis (MANOVA), followed by univariate analysis (ANOVA) using Statistica v.10.0. (StatSoft, Tulsa, USA). Furthermore, multilayered perceptron neural networks (MLPs) were developed for description of (i) phenolic composition (TP, TPA and TF) and (ii) volatile composition (TE, THA, TFA and TT) in white wine after both ultrasound experiments. The neural networks were developed by separating measured values for each analyzed variable in the ratio of 70:15:15 (learning:-training:validation). Back propagate error implemented into Statistica v.10.0. program was used to train ANNs. The optimal ANN architecture was selected by evaluating the root mean square errors (RMSE) and the linear correlation coefficients (R^2) calculate based on measured and predicted values. Moreover, one-way ANOVA and post-hoc Tukey's HSD test were used to evaluate the significant differences in aged wines.

3. Results and discussion

3.1. Changes in white wine quality induced by HPU processing

With a view to investigating the effect of HPU on the white wine quality characteristics, two different ultrasound experiments were performed. As shown in Table 1, white wine was treated by bath and probe ultrasonic systems at different operating conditions (inputs) and possible changes in analyzed parameters (outputs) were monitored. In supplementary Tables S3–S4 are shown the obtained results of these two experiments for color, phenolic and volatile composition of white wine, respectively. The results of statistical analysis (ANOVA) showed a significant effect of process parameters (inputs) and their interactions on measured parameters (outputs) in performed ultrasound experiments ($p < 0.0001$, $p < 0.001$, $p < 0.01$, $p < 0.05$, Wilk's lambda) (Table 2).

3.1.1. Phenolic composition

Regarding the first experiment with ultrasonic bath (Table 2 and Table S3), the bath temperature was the most important parameter affecting TP and TF, while amplitude had significantly higher impact on TPA (higher *F*-values), respectively. In general, HPU treatments obviously promoted changing of wine phenolic compounds, since the treated samples showed lower TP and TF content, whereas the slight increase or decrease of TPA was observed compared to unsonicated wine. Namely, increased bath temperature resulted in a higher TPA and TF content. Also, a combination of higher amplitude and bath temperature induced higher TPA content (Table 2 and Table S3). Table S3 shows that the experiments performed at the conditions of 100% and 40%, 80 kHz and 60 °C during 65 min of sonication resulted in the highest TPA and TF content. Contrary, the highest TP content was obtained under 100%, 80 kHz and 20 °C after 50 min of sonication (Table S3). Our earlier study showed that HPU treatment could initiate some chemical reactions in red wine due to effect of ultrasound cavitation [30]. Higher frequencies, larger amplitudes and intensities are necessary to generate cavitation [39], but this conditions may not always lead to the highest cavitation [40]. Interestingly, what can be observed is that the phenolic composition of red and white wines was affected by different HPU process parameters. In this study, the decisive factor was bath temperature. Namely, it is familiar that at higher temperatures, the vapor pressure increases leading to a less violent collapse, contributing to the reduced sonication effect [40]. Hence, the observed decrease in degradation rate of phenolic compounds with the temperature increase could be attributed to aforementioned phenomena. Moreover, the perceived differences in ultrasound influencing the phenolics in red and white wines may be due to the nature and amount of phenolics in given wines. Aside from that, the amount of specific phenolic groups, the concentrations of individual phenolics as well as potential interactions among them in wine are expected to provide some protection from free radical damage [41].

In the second experiment with ultrasonic probe (Table 2 and Table S4), ANOVA revealed that the probe diameter was the most important parameter affecting TPA and TF, while amplitude had significantly higher impact on TP (higher *F*-values), respectively. Moreover, the interaction of probe diameter and sonication time (X_1X_3) had significant impact on phenolic composition of treated wine. The highest TPA and TF content was determined at 100% amplitude after 3 and 9 min of sonication with a 19.1 mm probe, while the highest TP content was obtained with the same probe diameter at 50% amplitude after 9 min (Table S4). Similarly, as earlier presented in ultrasonic bath experiment, treated wines showed lower content of TP and TF, while TPA slightly increased or decreased, when compared to control wine. The observed changes in phenolic composition could be attributed to previous mentioned cavitation, which initiates some chemical reactions due to formation of free radicals [21]. Generally, wine phenolics are considered to act as antioxidants through chemical mechanisms such as free radical scavenging and metal chelation [42]. In white wine, phenolic acids and monomeric flavan-3-ols are the most important in terms of quantity and ability to participate in redox reactions [43]. When compared to our earlier research [30], it can be observed that partly different HPU process parameters have contributed to the changes in

Table 1
Experimental design for bath and probe ultrasonic processing.

	Inputs Operating process parameters	Outputs Quality characteristics
Bath ultrasonic experiment	Amplitude (%): 40, 60, 100	TP
	Frequency (kHz): 37, 80	TPA
	Bath temperature (°C): 20, 40, 60	TF
	Sonication time (min): 20, 50, 65, 90	Color properties
Probe ultrasonic experiment	Probe diameter (mm): 12.7, 19.1, 25.4	TE
	Amplitude (%): 25, 50, 75, 100	THA
	Sonication time (min): 3, 6, 9	TFA
		TT

Table 2
Summary of *F*-values of ANOVA for bath and probe ultrasonic effects on quality characteristics of white wine.

Bath ultrasonic experiment												
Source	TP	TPA	TF	L*	a*	b*	C*	H*	TE	THA	TFA	TT
Amplitude (X_1)	25.74[a]	**21.36**[a]	1.32	1.39	8.94[b]	136.52[a]	9.31[b]	47.21[a]	38.22[a]	4.19[d]	117.80[a]	**84.13**[a]
Frequency (X_2)	1.52	9.56[c]	12.54[b]	16.44[b]	1.06	188.45[a]	6.14[d]	**115.70**[a]	85.57[a]	4.36[d]	5.88[d]	41.05[a]
Bath temperature (X_3)	**98.29**[a]	9.84[b]	**593.43**[a]	**622.15**[a]	**158.58**[a]	**195.73**[a]	**63.40**[a]	33.82[a]	**983.59**[a]	**486.71**[a]	**178.40**[a]	3.96[d]
Sonication time (X_4)	3.86[d]	8.45[b]	17.44[a]	16.86[a]	16.21[a]	13.43[a]	45.44[a]	17.29[a]	587.98[a]	322.55[a]	44.01[a]	5.30[c]
X_1X_2	9.33[b]	36.05[a]	11.99[a]	10.70[b]	25.27[a]	5.98[c]	**63.44**[a]	2.80	17.61[a]	12.99[a]	1.43	6.94[c]
X_1X_3	3.37[d]	**90.86**[a]	**15.62**[a]	**18.21**[a]	**29.82**[a]	**142.97**[a]	6.62[b]	**139.04**[a]	6.31[b]	14.55[a]	**16.54**[a]	1.56
X_2X_3	8.07[b]	54.53[a]	0.87	1.65	28.74[a]	12.58[a]	2.14	1.65	22.28[a]	**27.22**[a]	5.62[c]	3.58[d]
X_1X_4	4.22[c]	4.03[c]	5.32[b]	5.31[b]	2.16	13.13[a]	7.92[a]	1.13	45.62[a]	19.88[a]	6.10[a]	7.73[a]
X_2X_4	9.52[a]	0.37	6.95[b]	7.20[b]	1.02	30.11[a]	1.71	4.59[c]	11.43[a]	9.46[a]	12.68[a]	4.82[c]
X_3X_4	4.80[b]	3.20[c]	6.95[a]	6.46[a]	3.79[c]	44.24[a]	5.97[a]	12.09[a]	20.55[a]	25.28[a]	13.00[a]	11.62[a]
$X_1X_2X_3$	**14.60**[a]	23.32[a]	2.35	2.59[d]	19.25[a]	9.68[a]	16.22[a]	15.87[a]	**73.91**[a]	17.03[a]	3.88[c]	**43.11**[a]
$X_1X_2X_4$	4.63[b]	1.07	0.87	0.91	0.88	45.73[a]	3.52[c]	6.39[a]	5.75[b]	6.61[a]	4.28[c]	3.05[d]
$X_1X_3X_4$	3.67[b]	2.11[d]	2.03[d]	1.99[d]	1.79	26.98[a]	2.75[c]	5.65[a]	12.58[a]	3.78[b]	2.54[c]	7.97[a]
$X_2X_3X_4$	4.13[c]	2.88[d]	2.83[d]	2.86[d]	1.56	65.87[a]	6.03[a]	6.97[a]	12.24[a]	8.30[a]	4.84[b]	12.44[a]
$X_1X_2X_3X_4$	4.16[b]	4.13[b]	1.13	1.33	2.61[c]	21.53[a]	2.11[d]	9.50[a]	17.64[a]	9.44[a]	4.96[a]	1.96[d]
Probe ultrasonic experiment												
Source	TP	TPA	TF	L*	a*	b*	C*	H*	TE	THA	TFA	TT
Probe diameter (X_1)	6.37[c]	**14.19**[a]	**59.59**[a]	36.91[a]	**35.36**[a]	117.54[a]	**38.47**[a]	3.91[d]	16.67[a]	**19.54**[a]	**15.93**[a]	**144.75**[a]
Amplitude (X_2)	**16.97**[a]	4.86[c]	6.24[c]	7.31[b]	7.76[b]	31.81[a]	21.15[a]	4.02[d]	**17.47**[a]	5.47[c]	7.18[b]	4.78[c]
Sonication time (X_3)	1.42	1.69	38.37[a]	**38.40**[a]	22.00[a]	**139.77**[a]	19.75[a]	**9.36**[b]	15.14[a]	8.23[c]	6.66[c]	34.72[a]
X_1X_2	19.41[a]	10.12[a]	16.33[a]	10.72[a]	13.02[a]	54.92[a]	**20.58**[a]	**3.99**[c]	**11.25**[a]	**16.91**[a]	7.95[a]	16.98[a]
X_1X_3	**44.12**[a]	**31.46**[a]	**52.89**[a]	**41.67**[a]	**55.98**[a]	155.49[a]	4.98[d]	2.01	11.06[a]	2.43	**13.81**[a]	**45.04**[a]
X_2X_3	5.96[b]	2.71[d]	5.73[b]	6.04[b]	4.99[b]	**158.46**[a]	6.07[b]	2.77[d]	8.75[a]	4.89[c]	4.85[c]	13.17[a]
$X_1X_2X_3$	17.97[a]	6.48[a]	7.44[a]	5.85[a]	8.34[a]	96.35[a]	4.22[b]	2.48[d]	9.02[a]	12.37[a]	8.53[a]	22.69[a]

[a] $p < 0.0001$, [b]$p < 0.001$, [c]$p < 0.01$, [d]$p < 0.05$. The significance of the parameters and their interactions that contribute most to the variation in the model outputs is marked bold. The degrees of freedom for the error terms for conducted experiments: df = 143 and df = 71.

phenolics of red and white wines, suggesting that different wine varieties can have different responses to treatment conditions, depending on the complexity of the wine matrix.

3.1.2. Color properties

Observing the effects of HPU process parameters as well as their interactions in first experiment on the color properties (Table 2 and Table S3) it can be stated that the bath temperature and the interaction amplitude × bath temperature (X_1X_3) were the most significant in influencing following values L*, a*, b* and C* (higher *F*-values). Generally, the wine samples obtained by this technique differed slightly in values of CIELab parameters from unsonicated (control) wine. Additionally, since the magnitude of color difference between the two samples is best represented by the total color difference value (ΔE*), this was also calculated. It is usually considered that the minimal detectable color difference is in the range of 1–2, while the values between 3.5 and 5 indicate that the difference is clearly perceptible [44]. The most of ΔE* values between sonicated and control wine samples ranged from 0.1 to 1.8, all being barely perceptible by the human eye. Only HPU treatments at conditions of 60% amplitude, 37 kHz and 60 °C after 50 and 90 min resulted in higher ΔE* values of 2.2 and 2.4 (Table S3). This means that color properties and phenolic compounds were both slightly altered by the same studied HPU parameters, namely bath temperature and amplitude. Apparently, the thermal effects in the bubble collapse and the formation of highly reactive species in HPU treatment triggered certain chemical reactions relevant for wine color. Additionally, the hydroxyl radicals produced by sonolysis of water can react with many wine components [45]. Namely, the phenolic composition is closely related to the sensory characteristics of wine, such as color, flavor and astringency [46]. In young white wines, oxidative processes are well known issue that influences their phenolic compounds and eventually their color [47]. It is thought that the oxidative browning of white wines is particularly related to the content of flavan-3-ols [42]. Especially, the oxidation of constituents like (+)-catechin, (-)-epicatechin, gallocatechin, gallic and caffeic acid, which are considered to be most easily oxidized in wine, contributes to previously mentioned chemical process [48].

In addition, among HPU process parameters in second experiment, the probe diameter and sonication time as well as their interaction had the most significant influence on the color properties (L*, a*, b* and C*) (Table 2). As can be seen in Table S4, in the most of HPU treated samples values L* and a* slightly decreased, whereas parameters b* and C* slightly increased, while H* stayed nearly equal in comparison to control wine. Furthermore, ΔE* between most of HPU treated and control samples ranged from of 0.2–3.8, all being very slight perceptible (Table S4). The greatest change in color (ΔE* = 4.6) was noticed at 100% amplitude after 6 min of sonication with 12.7 mm probe. Contrary, the sample sonicated with a larger probe diameter (25.4 mm) and 75% amplitude for 6 min showed the lowest value of ΔE*, meaning that it was the closest to the control sample (Table S4). It is important to highlight, that ΔE* values around 3 are considered as visually acceptable color tolerance level determined for red wines [49]. However, white wines are located in color space regions that are very different to those of red wines [50]. Hence, it is difficult to extrapolate this ΔE* value to our results and to determine its real significance. As previously explained, the slight color-related changes can be associated with those in phenolic compounds, primarily flavan-3-ols, which are generally assumed to contribute to the white wine color. Recently, Fu et al. [51] reported that the cavitation-generated free radicals might advance the formation of glyoxylic acid from tartaric acid in a model wine, and the subsequent reaction of glyoxylic acid and (+)-catechin, lastly resulting in the yellow pigments production. Comparing HPU effect on the color properties of red wine from our last study [30] with current results for the white wine, it is obvious that color of red wine was more affected by ultrasound, primarily because of the presence of anthocyanins which are known to be more sensitive and susceptible to degradation than other phenolic compounds. Finally, it is evident that HPU applied to wines with different chemical composition leads to different results.

3.1.3. Volatile composition

For bath ultrasonic treatments (Table 2 and Table S3), the obtained results demonstrated that among the four process variables, the bath temperature was the main parameter affecting TE, THA and TFA, whereas the amplitude had considerable impact on TT (higher *F*-

values). Also, the interaction effects of amplitude or frequency and bath temperature (X_1X_3, X_2X_3), and the one between amplitude, frequency and bath temperature ($X_1X_2X_3$) showed to have an important role in influencing the volatile composition of sonicated white wine. In general, HPU treated samples showed lower values in analyzed groups of volatile compounds depending on applied process conditions, when compared to control. Table S3 shows that the highest TE and THA content was observed at 100% amplitude, 37 kHz and 20 ˚C after 20 min of sonication. Moreover, as a result of an increase in the bath temperature and sonication time, particularly at highest conditions of 60 ˚C after 90 min, there was a decrease in contents of these groups of compounds, while opposite trend was observed in the content of TFA. Namely, esters are known to be more sensitive and susceptible to temperature changing than other volatile compounds. The changes in ester composition is due to the reaction of hydrolysis, which appears to be accelerated with the increase of temperature [52]. Interestingly, the HPU conditions of 60% amplitude, 80 kHz frequency, 60 ˚C and 90 min induced higher content of TFA and TT in treated wine. However, the results of TT showed no particular trend at different process operating conditions (Table S3). It is important to point out that, except color and phenolics, a certain volatile profile is also strongly associated with the sensory quality of wine, which loss might often be accelerated due to numerous factors. The observed changes in the volatile composition of sonicated wine could be due to the combination of the cavitation and thermal effects, which therefore results in a stronger sonochemical effect on the volatile compounds stability. It is known that the cavitation intensity will be decreased at the higher operating temperatures. However, the increase in temperature can lead to the increase of the reaction rate of oxidation [35]. In general, it is difficult to understand the effect of ultrasound temperature on the chemical reactions in complex solution such as wine, since the temperature can influence the gas solubility, surface tension and the vapor pressure of the solutes [35]. Recently, Zhang et al. [28] reported that the mechanical effects of ultrasound may also increase the molecular collisions and the diffusion coefficients, resulting in an increase of the degradation rate of the higher alcohols in a model wine. Moreover, the degassing effect of ultrasound was also considered as a possible explanation for the reduction of volatile esters in wines [26]. However, by subjecting wine to appropriate and controlled ultrasound treatment, positive sensory impacts could be achieved. Cui et al. [32] found that subjecting white wine to combined ultrasound (40 kHz, 20 min)/SO_2 (40 mg/L) treatment, resulted in wines with pleasant taste, specific variety flavor and aromas.

In second HPU experiment (Table 2 and Table S4), it was shown that the probe diameter was the most important parameter influencing THA, TFA and, in particular TT (higher *F*-values), while the amplitude had a greater impact on TE. Also, a certain part of variation in the volatile composition of sonicated wine was due to interactions between the probe diameter and amplitude or sonication time (X_1X_2, X_1X_3). Firstly, from Table S4 it can be seen that the lowest content of TE and THA was achieved at 50% amplitude after 6 and 9 min of sonication with a 12.7 mm probe. Secondly, ultrasound treatment of 50% amplitude and 3 min with a larger probe (19.1 mm) resulted in lower TFA and TT content compared to control wine. All together, these results demonstrated that HPU treated wines showed slightly lower values in volatile composition than unsonicated one. The application of HPU on wine can accelerate different chemical reactions (polymerization, condensation and oxidation) that involve compounds like esters, alcohols and aldehydes [53]. Researches Singleton and Draper et al. [26] conducted a study investigating the effect of direct contact ultrasound (90 kHz, 35 W, 60 min) on the phenolic and volatile profile of white wine, where a sensory panel confirmed the increase of tannin content and the formation of a negative “scorched” flavor. So, it seems that the changes occurring in volatiles of sonicated wine are apparently driven by oxidation reactions generated during HPU treatment. Namely, the collapse of cavitation bubbles formed during HPU produces free radicals, which can enhance the rate of oxidation reactions as discussed previously [53]. Furthermore, the main wine components like tartaric acid, ethanol, iron and copper ions, which are essential to the chemical reactions in wine, could fasten sonochemical degradation of higher alcohols, primarily due to ultrasound-generated free radicals and its subsequent reactions [28].

This confirms that it is still very difficult to interpret all the influencing variables when applying HPU on the complex wine matrix. In a whole, the results obtained in the above sections suggest that it is necessary to choose adequate HPU processing conditions, in order to produce the lowest degradation rate of phenolic and volatile compounds as well as to preserve the color and retain the overall quality of wine.

3.2. Modeling using ANN

ANN modeling approach was employed to describe and predict (i) the phenolic composition (TP, TPA and TF) and (ii) volatile composition (TE, THA, TFA and TT) in white wine after both ultrasound experiments (ultrasonic bath and ultrasonic probe). The models input variables were experimental conditions, as presented in Table 1. The similar approach was previously described by Lukić et al. [30], were ANNs were used to model the effects of bath and probe ultrasound processing on the young red wine chemical composition. Based on the presented results for the total color difference (ΔE) (Tables S3 and S4) it was concluded that the HPU treatments had no major effect on the color and that color properties will not be included into ANN modeling. For each HPU experiment two ANNs were developed, one for the prediction of the phenolic composition and one for the prediction of the volatile composition because of the different effect of the ultrasound on the analyzed model outputs. The architectures some of the proposed ANNs are presented in Table 3. In the case of ultrasonic bath experiment, results showed that there was higher linear correlation coefficients (R^2) for ANNs developed for prediction of volatile composition in comparison to those for prediction of phenolic composition. The optimum architecture was selected considering R^2, RMSE and amount of neurons (or nodes) in layer in between input layers and output layers (less neurons means simpler network). Based on that, MLP 4-8-3 was selected for the prediction of phenolic composition and MLP 4-9-4 was selected for prediction of volatile composition of white wine after ultrasonic bath treatments. The selected ANNs were defined by 4 nodes in the input layer, 8 (for phenolic composition) or 9 (for volatile composition) nodes in the hidden layer, and 3 (for phenolic composition) or 4 (for volatile composition) nodes in the output layer. For MLP 4-8-3 hidden and output activation function was Logistic, for MLP 4-9-4 hidden activation function was also Logistic, while the output activation function was Identity function. For the described ANNs correlation coefficients for training, test and validation were as follows: R^2_{training} (MLP 4-8-3) = 0.7889, R^2_{test} (MLP 4-8-3) = 0.7872, $R^2_{\text{validation}}$ (MLP 4-8-3) = 0.7804, R^2_{training} (MLP 4-9-4) = 0.8685, R^2_{test} (MLP 4-9-4) = 0.8711 and $R^2_{\text{validation}}$ (MLP 4-9-4) = 0.8305 (Table 3).

It is important to emphasize that ANN, developed for the prediction of both phenolic and volatile composition after treatments with ultrasonic probe, had higher R^2 values for training, test and validation and reduced RMSE values (Table 3) in comparison to ultrasonic bath experiment. This was opposite to the effect noticed by Lukić et al. [30] for red wine, where higher correlation coefficients were obtained for the ANN developed for the ultrasonic bath experiments. By analyzing the results in Table 3 it can also be noticed that there were higher linear correlation coefficients for ANNs developed for prediction of phenolic composition in comparison to those for prediction of volatile composition. For the prediction of phenolic composition, the highest value of correlation coefficients for training and validation and the lowest RMSE for validation were observed for MLP 3–5-3 (R^2_{training} = 0.9382, $R^2_{\text{validation}}$ = 0.9536, $\text{RMSE}_{\text{validation}}$ = 0.0145). Furthermore, for the prediction of volatile composition of white wine after ultrasonic probe treatments, the highest value of correlation coefficients for training and

Table 3
Architecture of ANN developed for prediction of phenolic and volatile composition of wine samples after HPU bath and probe experiments. The networks selected as the most suitable are marked bold.

Prediction		MLP network description	Training perf./ Training error	Test perf./ Test error	Validation perf./ Validation error	Hidden activation	Output activation
Bath ultrasonic experiment	Phenolic composition	4-9-3	0.7840 0.0223	0.7663 0.0338	0.7341 0.0387	Tanh	Identity
		4-10-3	0.7931 0.0215	0.7578 0.0342	0.7677 0.0361	Logistic	Logistic
		4-8-3	**0.7889** **0.0219**	**0.7872** **0.0338**	**0.7804** **0.0356**	**Logistic**	**Logistic**
		4-10-3	0.7987 0.0211	0.7527 0.0352	0.7689 0.0368	Logistic	Exponential
		4-9-3	0.7741 0.0228	0.7661 0.0331	0.7529 0.0374	Logistic	Exponential
	Volatile composition	4-8-4	0.8522 0.0288	0.8692 0.0268	0.8079 0.0526	Logistic	Identity
		4-8-4	0.8375 0.0314	0.8534 0.0320	0.8044 0.0520	Logistic	Exponential
		4-10-4	0.8828 0.0233	0.8689 0.0295	0.8099 0.0521	Logistic	Identity
		4-10-4	0.8568 0.0280	0.8674 0.0287	0.7959 0.0535	Logistic	Identity
		4-9-4	**0.8685** **0.0258**	**0.8711** **0.0294**	**0.8305** **0.0502**	**Logistic**	**Identity**
Probe ultrasonic experiment	Phenolic composition	3-9-3	0.9356 0.0134	0.9033 0.0215	0.9513 0.0151	Tanh	Logistic
		3-5-3	0.9258 0.0161	0.9047 0.0227	0.9431 0.0195	Tanh	Tanh
		3-9-3	0.9373 0.0131	0.9013 0.0223	0.9516 0.0146	Tanh	Tanh
		3-5-3	**0.9382** **0.0135**	**0.9014** **0.0219**	**0.9536** **0.0145**	**Tanh**	**Logistic**
		3-9-3	0.9362 0.0135	0.9062 0.0211	0.9510 0.0161	Tanh	Exponential
	Volatile composition	3-7-4	0.8216 0.0325	0.8367 0.0365	0.7888 0.0449	Logistic	Identity
		3-5-4	**0.8253** **0.0334**	**0.8398** **0.0361**	**0.8121** **0.0431**	**Logistic**	**Identity**
		3-10-4	0.8210 0.0325	0.8373 0.0364	0.7973 0.0433	Logistic	Logistic
		3-10-4	0.8031 0.0361	0.8448 0.0356	0.7781 0.0442	Tanh	Tanh
		3-9-4	0.8325 0.0307	0.8390 0.0355	0.7635 0.0478	Tanh	Identity

Table 4
Correlation coefficients for prediction of selected outputs after HPU bath and probe experiments.

Prediction		Output	Training	Test	Validation
Bath ultrasonic experiment	Phenolic composition	TP	0.7372	0.7564	0.7445
		TPA	0.7915	0.5683	0.5982
		TF	0.8381	0.8468	0.8984
	Volatile composition	TE	0.9161	0.9222	0.8677
		THA	0.8900	0.8645	0.8332
		TFA	0.8568	0.8725	0.8145
		TT	0.8112	0.8252	0.8070
Probe ultrasonic experiment	Phenolic composition	TP	0.9311	0.8600	0.7767
		TPA	0.9705	0.9512	0.9419
		TF	0.9778	0.9763	0.9879
	Volatile composition	TE	0.8394	0.7937	0.6580
		THA	0.9425	0.9672	0.9466
		TFA	0.7193	0.6983	0.5649
		TT	0.9413	0.9001	0.8974

validation and the lowest RMSE for validation were observed for MLP 3–5-4 ($R^2_{training}$ = 0.8253, $R^2_{validation}$ = 0.8121, $RMSE_{validation}$ = 0.0431). Based on the presented results, the described ANNs were selected as the optimal for the prediction: MLP 3–5-3 selected for the prediction of phenolic composition and MLP 3–5-4 selected for the prediction of phenolic composition. The performance of the selected ANN models to predict output variables (phenolic composition - TP, TPA and TF and volatile composition - TE, THA, TFA and TT) in both ultrasound experiments are given in Table 4 and Figs. 1 and 2.

Based on the presented results it can be stated that the highest correlations among experimental and ANN model predicted data in the case of phenolic composition after ultrasonic bath experiment were obtained for TF ($R^2_{training}$ = 0.8381, R^2_{test} = 0.8468, $R^2_{validation}$ = 0.8984) (Fig. 1.1c), followed by TP ($R^2_{training}$ = 0.7372, R^2_{test} = 0.7564, $R^2_{validation}$ = 0.7445) (Fig. 1.1a). On the other hand, the minimum acceptable outcome of ANN performance belonged to TPA (Fig. 1.1b). In the case of volatile composition prediction, the highest correlations among experimental and ANN model predicted data were obtained for TE ($R^2_{training}$ = 0.9161, R^2_{test} = 0.9222, $R^2_{validation}$ = 0.8677) (Fig. 1.1d), followed by THA ($R^2_{training}$ = 0.8900, R^2_{test} = 0.8645, $R^2_{validation}$ = 0.8332) (Fig. 1.1e) and TFA ($R^2_{training}$ = 0.8568, R^2_{test} = 0.8725, $R^2_{validation}$ = 0.8145) (Fig. 1.1f), while the biggest dispersion between data was noticed for TT ($R^2_{training}$ = 0.8112, R^2_{test} = 0.8252, $R^2_{validation}$ = 0.8070) (Fig. 1.1 g).

Further, the highest correlations among experimental and model predicted data in relation to phenolic composition after probe

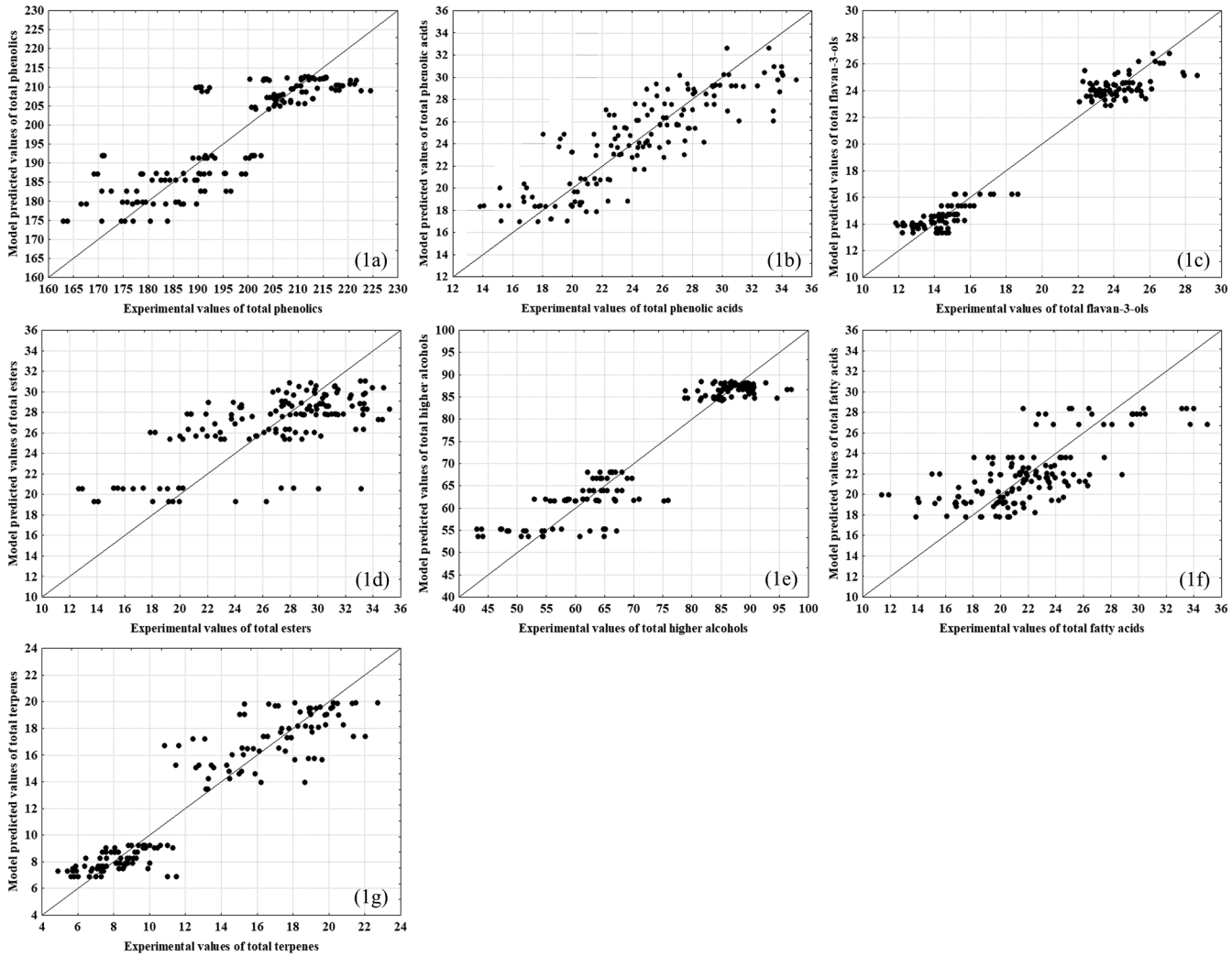

Fig. 1. Experimental and predicted results obtained by ANN for ultrasonic bath experiment: (1a) TP, (1b) TPA, (1c) TF, (1d) TE, (1e) THA, (1f) TFA and (1 g) TT.

ultrasonic experiment were obtained for TF ($R^2_{training}$ = 0.9778, R^2_{test} = 0.9763, $R^2_{validation}$ = 0.9879) (Fig. 2.2c), followed by TPA ($R^2_{training}$ = 0.9705, R^2_{test} = 0.9512, $R^2_{validation}$ = 0.9419) (Fig. 2.2b) and TP ($R^2_{training}$ = 0.9705, R^2_{test} = 0.9512, $R^2_{validation}$ = 0.9419) (Fig. 2.2a). In the case of volatile composition prediction, the highest correlations among experimental and ANN model predicted data were obtained for THA ($R^2_{training}$ = 0.9425, R^2_{test} = 0.9672, $R^2_{validation}$ = 0.9466) (Fig. 2.2e), followed by TT ($R^2_{training}$ = 0.9413, R^2_{test} = 0.9001, $R^2_{validation}$ = 0.8974) (Fig. 2.2 g) and TE ($R^2_{training}$ = 0.8394, R^2_{test} = 0.7937, $R^2_{validation}$ = 0.6580) (Fig. 2.2d), while the biggest dispersion between data was noticed for TFA ($R^2_{training}$ = 0.7193, R^2_{test} = 0.6983, $R^2_{validation}$ = 0.5649) (Fig. 2.2f). The presented results show the potential of usage of ANN modeling of wine composition because ANN methodology allows simultaneous analysis of the effect of the multiple variables at the multiple outputs. There is previously described usage of ANN methodology in wine composition analysis. For example, ANN modeling was used for classification of Slovak white wines [54] and the wines from South America [55], and to predict aging time in red wines [56].

3.3. The impact of HPU combined with sulfite and GSH treatments on the white wine quality during bottle storage

The results obtained for the chemical composition of white wine treated by HPU and antioxidants (sulfite and GSH) during 18 months of bottle storage are presented in Table 5. Firstly, there was a clear decline in TP and TF, whereas TPA content increased with time, independently from applied treatments. The reduction of flavan-3-ols during storage is well known, since these compounds can undergo oxidation and polymerization reactions [47]. The observed increase in the content of total phenolic acids can be primarily related to the increase in some of the individual phenolic acids (caffeic, *p*-coumaric and ferulic acid), which has been generally attributed to the hydrolysis of the corresponding hydroxycinnamic acid esters during wine aging [42,43]. In general, HPU treated samples showed slightly lower content of both total and individual phenolics along storage period compared to control wine. Particularly, HPU treatment after 18 months of storage significantly affected TP and TF, while there was no major difference in the content of TPA (Table 5). Moreover, after 6 months of storage, the statistical analysis revealed a significant difference ($p < 0.05$) among applied treatments with antioxidants (sulfite and GSH) of wines in TP and TPA content. After 18 months, these differences were also significant in the content of TP and TF, except in TPA (Table 5). In addition, the lowest concentrations of both total and individual phenolics were found in SLW. However, there were no significant differences in TPA and TF content among SSW and SLGW. Generally, the addition of antioxidants, such as sulfites and GSH, is the most common method used to protect wine from oxidation and to avoid forming of secondary characteristics, specific to wine aging process [57]. As already known, SO_2 can react with reduced form of oxygen (hydrogen peroxide) or remove or

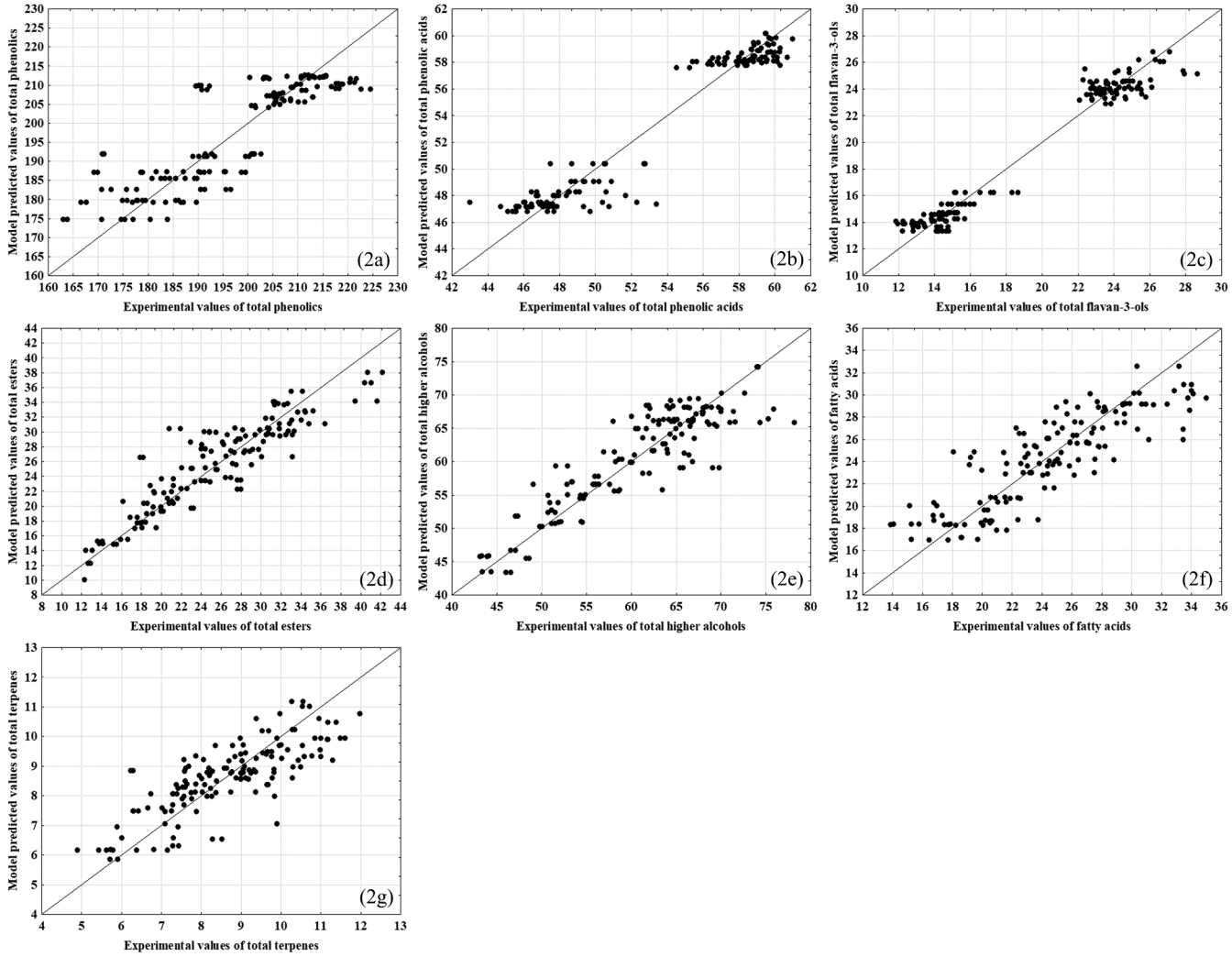

Fig. 2. Experimental and predicted results obtained by ANN for ultrasonic probe experiment: (2a) TP, (2b) TPA, (2c) TF, (2d) TE, (2e) THA, (2f) TFA and (2 g) TT.

stabilize the substrates susceptible to oxidation, such as phenolic and volatile compounds, while GSH has ability to scavenge quinones in wine acidic conditions and in this way may efficiently manage the oxidation mechanism [57].

Regarding the color properties, it was noticeable a decreasing trend in L* and a*, while parameters b* and C* slightly increased in the presented wine samples along the storage (Table 5). This indicates that there was a change in wine color from pale yellow to yellow-brown over the time. Also, the observed changes in color parameters are characteristic during the storage of white wines [42]. With respect to the effect of HPU treatment, there was no significant influence of this technique on the color properties of white wine after 18 months. Regarding the influence of sulfite and GSH treatments, there were no significant differences ($p < 0.05$) among the different treatments of wine samples, except in lightness in SLW compared to others. Namely, higher concentrations of sulfite and GSH resulted in higher L* value. Moreover, these samples had the total color difference (ΔE*) below 2.5 after 18 months of aging, meaning that the difference in color in these cases was slightly perceptible. In addition, SLW showed ΔE* higher than 5, but only after 12 and 18 months of storage, which means that the difference in color is noticeable (Table 5). In general, the appearance of browning due to oxidation reactions is considered as a decrease in the quality of white wine. Regarding the antioxidants used in this study, it was previously reported that SO_2 alone and GSH in the combination with SO_2 could reduce the oxidative color changes and the formation of xanthylium pigments [12]. Moreover, earlier researches also reported that the addition of GSH in the white wine production has a positive effect, specifically in view of protecting volatile compounds and color stability [10,11]. However, the role of this antioxidant and its complementary action with SO_2 is still not clear and has to be further investigated [57].

In relation to the volatile composition, a decreasing trend can be observed in TT content for all presented wines along the storage, whereas TE, TFA and THA content slightly increased, independently of treatments applied (Table 5). In addition, the slight increase in the content of TE was primarily due to increase of two individual volatile compounds, precisely diethyl succinate and ethyl acetate during the storage of wine, while other analyzed esters which were included for calculating the TE content decreased. Earlier studies on white wines also revealed changes in the volatile composition during their aging period [13,47,52]. Namely, esters are the main source of fruity aromas and their decrease during storage is due to hydrolysis reaction [52]. In relation to higher alcohols, these compounds are mostly stable over time, but their equally possible increment is considered to derive from hydrolysis of acetate esters [58]. Moreover, there was no clear trend established for volatile fatty acids during aging in the present literature, since some of these compounds can increase or decrease or stay unchanged [59]. In general, slightly lower TE, THA, TFA and TT content was found in HPU treated samples in comparison with CONW. As can be seen from Table 5, HPU treatment after 18 months of storage

Table 5
Impact of combined HPU and antioxidants (sulfite and GSH) treatments on the white wine quality during 18 months of storage.

Quality characteristics	Months	CONW	SSW	SLGW	SLW
TP (mg/L)	0	226.91 ± 0.39^{a}	223.32 ± 0.45^{a}	210.41 ± 1.22^{b}	206.91 ± 1.67^{b}
	3	221.88 ± 2.53^{a}	216.33 ± 0.71^{a}	206.38 ± 0.53^{b}	200.50 ± 1.30^{b}
	6	213.29 ± 0.18^{a}	206.71 ± 0.53^{b}	194.79 ± 0.53^{c}	181.63 ± 1.36^{d}
	12	198.05 ± 0.19^{a}	193.71 ± 0.65^{a}	178.27 ± 0.39^{b}	166.73 ± 3.40^{c}
	18	179.38 ± 0.61^{a}	175.55 ± 0.39^{b}	160.06 ± 1.30^{c}	154.99 ± 0.95^{d}
TPA (mg/L)	0	54.83 ± 0.13^{a}	53.92 ± 0.22^{ab}	52.95 ± 0.12^{b}	52.88 ± 0.51^{b}
	3	56.44 ± 0.16^{a}	55.65 ± 0.14^{b}	54.71 ± 0.06^{c}	54.41 ± 0.08^{c}
	6	60.21 ± 0.01^{a}	59.47 ± 0.14^{b}	56.98 ± 0.04^{c}	56.20 ± 0.26^{d}
	12	69.93 ± 0.18^{a}	69.62 ± 0.50^{a}	66.48 ± 0.31^{b}	65.88 ± 0.09^{b}
	18	66.94 ± 0.29^{a}	66.42 ± 0.25^{ab}	65.86 ± 0.12^{b}	65.54 ± 0.23^{b}
TF (mg/L)	0	24.66 ± 0.12^{a}	24.24 ± 0.04^{a}	22.51 ± 0.02^{b}	21.70 ± 0.13^{c}
	3	24.38 ± 0.07^{a}	23.94 ± 0.47^{a}	21.85 ± 0.26^{b}	21.53 ± 0.44^{b}
	6	23.27 ± 0.19^{a}	22.38 ± 0.13^{b}	21.16 ± 0.18^{c}	21.02 ± 0.11^{c}
	12	22.46 ± 0.30^{a}	21.56 ± 0.27^{ab}	19.92 ± 0.02^{bc}	19.38 ± 0.82^{c}
	18	21.97 ± 0.05^{a}	20.02 ± 0.32^{b}	19.34 ± 0.17^{bc}	18.42 ± 0.40^{c}
L*	0	99.75 ± 0.07^{a}	99.56 ± 0.24^{a}	99.78 ± 0.05^{a}	99.66 ± 0.43^{a}
	3	99.38 ± 0.09^{a}	99.11 ± 0.13^{ab}	98.78 ± 0.15^{b}	98.13 ± 0.03^{c}
	6	98.46 ± 0.19^{ab}	98.73 ± 0.34^{a}	98.29 ± 0.17^{ab}	97.58 ± 0.16^{b}
	12	97.55 ± 0.05^{b}	98.31 ± 0.14^{a}	97.09 ± 0.08^{c}	92.05 ± 0.09^{d}
	18	95.90 ± 1.26^{a}	97.51 ± 0.43^{a}	94.02 ± 0.16^{a}	90.00 ± 1.20^{b}
a*	0	−0.90 ± 0.10^{ab}	−1.06 ± 0.02^{b}	−0.90 ± 0.03^{ab}	−0.79 ± 0.02^{a}
	3	−0.84 ± 0.02^{a}	−0.84 ± 0.06^{a}	−0.85 ± 0.04^{a}	−0.65 ± 0.13^{a}
	6	−0.79 ± 0.19^{ab}	−0.93 ± 0.34^{b}	−0.91 ± 0.17^{b}	−0.54 ± 0.16^{a}
	12	−0.91 ± 0.05^{b}	−0.93 ± 0.14^{b}	−0.70 ± 0.08^{a}	−0.95 ± 0.09^{b}
	18	−1.07 ± 1.26^{a}	−0.91 ± 0.43^{a}	−0.85 ± 0.16^{a}	−0.97 ± 1.20^{a}
b*	0	4.66 ± 0.03^{b}	5.15 ± 0.10^{a}	5.22 ± 0.13^{a}	5.12 ± 0.16^{ab}
	3	4.75 ± 0.33^{b}	5.04 ± 0.01^{ab}	5.24 ± 0.01^{ab}	5.55 ± 0.12^{a}
	6	4.65 ± 0.23^{b}	5.24 ± 0.07^{b}	6.52 ± 0.19^{a}	6.22 ± 0.14^{a}
	12	5.86 ± 0.22^{a}	5.65 ± 0.12^{a}	5.82 ± 0.36^{a}	5.68 ± 0.15^{a}
	18	5.18 ± 0.74^{a}	5.14 ± 0.09^{a}	6.39 ± 0.52^{a}	5.70 ± 0.88^{a}
C*	0	4.75 ± 0.05^{b}	5.26 ± 0.10^{a}	5.30 ± 0.12^{a}	5.18 ± 0.16^{ab}
	3	4.83 ± 0.33^{b}	5.11 ± 0.02^{ab}	5.30 ± 0.02^{ab}	5.58 ± 0.14^{a}
	6	4.72 ± 0.24^{b}	5.32 ± 0.05^{b}	6.59 ± 0.19^{a}	6.25 ± 0.13^{a}
	12	5.90 ± 0.21^{a}	5.73 ± 0.11^{a}	5.86 ± 0.36^{a}	5.76 ± 0.15^{a}
	18	5.30 ± 0.68^{a}	5.22 ± 0.09^{a}	6.44 ± 0.54^{a}	5.79 ± 0.82^{a}
H*	0	−1.40 ± 0.00^{b}	−1.37 ± 0.01^{a}	−1.40 ± 0.01^{b}	−1.42 ± 0.00^{b}
	3	−1.30 ± 0.13^{a}	−1.41 ± 0.01^{a}	−1.41 ± 0.01^{a}	−1.45 ± 0.02^{a}
	6	−1.39 ± 0.00^{a}	−1.40 ± 0.02^{a}	−1.43 ± 0.00^{a}	−1.48 ± 0.02^{b}
	12	−1.28 ± 0.20^{a}	−1.41 ± 0.01^{a}	−1.45 ± 0.01^{a}	−1.41 ± 0.00^{a}
	18	−1.36 ± 0.07^{a}	−1.38 ± 0.02^{a}	−1.45 ± 0.03^{a}	−1.40 ± 0.07^{a}
ΔE*	0	−	0.57 ± 0.16^{a}	0.56 ± 0.12^{a}	0.58 ± 0.06^{a}
	3	−	0.40 ± 0.08^{c}	0.78 ± 0.11^{b}	1.50 ± 0.02^{a}
	6	−	0.70 ± 0.09^{b}	1.89 ± 0.18^{a}	1.82 ± 0.21^{a}
	12	−	0.79 ± 0.16^{b}	0.56 ± 0.07^{b}	5.50 ± 0.08^{a}
	18	−	1.61 ± 0.42^{b}	2.28 ± 0.13^{b}	5.96 ± 1.27^{a}
TE (mg/L)	0	29.99 ± 0.68^{a}	29.23 ± 0.85^{ab}	27.19 ± 0.11^{b}	24.94 ± 0.09^{c}
	3	30.53 ± 0.01^{a}	29.47 ± 0.29^{b}	27.80 ± 0.26^{c}	25.21 ± 0.01^{d}
	6	31.18 ± 0.90^{a}	30.87 ± 0.77^{ab}	28.57 ± 0.04^{b}	25.36 ± 0.01^{c}
	12	31.43 ± 0.39^{a}	30.99 ± 0.89^{a}	28.58 ± 0.14^{b}	27.31 ± 0.64^{b}
	18	35.47 ± 0.77^{a}	33.36 ± 1.30^{a}	29.12 ± 0.32^{b}	28.83 ± 0.08^{b}
THA (mg/L)	0	81.30 ± 0.57^{a}	78.67 ± 0.06^{b}	77.60 ± 0.77^{b}	74.88 ± 0.05^{c}
	3	82.71 ± 0.15^{a}	80.64 ± 0.31^{b}	80.06 ± 0.00^{b}	77.30 ± 0.65^{c}
	6	93.26 ± 0.51^{a}	83.95 ± 0.02^{b}	83.67 ± 0.01^{b}	80.37 ± 0.41^{c}
	12	94.71 ± 0.58^{a}	85.50 ± 0.10^{b}	85.41 ± 0.86^{b}	84.73 ± 0.08^{b}
	18	96.92 ± 0.15^{a}	96.75 ± 0.42^{a}	95.54 ± 0.03^{ab}	95.05 ± 0.57^{b}
TFA (mg/L)	0	13.66 ± 0.06^{a}	12.95 ± 0.02^{a}	11.67 ± 0.00^{b}	11.50 ± 0.37^{b}
	3	14.51 ± 0.08^{a}	14.47 ± 0.23^{a}	14.40 ± 0.13^{a}	13.08 ± 0.13^{b}
	6	18.55 ± 0.03^{a}	16.02 ± 0.11^{b}	15.61 ± 0.06^{ab}	15.26 ± 0.23^{c}
	12	20.15 ± 0.16^{a}	17.42 ± 0.04^{b}	16.82 ± 0.07^{c}	15.67 ± 0.12^{d}
	18	24.34 ± 0.00^{a}	23.42 ± 0.17^{b}	21.24 ± 0.01^{c}	21.21 ± 0.11^{c}
TT (μg/L)	0	23.82 ± 0.02^{a}	23.80 ± 0.08^{a}	23.44 ± 0.11^{b}	23.24 ± 0.17^{b}
	3	21.83 ± 0.06^{a}	20.06 ± 0.59^{b}	19.44 ± 0.12^{bc}	18.55 ± 0.30^{c}
	6	18.80 ± 0.18^{a}	17.34 ± 0.05^{b}	17.04 ± 0.00^{b}	15.78 ± 0.16^{c}
	12	16.55 ± 0.31^{a}	14.92 ± 0.11^{b}	14.89 ± 0.05^{b}	14.83 ± 0.16^{b}
	18	10.61 ± 0.39^{a}	9.12 ± 0.09^{b}	8.77 ± 0.05^{b}	8.28 ± 0.15^{b}

CONW-control wine, SSW-sonicated standard-sulfite wine, SLGW-sonicated low-sulfite-GSH wine and SLW-sonicated low-sulfite wine. Results are expressed as mean value ± standard deviation (N = 6). For each quality characteristic, values with different letters are significantly different between the samples at the same time (ANOVA, $p < 0.05$).

significantly influenced the content of TFA and TT, whereas there were no significant differences in TE and THA content compared with CONW. Regarding the influence of sulfite and GSH treatments, the lowest content of all analyzed volatile compounds was found in SLW. Additionally, there was no important distinction in THA and TT content among SSW and SLGW samples. In a whole, these results suggest that finding suitable ultrasound treatment (particularly proper temperature and exposure time) is crucial for the maintenance of fresh fruity aromas

and that in this way the volatile composition would not be compromised by the application of HPU at long term.

4. Conclusions

The applied HPU process parameters and their interactions in bath and probe ultrasonic experiments influenced differently the phenolic and volatile composition of white wine, while there were no major changes in the color properties. In bath ultrasonic experiment, temperature of bath was the most significant parameter, where higher bath temperature (60 °C) primarily affected the volatile composition, precisely TE and THA, and to a much lesser extent phenolic composition. Regarding probe ultrasonic experiment, the selection of the probe diameter, followed by amplitude were the most influencing parameters, where especially a larger probe diameter (19.1 mm) in combination with higher ultrasound amplitude (50–100%) resulted in a more favorable effect on the phenolic and volatile composition. When considering ANN predictions, the best agreements between experimental and model predicted results were obtained for the TF, TE and THA, respectively. Furthermore, HPU treatment did not affect color properties after 18 months of aging, but it showed impact on phenolic and volatile compounds. Particularly, the lower content of these compounds was found in HPU treated samples with lower concentration of antioxidants. On the other hand, sonicated standard-sulfite and low-sulfite-GSH wines showed similar content of phenolic and volatile compounds. Therefore, HPU combined with lower sulfite content and GSH could allow a reduction in the addition of sulfites to wine.

CRediT authorship contribution statement

Katarina Lukić: Conceptualization, Methodology, Formal analysis, Investigation, Visualization, Writing - original draft, Writing - review & editing. **Mladen Brnčić:** Resources, Supervision. **Natka Ćurko:** Conceptualization, Methodology, Formal analysis, Investigation, Visualization, Writing - original draft, Writing - review & editing, Writing - review & editing. **Marina Tomašević:** Writing - review & editing. **Ana Jurinjak Tušek:** Formal analysis, Investigation, Visualization. **Karin Kovačević Ganić:** Resources, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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Table S1 Chromatographic and calibration parameters for all analyzed phenolic compounds

Compounds name	Retention time (min)	λ_{max} (nm)	$[M-H]^-$ (m/z)	Concentration range (N=6) (mg/L)	r^2	Linear equation	
						Slope (a)	Intercept (b)
Gallic acid	6.68	280	169	0.22-121.2	0.999	72.4212233	-209.09613
Protocatechuic acid	11.09	280	153	0.23-124.4	0.999	40.6025114	-63.999362
Vanillic acid	18.73	280	167	0.25-136.4	0.999	42.4728047	-137.9969
Syringic acid	20.39	280	197	0.23-128.4	0.999	70.2483108	-114.19083
Caftaric acid	14.88	320	311	0.41-99	0.999	67.3614002	-61.173466
Chlorogenic acid	18.39	320	353	0.26-140.8	0.999	68.2724895	-135.00605
Caffeic acid	19.31	320	179	0.23-125.6	0.999	129.555693	-235.94176
p-coumaric acid	24.58	320	163	0.24-129.6	0.999	152.432194	-130.69723
Ferulic acid	26.00	320	193	0.22-120	0.999	137.942403	-320.60336
(+)-catechin	15.83	280	289	0.43-102	0.999	19.1179056	-48.598441
(-)-epicatechin	20.02	280	289	0.25-60	0.999	15.3520835	-23.815175
Procyanidin B1	14.07	280	577	0.42-100	0.999	16.5445368	-22.915447
Procyanidin B2	17.23	280	577	0.36-85.71	0.998	14.958869	-19.540889

Table S2 Chromatographic and calibration parameters for all analyzed volatile compounds

Compounds name	Retention time (min)	Retention index	Target and qualifier ions (m/z)	Concentration range (N=6) (mg/L)	r^2	Linear equation[1]	
						Slope (a)	Intercept (b)
Ethyl acetate	5.04	881	**43**, 103, 88	12.7-253.8	0.998	0.115	0.0506
i-butyl acetate	8.62	998	**43**, 56, 73	0.02-0.6	0.999	0.0159	0.0127
i-amyl acetate	13.54	1126	**43**, 70, 55, 87	0.1-6	0.999	39.4	0.0124
amyl alcohol	17.17	1217	**55**, 42, 70, 57	30-620	0.999	0.918	0.325
Ethyl hexanoate	18.56	1239	**88**, 99, 43	0.04-1.2	0.999	162	-0.0825
Hexyl acetate	20.30	1279	**43**, 56, 55, 84	0.17-5.1	0.999	112	-0.0182
1-hexanol	23.93	1346	**56**, 43, 55, 69	0.06-1.8	0.999	4.23	0.0663
cis-3-hexenol	25.34	1376	**67**, 41, 82	0.02-0.6	0.999	0.0013	0.000002
Ethyl octanoate	27.96	1434	**88**, 101, 127, 57	0.1-3	0.999	708	-0.609
Linalool	32.90	1537	**71**, 93, 55, 121	1.4-105.3[2]	0.999	0.0158	0.0012
Ethyl decanoate	36.35	1638	**88**, 101, 155	0.018-3.24	0.998	728	0.508
Diethyl succinate	37.54	1670	**101**, 129, 73, 55	0.7-21	0.999	2.30	-0.0048
α-terpineol	38.80	1683	**59**, 121, 136, 93	0.8-96.9[2]	0.999	0.016	0.0028
2-phenylethyl acetate	42.67	1805	**104**, 43, 91	0.2-4	0.999	88.5	0.371
Hexanoic acid	43.62	1863	**60,** 73, 87	0.6-21.3	0.998	3.005	0.000
Phenylethyl alcohol	46.95	1899	**91**, 92, 122	6-120	0.999	1.05	-0.180
Octanoic acid	50.86	2083	**60,** 73, 101, 43	0.19-6.2	0.998	9.193	0.000
Decanoic acid	57.43	2296	**73,** 60, 129	0.09-3.17	0.998	25.84	0.000

[1] Equation: $A_C/A_{IS} = a (C_C/C_{IS}) + b$, where A_C- area of aroma compound, A_{IS}- area of internal standard, C_C- concentration of aroma compound and C_{IS}- concentration of internal standard; [2] expressed in µg/L

Table S3 Impact of ultrasonic bath processing on the white wine quality

Bath ultrasonic experiment															
	Inputs*				Outputs											
Run	X_1	X_2	X_3	X_4	TP (mg GAE/L)	TPA (mg/L)	TF (mg/L)	L^*	a^*	b^*	C^*	H^*	ΔE^*	TE (mg/L)	THA (mg/L)	TFA (mg/L)	TT (μg/L)
0	0	0	0	0	206.14±0.32	48.29±0.80	17.97±0.41	98.67±0.34	-0.89±0.02	6.01±0.19	6.07±0.19	-1.42±0.01	–	44.43±0.64	69.93±2.04	37.21±0.38	11.66±0.26
1	100	80	20	20	198.36±0.13	47.64±0.00	16.62±0.81	98.67±0.00	-0.85±0.02	5.90±0.02	5.96±0.02	-1.43±0.00	0.11±0.02	40.68±0.46	68.35±0.03	24.68±0.30	10.37±0.67
2	100	80	20	50	204.59±0.96	47.99±0.08	16.33±0.00	98.56±0.24	-0.83±0.01	5.97±0.09	6.02±0.09	-1.43±0.00	0.21±0.11	34.04±0.56	64.95±0.94	25.71±2.98	10.16±1.11
3	100	80	20	65	189.73±1.29	48.50±0.16	17.26±0.12	98.68±0.03	-0.83±0.01	5.97±0.04	6.03±0.04	-1.43±0.00	0.08±0.02	31.32±0.70	62.41±0.13	26.73±0.54	10.31±0.04
4	100	80	20	90	189.77±0.45	49.15±0.15	16.27±0.00	98.62±0.06	-0.81±0.00	6.08±0.00	6.13±0.00	-1.44±0.00	0.12±0.03	24.20±0.27	56.10±0.30	26.42±0.77	9.44±0.54
5	100	80	40	20	190.91±0.64	48.22±0.11	14.09±0.27	98.61±0.09	-0.84±0.00	5.98±0.03	6.04±0.03	-1.43±0.00	0.10±0.05	33.59±0.78	64.28±0.49	23.47±0.57	8.85±0.23
6	100	80	40	50	189.23±1.35	49.34±0.35	14.41±1.01	98.24±0.28	-0.80±0.02	6.24±0.07	6.29±0.06	-1.44±0.00	0.50±0.28	27.79±0.17	57.49±0.82	26.90±2.96	10.25±0.41
7	100	80	40	65	189.55±0.39	49.97±0.31	14.55±0.03	98.39±0.05	-0.81±0.01	6.33±0.01	6.38±0.01	-1.44±0.00	0.43±0.02	23.03±0.05	53.35±0.07	28.52±0.59	9.32±0.31
8	100	80	40	90	186.64±1.29	50.78±0.33	14.99±0.11	98.34±0.03	-0.80±0.01	6.49±0.01	6.54±0.01	-1.45±0.00	0.60±0.00	20.98±0.30	49.90±0.18	27.95±0.27	9.09±0.14
9	100	80	60	20	196.09±0.64	43.12±0.87	13.82±0.50	98.15±0.07	-0.85±0.00	6.77±0.11	6.82±0.11	-1.45±0.00	0.93±0.05	31.81±0.70	61.78±0.15	24.42±1.99	7.83±0.57
10	100	80	60	50	194.59±0.58	45.18±0.06	16.70±0.12	97.42±0.37	-0.73±0.01	6.82±0.90	6.84±0.91	-1.46±0.01	1.56±0.76	28.26±1.07	69.42±0.50	25.19±1.40	9.00±0.02
11	100	80	60	65	190.05±0.84	54.21±0.44	14.69±0.03	97.78±0.08	-0.83±0.03	7.19±0.09	7.22±0.10	-1.48±0.02	1.48±0.02	18.39±0.28	51.10±0.02	30.79±0.21	7.93±0.49
12	100	80	60	90	190.27±0.26	48.36±0.76	12.92±0.21	97.71±0.29	-0.76±0.05	7.39±0.00	7.42±0.01	-1.48±0.02	1.70±0.17	12.71±0.48	46.28±0.36	33.41±0.81	8.07±0.18
13	100	37	20	20	197.00±0.26	43.33±0.09	16.47±0.83	98.44±0.31	-0.73±0.01	5.87±0.08	5.92±0.08	-1.45±0.00	0.36±0.17	41.40±1.04	74.08±0.08	26.18±0.15	7.64±0.16
14	100	37	20	50	186.59±0.32	43.36±0.56	14.71±0.50	98.64±0.08	-0.73±0.01	5.87±0.08	5.91±0.07	-1.45±0.00	0.22±0.03	32.18±0.64	66.97±0.73	23.18±0.44	9.07±0.42
15	100	37	20	65	189.09±0.64	43.89±0.09	14.83±0.19	98.47±0.07	-0.70±0.04	6.02±0.08	6.06±0.07	-1.46±0.01	0.28±0.08	24.55±0.37	61.05±0.82	27.98±0.32	8.38±0.99
16	100	37	20	90	191.59±0.19	45.37±1.46	13.96±0.30	97.81±0.15	-0.71±0.16	6.70±0.41	6.74±0.43	-1.47±0.02	1.16±0.11	24.20±0.27	56.10±0.30	26.42±0.77	9.44±0.54
17	100	37	40	20	176.64±4.50	44.44±1.53	14.13±0.25	98.97±0.03	-0.86±0.01	6.32±0.00	6.38±0.00	-1.44±0.00	0.43±0.02	34.79±0.60	71.29±1.82	31.06±4.00	9.31±2.06
18	100	37	40	50	182.50±5.85	45.77±0.40	14.48±0.26	98.48±0.36	-0.73±0.18	6.49±0.37	6.53±0.39	-1.46±0.02	0.66±0.13	32.15±1.40	76.24±2.67	31.92±2.16	11.16±0.01
19	100	37	40	65	175.45±1.54	46.61±0.91	15.13±1.31	98.56±0.31	-0.85±0.02	6.90±0.14	6.95±0.14	-1.45±0.00	0.93±0.17	26.27±1.25	63.38±4.27	22.63±0.23	10.29±1.86
20	100	37	40	90	187.77±0.19	45.33±0.54	14.64±0.12	98.61±0.10	-0.84±0.02	7.04±0.04	7.09±0.04	-1.45±0.00	1.03±0.04	18.26±0.23	53.27±1.57	26.19±1.62	9.71±0.07
21	100	37	60	20	181.63±0.40	45.69±0.53	14.77±0.29	98.31±0.18	-0.81±0.00	7.01±0.01	7.06±0.01	-1.46±0.00	1.07±0.06	25.30±1.63	68.04±2.11	30.65±4.88	10.97±1.41
22	100	37	60	50	186.32±1.48	48.36±0.92	13.62±0.46	98.20±0.02	-0.80±0.00	7.48±0.07	7.53±0.07	-1.46±0.00	1.55±0.07	20.05±1.63	52.22±0.97	26.65±1.48	10.40±0.20
23	100	37	60	65	196.91±0.26	48.56±0.29	15.34±0.39	98.44±0.06	-0.80±0.00	7.45±0.18	7.49±0.18	-1.46±0.00	1.46±0.18	17.23±0.49	51.36±0.63	30.88±2.11	10.62±0.12
24	100	37	60	90	195.77±0.96	47.61±1.16	14.27±0.93	98.47±0.01	-0.80±0.01	7.54±0.00	7.58±0.00	-1.47±0.00	1.55±0.00	12.28±0.01	43.88±0.68	30.43±1.45	9.61±0.12
25	60	80	20	20	171.32±0.06	44.49±0.24	15.82±0.40	98.79±0.12	-0.84±0.03	6.25±0.13	6.30±0.14	-1.44±0.00	0.30±0.06	40.52±1.58	70.65±0.98	17.07±0.88	10.57±0.58
26	60	80	20	50	176.32±5.72	45.25±0.37	15.89±0.40	98.82±0.25	-0.87±0.00	6.29±0.11	6.35±0.10	-1.43±0.00	0.38±0.02	33.54±0.67	61.37±4.79	15.51±0.36	7.75±0.16
27	60	80	20	65	172.14±1.09	42.82±1.07	16.33±0.28	98.87±0.22	-0.83±0.06	6.08±0.41	6.14±0.41	-1.44±0.00	0.40±0.04	32.32±0.72	70.01±2.20	23.05±0.95	8.99±1.19
28	60	80	20	90	173.77±0.45	44.00±0.27	14.65±0.50	98.84±0.24	-0.66±0.01	5.85±0.11	5.89±0.11	-1.46±0.00	0.36±0.16	35.59±1.12	68.93±0.45	18.29±0.76	7.48±0.56
29	60	80	40	20	175.14±0.32	45.98±0.10	15.74±0.43	98.84±0.09	-0.61±0.01	6.15±0.10	6.18±0.09	-1.47±0.01	0.36±0.00	30.78±0.47	67.93±0.06	19.12±1.27	8.50±0.69
30	60	80	40	50	198.09±3.99	46.44±0.13	16.28±0.12	98.81±0.06	-0.60±0.00	6.22±0.00	6.25±0.00	-1.48±0.00	0.39±0.02	23.23±1.68	64.96±1.29	24.49±0.44	9.05±0.44
31	60	80	40	65	191.09±1.54	46.07±0.03	16.85±0.12	98.73±0.43	-0.56±0.07	6.23±0.24	6.25±0.23	-1.48±0.01	0.52±0.09	24.10±1.06	65.57±0.22	25.08±1.50	7.57±0.05
32	60	80	40	90	186.77±0.84	48.07±1.28	15.80±0.43	98.85±0.15	-0.65±0.00	6.00±0.02	6.04±0.02	-1.46±0.00	0.31±0.09	20.11±1.25	61.07±3.36	25.32±3.05	6.26±0.04
33	60	80	60	20	204.05±0.45	45.89±0.01	14.69±0.07	98.62±0.18	-0.91±0.01	7.11±0.05	7.17±0.05	-1.44±0.00	1.12±0.05	25.13±0.45	60.93±1.29	16.04±1.27	6.30±0.01
34	60	80	60	50	195.05±4.82	48.33±0.17	14.86±0.31	98.24±0.39	-0.89±0.00	7.55±0.04	7.60±0.04	-1.45±0.00	1.62±0.14	18.59±3.47	50.91±2.68	21.04±2.60	8.05±0.44
35	60	80	60	65	173.91±0.77	48.08±1.90	14.78±0.01	98.46±0.37	-0.88±0.02	7.41±0.29	7.46±0.29	-1.45±0.01	1.44±0.34	17.73±0.22	51.09±0.55	32.29±1.63	10.39±0.13
36	60	80	60	90	197.68±0.32	50.34±0.52	14.54±0.29	98.54±0.29	-0.88±0.02	7.44±0.08	7.49±0.08	-1.45±0.00	1.45±0.11	13.80±0.34	46.79±0.37	37.21±2.17	11.28±0.14
37	60	37	20	20	179.23±6.62	47.00±0.14	15.48±0.28	98.89±0.01	-0.86±0.02	6.17±0.08	6.23±0.08	-1.43±0.00	0.28±0.04	24.51±2.27	64.89±0.73	15.97±2.68	8.19±0.07
38	60	37	20	50	178.73±2.44	47.42±0.24	15.20±0.10	99.05±0.11	-0.89±0.01	6.06±0.01	6.13±0.01	-1.43±0.00	0.39±0.11	29.45±0.42	67.56±0.54	17.02±0.42	9.52±0.68
39	60	37	20	65	173.05±3.28	46.65±0.31	14.36±0.08	98.88±0.09	-0.80±0.04	6.11±0.14	6.16±0.13	-1.44±0.01	0.28±0.01	27.66±1.01	62.73±0.44	20.24±0.18	8.57±0.31
40	60	37	20	90	163.41±0.58	50.41±4.19	14.05±0.30	98.68±0.18	-0.84±0.01	6.29±0.13	6.34±0.13	-1.44±0.00	0.31±0.12	18.01±0.24	63.01±2.52	18.29±2.18	8.42±0.32
41	60	37	40	20	178.91±2.96	46.73±0.08	14.97±0.30	98.75±0.42	-0.87±0.02	6.22±0.11	6.28±0.10	-1.43±0.01	0.38±0.03	26.93±2.71	72.93±4.15	21.63±1.07	9.65±0.02
42	60	37	40	50	181.93±4.37	47.68±0.05	15.19±0.14	99.21±0.05	-0.86±0.02	5.93±0.11	5.99±0.10	-1.43±0.01	0.55±0.07	27.77±0.25	63.62±0.18	22.96±1.98	10.87±0.61
43	60	37	40	65	167.05±0.71	47.82±0.20	14.36±0.13	98.67±0.43	-0.83±0.01	6.14±0.16	6.20±0.16	-1.44±0.01	0.36±0.06	19.75±0.68	62.53±6.07	21.92±3.97	7.81±0.35
44	60	37	40	90	186.77±0.32	50.01±2.32	15.04±0.28	99.03±0.25	-0.84±0.02	6.13±0.08	6.19±0.08	-1.44±0.01	0.40±0.19	23.08±0.19	58.33±0.31	26.28±1.75	10.03±0.35
45	60	37	60	20	201.95±0.96	46.56±0.22	14.51±0.22	98.53±0.01	-0.94±0.02	6.96±0.06	7.02±0.06	-1.44±0.00	0.96±0.06	31.58±2.19	64.92±0.21	23.01±0.37	6.84±0.58
46	60	37	60	50	171.00±0.26	48.51±0.05	14.31±0.19	97.27±0.47	-0.84±0.01	7.69±0.05	7.73±0.04	-1.46±0.00	2.20±0.34	18.81±0.39	56.82±1.07	28.07±2.04	7.98±0.56
47	60	37	60	65	200.82±0.13	48.53±0.82	13.91±0.11	98.38±0.18	-0.91±0.01	7.42±0.36	7.48±0.35	-1.45±0.00	1.45±0.38	16.26±0.51	47.22±0.19	29.73±0.19	9.00±0.36
48	60	37	60	90	192.09±0.90	49.75±1.19	13.82±0.62	97.09±0.60	-0.76±0.03	7.76±0.33	8.02±0.01	-1.47±0.01	2.37±0.64	12.78±0.17	43.50±0.58	30.30±0.25	8.99±0.17

Table S3 *(continued)*

Bath ultrasonic experiment																	
	Inputs*				Outputs												
Run	X_1	X_2	X_3	X_4	TP (mg GAE/L)	TPA (mg/L)	TF (mg/L)	L*	a*	b*	C*	H*	ΔE*	TE (mg/L)	THA (mg/L)	TFA (mg/L)	TT (μg/L)
49	40	80	20	20	191.23±1.48	45.25±0.19	14.72±0.11	97.73±0.20	-0.77±0.03	6.35±0.08	6.40±0.07	-1.45±0.01	1.01±0.16	31.27±0.09	69.30±0.57	18.59±0.04	7.06±0.46
50	40	80	20	50	185.41±2.38	45.54±0.06	14.30±0.16	98.49±0.13	-0.81±0.00	6.23±0.06	6.28±0.06	-1.44±0.00	0.30±0.12	21.32±0.80	63.87±0.39	15.66±2.56	5.16±0.38
51	40	80	20	65	184.32±3.92	47.50±0.24	14.16±0.08	98.87±0.11	-0.82±0.02	6.03±0.10	6.09±0.10	-1.44±0.01	0.23±0.08	28.53±2.78	64.46±0.01	20.58±0.08	5.81±0.14
52	40	80	20	90	195.36±0.13	47.94±2.47	12.52±0.43	98.94±0.16	-0.83±0.00	5.93±0.02	5.99±0.02	-1.43±0.00	0.29±0.15	30.13±2.44	64.22±1.69	20.23±0.43	7.36±0.10
53	40	80	40	20	171.59±1.35	46.02±0.06	14.64±0.04	97.59±0.01	-0.79±0.02	6.57±0.14	6.62±0.14	-1.45±0.00	1.23±0.07	33.02±0.39	68.80±3.04	22.20±0.46	8.49±1.99
54	40	80	40	50	176.68±1.48	47.13±0.23	13.31±0.75	98.76±0.14	-0.79±0.03	6.24±0.13	6.29±0.13	-1.44±0.01	0.29±0.07	26.68±0.36	58.76±0.24	19.97±0.01	6.27±0.77
55	40	80	40	65	196.09±0.64	44.91±2.70	12.04±0.26	98.61±0.07	-0.80±0.01	6.23±0.07	6.28±0.07	-1.44±0.00	0.25±0.08	27.78±0.22	61.59±0.52	27.62±1.68	8.40±0.17
56	40	80	40	90	190.86±0.58	51.34±1.35	13.62±0.53	98.29±0.06	-0.74±0.03	6.50±0.06	6.54±0.07	-1.46±0.00	0.64±0.07	21.12±0.71	53.88±1.39	23.35±2.71	7.29±0.00
57	40	80	60	20	192.27±1.54	48.66±0.01	15.15±0.05	98.08±0.15	-0.76±0.01	6.81±0.02	6.85±0.02	-1.46±0.00	1.01±0.07	27.78±0.65	64.73±3.21	23.20±0.86	5.70±0.12
58	40	80	60	50	190.91±1.16	48.66±1.69	16.12±0.57	98.05±0.34	-0.71±0.02	7.04±0.09	7.07±0.08	-1.47±0.00	1.23±0.25	20.06±0.19	54.45±0.32	27.75±0.42	6.65±0.92
59	40	80	60	65	190.41±1.99	50.51±0.04	18.49±0.23	97.97±0.18	-0.69±0.01	7.21±0.07	7.25±0.07	-1.48±0.00	1.41±0.15	17.33±0.02	51.29±0.30	27.58±2.70	6.83±0.25
60	40	80	60	90	199.84±0.55	52.74±0.04	17.23±0.12	98.15±0.02	-0.66±0.00	7.21±0.02	7.24±0.02	-1.48±0.00	1.33±0.01	15.36±0.21	48.42±0.22	34.36±0.88	11.23±0.35
61	40	37	20	20	199.05±0.58	45.12±0.60	14.11±0.91	98.75±0.19	-0.85±0.02	6.10±0.11	6.15±0.10	-1.43±0.01	0.20±0.03	30.95±2.20	66.05±0.21	17.47±3.13	9.31±0.46
62	40	37	20	50	178.68±0.32	46.43±0.97	13.62±0.72	98.61±0.22	-0.86±0.00	6.08±0.00	6.14±0.00	-1.43±0.00	0.18±0.08	28.99±1.23	67.24±1.07	21.28±0.48	10.79±0.29
63	40	37	20	65	190.86±0.45	45.99±0.47	14.25±0.15	98.57±0.14	-0.85±0.00	6.12±0.05	6.18±0.05	-1.43±0.00	0.18±0.04	29.01±2.31	63.15±1.51	18.48±2.41	9.30±0.65
64	40	37	20	90	169.50±0.45	49.20±2.18	12.93±0.19	97.92±0.01	-0.81±0.01	6.44±0.12	6.49±0.12	-1.45±0.00	0.87±0.07	30.69±0.12	66.61±0.36	21.32±0.40	9.70±0.45
65	40	37	40	20	185.82±0.39	46.77±0.90	12.10±0.23	98.74±0.15	-0.84±0.01	6.17±0.09	6.22±0.08	-1.44±0.00	0.22±0.02	28.82±1.94	71.03±5.92	21.03±0.65	7.91±0.49
66	40	37	40	50	177.14±3.28	47.12±0.99	13.24±0.22	98.43±0.06	-0.83±0.01	6.32±0.11	6.37±0.11	-1.44±0.00	0.40±0.13	25.53±0.05	63.97±0.00	24.37±0.00	7.94±0.12
67	40	37	40	65	176.68±1.35	46.91±0.63	12.83±0.13	98.80±0.03	-0.85±0.01	6.20±0.04	6.26±0.03	-1.44±0.00	0.23±0.01	20.58±0.84	59.97±0.12	18.74±0.98	7.73±0.51
68	40	37	40	90	178.36±0.64	48.18±1.60	12.67±0.45	98.64±0.24	-0.83±0.00	6.21±0.10	6.22±0.15	-1.44±0.00	0.28±0.04	22.27±0.43	56.04±0.50	24.37±0.09	9.26±0.09
69	40	37	60	20	186.41±1.35	49.13±0.35	15.86±0.51	98.19±0.10	-0.53±0.02	6.60±0.13	6.83±0.17	-1.49±0.00	0.85±0.05	25.14±1.56	62.83±2.99	23.40±2.48	6.76±0.55
70	40	37	60	50	183.82±0.64	48.99±0.49	15.14±0.24	98.35±0.12	-0.69±0.00	7.02±0.04	7.10±0.03	-1.48±0.00	1.08±0.07	19.58±0.54	52.51±2.58	25.68±1.00	6.65±1.08
71	40	37	60	65	181.64±1.29	49.77±0.58	15.85±0.18	97.99±0.04	-0.66±0.02	7.37±0.15	7.32±0.05	-1.49±0.01	1.54±0.15	18.74±1.02	53.20±1.82	33.73±0.36	7.42±0.20
72	40	37	60	90	189.55±0.26	50.39±0.69	14.56±0.26	97.82±0.10	-0.55±0.04	7.51±0.05	7.52±0.07	-1.52±0.03	1.76±0.01	13.93±0.23	43.71±0.58	31.74±1.98	8.09±0.69

*X_1 = amplitude (%), X_2 = frequency (kHz), X_3 = bath temperature (°C), X_4 = sonication time (min). Experimental results are presented as means ± S.D.

Table S4 Impact of ultrasonic probe processing on the white wine quality

Probe ultrasonic experiment															
	Inputs*			Outputs											
Run	X_1	X_2	X_3	TP (mg GAE/L)	TPA (mg/L)	TF (mg/L)	L*	a*	b*	C*	H*	ΔE*	TE (mg/L)	THA (mg/L)	TFA (mg/L)	TT (μg/L)
0	0	0	0	224.71±0.53	59.67±0.08	27.77±0.79	100.54±0.32	-1.10±0.02	4.23±0.05	4.38±0.04	-1.32±0.01	–	35.74±1.70	86.42±1.97	25.73±1.21	24.12±0.59
1	12.7	25	3	213.38±3.24	58.33±0.67	23.75±0.37	98.79±0.01	-0.85±0.03	5.65±0.05	5.71±0.05	-1.42±0.01	2.26±0.03	33.39±0.22	90.22±0.56	23.95±0.39	19.01±0.23
2	12.7	50	3	212.92±0.12	58.87±0.00	23.56±0.28	98.69±0.34	-0.84±0.02	5.73±0.16	5.79±0.16	-1.43±0.01	2.40±0.36	25.85±3.02	88.00±1.65	21.10±0.81	14.78±0.50
3	12.7	75	3	215.63±0.06	58.69±0.02	24.17±2.66	99.33±0.13	-0.89±0.03	5.59±0.07	5.66±0.07	-1.41±0.00	1.83±0.13	34.52±0.22	85.06±0.57	21.76±1.05	17.43±1.72
4	12.7	100	3	211.75±0.47	58.94±0.19	23.60±1.75	98.55±0.45	-0.83±0.01	5.70±0.02	5.76±0.02	-1.43±0.00	2.49±0.37	23.26±1.04	83.26±2.69	17.88±2.50	13.19±0.08
5	12.7	25	6	201.96±2.30	59.15±0.43	23.96±0.22	99.51±0.42	-0.91±0.01	5.46±0.09	5.53±0.09	-1.41±0.00	1.62±0.33	28.23±1.82	83.48±1.63	19.25±0.81	14.90±0.45
6	12.7	50	6	213.88±0.65	59.08±0.09	23.60±1.28	98.68±0.28	-0.87±0.01	5.63±0.04	5.70±0.04	-1.42±0.00	2.33±0.20	20.66±0.19	84.21±0.14	14.78±1.08	13.88±0.61
7	12.7	75	6	215.13±0.29	59.74±0.25	26.05±2.57	97.47±0.09	-0.74±0.00	5.97±0.05	6.02±0.05	-1.45±0.00	3.08±0.60	26.31±1.54	83.83±0.06	17.09±0.56	15.43±0.63
8	12.7	100	6	209.25±2.00	59.86±0.29	25.84±0.66	96.35±0.21	-0.76±0.02	6.03±0.02	6.07±0.01	-1.44±0.00	4.57±0.20	24.12±0.57	84.10±0.40	18.12±1.92	13.87±0.84
9	12.7	25	9	203.67±0.82	60.00±0.35	24.71±1.02	98.86±0.77	-0.93±0.02	5.77±0.09	5.85±0.09	-1.41±0.01	2.31±0.62	31.09±0.51	92.71±2.72	22.58±1.32	16.83±1.03
10	12.7	50	9	221.08±0.82	59.25±0.59	24.46±0.56	99.39±0.22	-0.92±0.01	5.67±0.05	5.74±0.05	-1.41±0.00	1.85±0.17	28.95±0.11	78.98±0.34	21.08±0.34	18.86±1.05
11	12.7	75	9	211.46±1.12	60.38±0.83	26.66±0.13	99.16±0.07	-0.91±0.00	5.59±0.03	5.66±0.03	-1.41±0.00	1.94±0.07	31.57±2.39	83.34±2.47	19.54±1.29	12.11±0.91
12	12.7	100	9	203.50±0.47	59.48±0.02	26.64±0.64	98.64±0.04	-0.89±0.01	5.78±0.11	5.85±0.11	-1.42±0.01	2.46±0.04	29.85±1.58	84.30±0.75	16.92±0.03	13.08±0.71
13	19.1	25	3	220.88±0.65	54.88±0.51	23.68±0.21	98.90±0.61	-0.88±0.02	5.85±0.25	5.87±0.18	-1.41±0.00	2.32±0.61	25.97±2.16	88.42±0.37	19.54±1.80	12.74±0.45
14	19.1	50	3	217.88±0.29	59.32±1.36	22.42±0.50	99.10±0.08	-0.92±0.01	5.73±0.07	5.80±0.06	-1.41±0.00	2.08±0.11	24.40±0.10	79.96±1.50	11.64±0.36	11.22±0.57
15	19.1	75	3	218.79±0.18	55.88±0.67	22.99±0.41	99.55±0.02	-0.96±0.03	5.54±0.01	5.62±0.02	-1.40±0.01	1.64±0.02	34.27±1.30	89.79±0.17	20.06±0.17	15.62±0.23
16	19.1	100	3	210.08±0.59	60.39±0.40	24.07±1.71	98.80±0.14	-0.92±0.02	5.55±0.01	5.62±0.01	-1.41±0.00	2.19±0.12	29.57±0.49	96.71±0.55	23.32±1.75	16.18±1.44
17	19.1	25	6	190.42±0.35	56.84±0.48	23.72±1.33	98.34±0.16	-0.82±0.03	5.68±0.06	5.74±0.05	-1.43±0.01	2.64±0.17	29.04±1.65	87.48±4.14	21.41±4.66	18.18±1.25
18	19.1	50	6	190.88±1.94	58.99±0.74	24.84±0.97	98.11±0.06	-0.84±0.00	5.82±0.11	5.63±0.46	-1.43±0.00	2.91±0.11	33.19±0.19	89.58±0.39	25.60±0.99	21.69±0.46
19	19.1	75	6	215.29±1.94	59.34±0.14	22.82±0.15	97.92±0.12	-0.74±0.04	6.19±0.30	6.24±0.30	-1.45±0.00	3.29±0.26	28.13±0.95	85.78±0.46	23.17±0.57	17.78±0.17
20	19.1	100	6	208.83±0.24	59.79±0.27	24.23±0.93	100.02±0.02	-0.94±0.01	5.45±0.13	5.53±0.12	-1.40±0.00	1.33±0.11	22.96±1.27	89.76±1.19	16.96±0.01	16.46±0.16
21	19.1	25	9	217.88±0.53	55.51±0.15	23.09±0.11	99.47±0.57	-0.92±0.06	5.57±0.20	5.64±0.19	-1.41±0.02	1.73±0.51	30.24±0.16	85.61±0.74	21.61±0.34	18.48±0.32
22	19.1	50	9	223.63±1.36	56.70±0.23	23.04±0.24	98.35±0.15	-0.82±0.02	5.96±0.16	6.02±0.16	-1.43±0.01	2.81±0.22	27.49±0.18	86.62±1.92	21.50±0.63	17.56±0.28
23	19.1	75	9	191.29±0.77	57.75±0.53	24.86±0.29	97.45±0.27	-0.74±0.03	6.36±0.42	6.40±0.41	-1.46±0.01	3.77±0.46	31.34±1.41	86.09±0.90	23.67±0.41	19.22±0.28
24	19.1	100	9	211.17±0.59	59.76±0.76	28.28±0.50	98.49±0.56	-0.86±0.05	5.56±0.01	5.63±0.00	-1.42±0.01	2.46±0.48	31.47±1.30	87.56±0.62	23.06±0.37	20.31±0.71
25	25.4	25	3	206.13±1.24	58.18±0.38	25.21±0.81	99.08±0.11	-0.90±0.01	5.53±0.07	5.60±0.06	-1.41±0.00	1.96±0.13	29.41±1.69	88.26±1.78	21.75±0.11	19.43±0.62
26	25.4	50	3	206.25±0.12	56.57±0.44	22.89±0.58	98.68±0.19	-0.86±0.01	5.61±0.04	5.67±0.04	-1.42±0.01	2.32±0.13	31.87±1.99	89.34±0.89	25.39±1.06	20.16±0.52
27	25.4	75	3	204.04±0.41	56.99±0.26	23.20±0.19	98.52±0.33	-0.84±0.02	5.83±0.13	5.89±0.13	-1.43±0.01	2.59±0.34	31.60±2.56	87.38±2.43	23.97±2.46	15.16±0.16
28	25.4	100	3	205.50±0.47	58.60±0.01	24.18±0.57	97.38±0.38	-0.78±0.01	6.07±0.15	6.12±0.14	-1.44±0.00	3.67±0.26	29.55±2.59	85.33±2.05	21.36±2.93	18.65±0.37
29	25.4	25	6	213.00±0.12	58.76±0.72	23.69±0.30	99.75±0.07	-0.97±0.20	4.44±0.29	4.55±0.24	-1.35±0.06	0.86±0.16	28.22±2.13	88.74±1.92	22.07±0.74	19.14±0.21
30	25.4	50	6	207.71±1.12	59.06±1.50	25.51±0.01	100.83±0.14	-1.14±0.01	4.08±0.06	4.23±0.06	-1.30±0.01	0.33±0.15	30.63±1.18	88.96±2.27	23.95±0.86	19.50±0.85
31	25.4	75	6	207.88±0.88	59.32±0.32	25.18±1.28	100.40±0.27	-1.12±0.00	4.18±0.03	4.33±0.03	-1.31±0.00	0.20±0.20	34.35±0.60	91.11±2.24	21.49±3.14	19.86±0.51
32	25.4	100	6	205.46±0.06	58.00±0.01	23.88±0.32	98.20±0.02	-0.85±0.01	5.62±0.33	5.68±0.33	-1.42±0.01	2.74±0.19	29.90±1.82	81.58±0.02	15.30±0.38	17.08±0.12
33	25.4	25	9	210.75±0.94	59.22±1.01	24.23±1.05	97.60±0.53	-0.83±0.05	5.91±0.15	5.97±0.14	-1.43±0.01	3.39±0.53	28.16±1.46	86.05±0.15	20.10±0.99	15.97±0.94
34	25.4	50	9	205.79±0.77	58.15±0.21	25.09±0.38	99.01±0.01	-0.94±0.04	5.41±0.29	5.50±0.28	-1.40±0.02	1.94±0.17	30.55±0.98	87.79±1.11	23.58±0.45	20.88±0.59
35	25.4	75	9	201.08±0.47	57.26±0.07	23.19±0.54	98.89±0.15	-0.90±0.01	5.57±0.03	6.03±0.15	-1.43±0.01	2.13±0.09	28.70±1.08	84.28±0.97	22.22±2.04	19.16±1.52
36	25.4	100	9	202.88±1.83	59.35±0.83	23.77±1.06	98.66±0.07	-0.87±0.02	5.63±0.14	5.69±0.14	-1.42±0.01	2.35±0.14	33.26±0.24	86.98±0.52	21.82±0.28	22.10±0.86

*X_1 = probe diameter (mm), X_2 = amplitude (%), X_3 = sonication time (min). Experimental results are presented as means ± S.D.

Paper 3

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ORIGINAL SCIENTIFIC PAPER

Influence of non-thermal processing techniques on sulfur dioxide and oxygen concentrations in young and aged wines

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Abstract

The application of innovative techniques like high hydrostatic pressure (HHP) and high power ultrasound (HPU) for food processing and preservation is one of the current topics in food science. In the enological field, these techniques have been identified as alternative methods for wine microbial stabilization and acceleration of wine aging process. Due to lack of available information about their influence on physicochemical characteristics, the aim of this work was to study the effect of HHP and HPU on sulfur dioxide and dissolved oxygen concentrations in red and white wines. The effect was evaluated immediately after the treatment and after 3, 6 and 12 months of aging in bottles. Moreover, the synergistic effect of mentioned techniques along with antioxidants additions (glutathione and SO_2) was also evaluated. The results showed that the concentrations of free and total SO_2 did not change immediately after HHP treatments, while after HPU processing there was no clear trend in analyzed parameters. As expected, results showed that both, free and total SO_2 decreased during storage period of red and white wines. Regarding both applied techniques, slightly higher concentrations of free SO_2 were observed in samples treated by HHP after 12 months of storage. Oxygen concentration slightly increased immediately after the treatments, with the highest concentration determined after HPU processing. During aging, its concentrations decreased and were similar or slightly higher than of those determined in untreated samples. Regarding the antioxidants additions, better protective effect was obtained by addition of higher concentration of SO_2 than glutathione, since these samples were characterized by lower concentrations of dissolved oxygen.

***Keywords**: high hydrostatic pressure, high power ultrasound, wine, sulfur dioxide, dissolved oxygen*

Sažetak

Primjena inovativnih tehnika kao što su visoki hidrostatski tlak (HHP) i ultrazvuk visokih snaga (HPU) u preradi i konzerviranju hrane jedna je od aktualnih tema u znanosti o hrani. U enološkom području, ove su tehnike prepoznate kao alternativne metode za mikrobiološku stabilizaciju vina i ubrzavanje procesa starenja vina. Uslijed nedostatka dostupnih informacija o utjecaju navedenih tehnika na fizikalno-kemijske karakteristike vina, cilj ovog rada bio je istražiti utjecaj HHP i HPU tretmana na koncentraciju sumporovog dioksida i otopljenog kisika u crnom i bijelom vinu. Utjecaj ovih tehnika utvrđen je neposredno nakon tretmana te nakon 3, 6 i 12 mjeseci starenja u bocama. Nadalje, ispitan je i sinergistički učinak navedenih tehnika i dodatka antioksidansa (glutation i SO_2). Rezultati su pokazali da se koncentracija slobodnog i ukupnog SO_2 nije promijenila odmah nakon HHP tretmana, dok nakon obrade HPU nema jasnog trenda u analiziranim parametrima. Kao što je bilo očekivano, koncentracija slobodnog i ukupnog SO_2 se smanjila tijekom perioda starenja crnog i bijelog vina. Obzirom na primijenjene tehnike, najveće koncentracije slobodnog SO_2 određene su u uzorcima tretiranim HHP-om, posebice nakon 12 mjeseci starenja. Odmah nakon tretmana, koncentracija kisika je lagano porasla, pri čemu je najveća koncentracija utvrđena nakon HPU tretmana. Tijekom starenja utvrđeno je smanjenje koncentracije kisika, čije su vrijednosti bile slične ili neznatno veće od onih utvrđenih u netretiranim uzorcima. Što *se tiče dodatka antioksidansa, bolji zaštitni učinak postignut je dodatkom više koncentracije SO_2 nego glutationa, obzirom da te uzorke karakteriziraju niže koncentracije otopljenog kisika.*

***Ključne riječi**: visoki hidrostatski tlak, ultrazvuk visokih snaga, vino, sumporov dioksid, otopljeni kisik*

Introduction

During last several years, physical techniques like high hydrostatic pressure (HHP) and high power ultrasound (HPU) have become of great interest in wine sector. Namely, the main advantage of their application is the reduction or even removal of chemical additives during wine production that may affect human health. The use of these techniques on wine should provide the antimicrobial effect and the preservation of aroma, taste and color properties at the same time.

Previous studies have already reported that HHP is able to inactivate undesirable microorganisms in red and white wines without affecting the sensory characteristics (Buzrul et al., 2012; Mok et al., 2006; Morata et al., 2012; Puig et al., 2008; van Wyk et al., 2018), suggesting that HHP might be a suitable alternative to reduce or replace SO_2 addition in wine production. In addition, HHP has also been proposed as a rapid and easy method for initiating the chemical reactions in wine by providing the activation energy (Liu et al., 2018; Norton and Son, 2008). With regard to HPU, this technique has been highlighted as a promising method for wine processing, since cavitation phenomena generated by ultrasonic waves in liquid medium can induce certain chemical reactions and accelerate reaction rates (Chemat et al., 2011; García and Sun, 2013; Zhang et

al., 2015). Herein, the use of HPU in wine technology has been often emphasized in terms of acceleration of wine aging process (Liu et al., 2015; Tao et al., 2014; Zhang and Wang, 2017), extraction improvement (Cabredo-Pinillos et al., 2006; Clodoveo et al., 2016; Plaza et al., 2019) and microbial stabilization (Cui et al., 2012; Luo et al., 2012; Jiranek et al., 2008).

Despite mentioned benefits of presented physical techniques, the replacement of antioxidant and antimicrobial effect of SO_2 is a difficult task. However, the combination of physical technique and lower concentration of SO_2 could help to reduce its use during the wine production. As a first step to determine the possibilities of using physical techniques in this field, it is necessary to evaluate their short- and long-term impact on sulfur dioxide and oxygen concentrations, as one of the main parameters employed for the assessment of wine quality. To date, there is still little information available regarding the influence of HHP and HPU on sulfur dioxide and dissolved oxygen in wines. Although their quantities depend on numerous factors, sulfur dioxide and oxygen concentrations in wine are still useful parameters in analyzing its condition.

During winemaking, the excessive contact of wine with oxygen may lead to oxidation (Fracassetti et al., 2013). Additionally, when wine is exposed to oxygen, the reactions between oxygen and wine antioxidants (phenolics, sulfur dioxide and glutathione) take place (Dimkou et al., 2013; Fracassetti et al., 2013). As a consequence, numerous modifications can occur in wine, such as decrease in dissolved oxygen and sulfur dioxide content. The moderate contact between wine and air is viewed as potentially favorable to improve color and flavor stability, particularly for red wines, but too much oxygen can lead to many problems, such as oxidative browning and loss of fresh and fruity aromas (Tomašević, 2017). Hence, the oxygen and SO_2 control during wine production process must be considered since they have an important impact on the sensory characteristics of wine. The aim of this study was (i) to evaluate the short-term effects of HHP and HPU treatments on the sulfur dioxide and oxygen concentrations in young red and white wines, and (ii) to study the long-term effects of HHP and HPU along with antioxidants additions (SO_2 and glutathione) on sulfur dioxide and oxygen concentrations in red and white wines during 12 months of storage.

Materials and methods

Wines

The young wines comprised the varieties of Cabernet Sauvignon and Graševina, and were obtained from winery Erdutski vinogradi, Erdut, Croatia, during vintage 2016. The physicochemical composition of red wine Cabernet Sauvignon was: alcoholic strength, by volume 13.1%, pH 3.46, total acidity 5.3 g/L as tartaric acid and volatile acidity 0.61 g/L as acetic acid, free SO_2 10 mg/L and total SO_2 20 mg/L, while those of white wine Graševina were: alcoholic strength, by volume 11.4%, pH 3.37, total acidity 5.1 g/L as tartaric acid and volatile acidity 0.31 g/L as acetic acid, free SO_2 25 mg/L and total SO_2 70 mg/L.

High hydrostatic pressure (HHP) treatments

The 100 mL of wine was poured into plastic bottle, packed in individual plastic bag and placed in the pressure chamber with maximum capacity of 2 L with propylene glycol as the compression fluid. A high hydrostatic pressure system FPG7100 (Stansted Fluid Power, Harlow, UK) was used for HHP treatments. The combination of following process parameters: pressures (200, 400 and 600 MPa) and pressure holding times (5, 15 and 25 min), was applied to assess the possible effects of the HHP treatment. All the treatments were carried out in triplicate and at room temperature (25 °C). Control sample represents the untreated wine sample. Samples were analyzed immediately after the HHP treatments.

High power ultrasound (HPU) treatments

For HPU treatments, an ultrasonic bath (Elmasonic P, Elma Schmidbauer GmbH, Singen, Germany) and an ultrasonic probe (Q700, Qsonica Sonicators, Newton, CT, USA) were used for various process conditions as described below.

To study the effects of ultrasonic bath treatment, the wine samples were sonicated at different combinations of following process parameters: ultrasound frequencies (37 and 80 kHz), ultrasound amplitudes (40, 60 and 100%), bath temperatures (20, 40 and 60 °C) and treatment durations (20, 50, 65 and 90 min) (Table 1). The wine samples (200 mL) were placed in a round-bottom glass vessel (400 mL), which served as a treatment chamber, and then immersed in the ultrasonic bath. The constant temperature of water inside the bath was kept by addition of cold water.

To study the effects of ultrasonic probe treatment, the combination of following process parameters, diameters of probes (12.7, 19.1 and 25.4 mm), ultrasound amplitudes (25, 50, 75 and 100%) and treatment durations (3, 6 and 9 min), was applied (Table 1). Each HPU probe was centered and immersed (2 cm) in a glass reactor (400 mL) containing 300 mL of the sample. The system was set at nominal power of 700 W and a constant frequency of 20 kHz. The wine samples were kept at 25 °C by cooling the reactor with cold water during the treatments.

All experimental trials of both HPU treatments were performed in triplicate. The control samples in both HPU treatments were untreated wines. Samples were analyzed immediately after the HPU treatments.

Table 1. *Experimental trials for ultrasonic bath and ultrasonic probe treatments*

Ultrasonic bath treatments					
Run	A (%)–f (kHz)–T (°C)–t (min)	Run	A (%)–f (kHz)–T (°C)–t (min)	Run	A (%)–f (kHz)–T (°C)–t (min)
1	100 – 80 – 20 – 20	25	60 – 80 – 20 – 20	49	40 – 80 – 20 – 20
2	100 – 80 – 20 – 50	26	60 – 80 – 20 – 50	50	40 – 80 – 20 – 50
3	100 – 80 – 20 – 65	27	60 – 80 – 20 – 65	51	40 – 80 – 20 – 65
4	100 – 80 – 20 – 90	28	60 – 80 – 20 – 90	52	40 – 80 – 20 – 90
5	100 – 80 – 40 – 20	29	60 – 80 – 40 – 20	53	40 – 80 – 40 – 20
6	100 – 80 – 40 – 50	30	60 – 80 – 40 – 50	54	40 – 80 – 40 – 50
7	100 – 80 – 40 – 65	31	60 – 80 – 40 – 65	55	40 – 80 – 40 – 65
8	100 – 80 – 40 – 90	32	60 – 80 – 40 – 90	56	40 – 80 – 40 – 90
9	100 – 80 – 60 – 20	33	60 – 80 – 60 – 20	57	40 – 80 – 60 – 20
10	100 – 80 – 60 – 50	34	60 – 80 – 60 – 50	58	40 – 80 – 60 – 50
11	100 – 80 – 60 – 65	35	60 – 80 – 60 – 65	59	40 – 80 – 60 – 65
12	100 – 80 – 60 – 90	36	60 – 80 – 60 – 90	60	40 – 80 – 60 – 90
13	100 – 37 – 20 - 20	37	60 – 37 – 20 – 20	61	40 – 37 – 20 – 20
14	100 – 37 – 20 – 50	38	60 – 37 – 20 – 50	62	40 – 37 – 20 – 50
15	100 – 37 – 20 – 65	39	60 – 37 – 20 – 65	63	40 – 37 – 20 – 65
16	100 – 37 – 20 – 90	40	60 – 37 – 20 – 90	64	40 – 37 – 20 – 90
17	100 – 37 – 40 – 20	41	60 – 37 – 40 – 20	65	40 – 37 – 40 – 20
18	100 – 37 – 40 – 50	42	60 – 37 – 40 – 50	66	40 – 37 – 40 – 50
19	100 – 37 – 40 – 65	43	60 – 37 – 40 – 65	67	40 – 37 – 40 – 65
20	100 – 37 – 40 – 90	44	60 – 37 – 40 – 90	68	40 – 37 – 40 – 90
21	100 – 37 – 60 – 20	45	60 – 37 – 60 – 20	69	40 – 37 – 60 – 20
22	100 – 37 – 60 – 50	46	60 – 37 – 60 – 50	70	40 – 37 – 60 – 50
23	100 – 37 – 60 – 65	47	60 – 37 – 60 – 65	71	40 – 37 – 60 – 65
24	100 – 37 – 60 – 90	48	60 – 37 – 60 – 90	72	40 – 37 – 60 – 90
Ultrasonic probe treatments					
Run	d (mm)–A (%)–t (min)	Run	d (mm)–A (%)–t (min)	Run	d (mm)–A (%)–t (min)
1	12.7 – 25 – 3	13	19.1 – 25 – 3	25	25.4 – 25 – 3
2	12.7 – 50 – 3	14	19.1 – 50 – 3	26	25.4 – 50 – 3
3	12.7 – 75 – 3	15	19.1 – 75 – 3	27	25.4 – 75 – 3
4	12.7 – 100 – 3	16	19.1 – 100 – 3	28	25.4 – 100 – 3
5	12.7 – 25 – 6	17	19.1 – 25 – 6	29	25.4 – 25 – 6
6	12.7 – 50 – 6	18	19.1 – 50 – 6	30	25.4 – 50 – 6
7	12.7 – 75 – 6	19	19.1 – 75 – 6	31	25.4 – 75 – 6
8	12.7 – 100 – 6	20	19.1 – 100 – 6	32	25.4 – 100 – 6
9	12.7 – 25 – 9	21	19.1 – 25 – 9	33	25.4 – 25 – 9
10	12.7 – 50 – 9	22	19.1 – 50 – 9	34	25.4 – 50 – 9
11	12.7 – 75 – 9	23	19.1 – 75 – 9	35	25.4 – 75 – 9
12	12.7 – 100 – 9	24	19.1 – 100 – 9	36	25.4 – 100 – 9

** A – Ultrasound amplitude, f – Ultrasound frequency, T – Bath temperature, t – Treatment duration, d – Diameter of the ultrasonic probe*
*** Control sample is untreated sample marked as experimental run 0*

Wine treatments and bottle storage

In order to study the long-term effects of HHP and HPU, the following process conditions for each technique and wine were applied: (i) pressure of 200 MPa and treatment duration of 5 min for HHP treatment in the case of both red and white wines; (ii) ultrasound amplitude of 100 %, ultrasound frequency of 80 kHz, bath temperature of 27 °C and treatment duration of 30 min for HPU treatment (ultrasonic bath) in the case of white wine, and (iii) probe diameter of 25.4 mm, ultrasound amplitude of 25% and treatment duration of 6 min for HPU treatment (ultrasonic probe) in the case of red wine. Also, the experiment consisted of antioxidants additions (SO_2 and GSH) in wines before processing to investigate the synergistic effect of their use along with mentioned techniques during 12 months of bottle aging (time 0, 3, 6 and 12 months). Treated red and white wine samples were: (i) wine with standard SO_2 concentration (25 mg/L of free SO_2 for red wine; 45 mg/L of free SO_2 for white wine), (ii) wine with low SO_2 concentration and addition of GSH (10 mg/L of free SO_2 with 20 mg/L of GSH for red wine; 25 mg/L of free SO_2 with 20 mg/L of GSH for white wine), and (iii) wine with low SO_2 concentration (10 mg/L of free SO_2 for red wine; 25 mg/L of free SO_2 for white wine). Control wines were untreated wines with standard concentration of SO_2. After HHP and HPU processing, the treated and untreated wines were bottled and sealed with natural corks in 750 mL glass wine bottles and stored under controlled conditions at 12 °C for 12 months. All treatments were performed in triplicate and analyses of sulfur dioxide and oxygen concentrations in wine were conducted after 0, 3, 6 and 12 months of aging.

Analyses of sulfur dioxide and oxygen concentrations in wine

The dissolved oxygen measurements were performed using a luminescence-based technology (NomaSense™ O_2 P6000, Nomacorc, Belgium). This trace oxygen meter generates a blue light which is sent to the oxygen sensor via the optical fiber. The system corrects the concentration of oxygen in terms of sugar and alcohol content and wine temperature. To measure the oxygen concentration in wines, an immersion probe with a detection limit of 15 µg/L of oxygen was used.

Total and free SO_2 were analyzed using a sulfur dioxide measurement device (LDS Sulfilyser, Laboratoires Dujardin-Salleron, Noizay, France). The measurement of SO_2 is based on potentiometric titration which includes adding iodine until the electrode measures a change in redox potential.

Data analysis

The experimental results were presented as the mean values ± standard deviation of six analytical repetitions. The statistical data analysis was performed by analysis of variance (ANOVA) using the Statistica V.10 software (StatSoft Inc., Tulsa, USA). Tukey's HSD Test was used as comparison test when samples analyses showed significant differences after ANOVA ($p < 0.05$).

Results and discussion

To investigate the possibility of using HHP and HPU techniques for managing the wine quality, the various experiments were performed on red and white wines. The results of the effects of different HHP treatments on the sulfur dioxide and oxygen concentrations in red and white wines are presented in Table 2. As it can be observed, only slight changes occurred in the concentration of dissolved oxygen in both HHP treated red and white wines compared to control samples, while there were no significant changes ($p < 0.05$) in the concentrations of total and free SO_2 in all experiments performed. Our results are in accordance with previous studies that showed that HHP treated and untreated wines maintained similar concentrations of free and total SO_2, indicating that HHP did not affect the main wine quality parameters (Santos et al., 2016; Tabilo-Munizaga et al., 2014). Furthermore, the concentration of dissolved oxygen slightly increased in both wines after HHP, but there was no clear trend among applied treatments. This effect seems to be a combination of HHP treatments with a high permeability to oxygen of the polyethylene bottles that need to be used during HHP processing (Dombre et al., 2015; Santos et al., 2019). In addition, very little is known about the effect of HHP on the oxygen concentration in wine. Delfini et al. (1995) studied this effect and found that there was a decrease in dissolved oxygen concentration in HHP treated samples of Moscato wine, but only in the first 5 minutes after contact with air, whereas the oxygen concentration determined 6 hours after treatment was higher in the untreated samples than in the treated ones, but the same trend was not confirmed after 24 hours of the treatment.

Table 2. *Effect of High Hydrostatic Pressure (HHP) treatments on the concentrations of dissolved oxygen, total and free SO_2 in red and white wines*

Red wine			
Treatments	Dissolved oxygen (mg/L)	Total SO_2 (mg/L)	Free SO_2 (mg/L)
Control (untreated)	1.21 ± 0.00^{d}	20 ± 0^{a}	10 ± 1^{a}
200 MPa/5min	1.90 ± 0.16^{c}	20 ± 0^{a}	10 ± 1^{a}
200 MPa/15min	1.99 ± 0.08^{bc}	20 ± 1^{a}	10 ± 0^{a}
200 MPa/25min	2.04 ± 0.06^{bc}	19 ± 1^{a}	10 ± 1^{a}
400 MPa/5min	1.89 ± 0.13^{c}	20 ± 0^{a}	10 ± 1^{a}
400 MPa/15min	2.13 ± 0.19^{abc}	20 ± 0^{a}	11 ± 1^{a}
400 MPa/25min	1.96 ± 0.16^{bc}	20 ± 1^{a}	11 ± 1^{a}
600 MPa/5min	2.47 ± 0.36^{ab}	19 ± 0^{a}	10 ± 1^{a}
600 MPa/15min	2.62 ± 0.21^{a}	19 ± 1^{a}	10 ± 0^{a}
600 MPa/25min	2.14 ± 0.18^{abc}	19 ± 1^{a}	10 ± 1^{a}
White wine			
Treatments	Dissolved oxygen (mg/L)	Total SO_2 (mg/L)	Free SO_2 (mg/L)
Control (untreated)	1.98 ± 0.01^{d}	70 ± 0^{a}	25 ± 1^{a}

200 MPa/5min	2.07 ± 0.03^{cd}	70 ± 0^{a}	25 ± 1^{a}
200 MPa/15min	2.14 ± 0.10^{bcd}	70 ± 0^{a}	24 ± 1^{a}
200 MPa/25min	2.20 ± 0.01^{bcd}	70 ± 0^{a}	24 ± 1^{a}
400 MPa/5min	2.29 ± 0.15^{abc}	70 ± 0^{a}	25 ± 1^{a}
400 MPa/15min	2.41 ± 0.03^{ab}	70 ± 0^{a}	24 ± 1^{a}
400 MPa/25min	2.37 ± 0.03^{ab}	70 ± 0^{a}	24 ± 1^{a}
600 MPa/5min	2.25 ± 0.06^{abcd}	70 ± 0^{a}	25 ± 1^{a}
600 MPa/15min	2.29 ± 0.02^{abc}	70 ± 0^{a}	25 ± 1^{a}
600 MPa/25min	2.52 ± 0.22^{a}	70 ± 0^{a}	24 ± 1^{a}

Regarding HPU, the effects of this technique on the sulfur dioxide and oxygen concentrations in red and white wines are shown in Figure 1. In general, the results showed that ultrasonic probe (Figure 1a and 1c) and ultrasonic bath (Figure 1b and 1d) treatments slightly influenced the concentrations of sulfur dioxide and dissolved oxygen in both, red and white wines. Compared to control samples, the concentration of dissolved oxygen slightly increased immediately after HPU treatments, while there was no clear trend in results for free and total SO_2. This result is probably a consequence of dissolution of a certain amount of oxygen in wine during the measurement. In general, the quantity of dissolved oxygen in wine depends on various factors, such as temperature, pH, atmospheric pressure and air exposure. Any operation involving contact with air, such as transferring, pumping or mixing, significantly accelerates the dissolution of oxygen (Ribéreau-Gayon et al., 2000). Regarding the effect of HPU on oxygen in wine, Singleton and Draper (1963) investigated the degree of oxidation after ultrasound in different atmospheres (air, nitrogen, and oxygen) and concluded that the use of ultrasound in the presence of air and nitrogen did not result in an increased oxidation while the oxygen atmosphere accelerated this process. Among different applied HPU techniques, slightly higher oscillations in concentration of sulfur dioxide and dissolved oxygen in both wines were observed after ultrasonic bath treatments. Our results are in agreement with the study of García et al. (2016) who also reported inconsistent data for the changes of sulfur dioxide in wine after ultrasound treatments. These authors suggested that degasification effect of ultrasound could be responsible for changes in the concentration of sulfur dioxide, particularly for the decrease of free SO_2 in wine. On the other hand, Cui et al. (2012) found that ultrasound treatment did not affect the concentrations of free and total SO_2 in white wine.

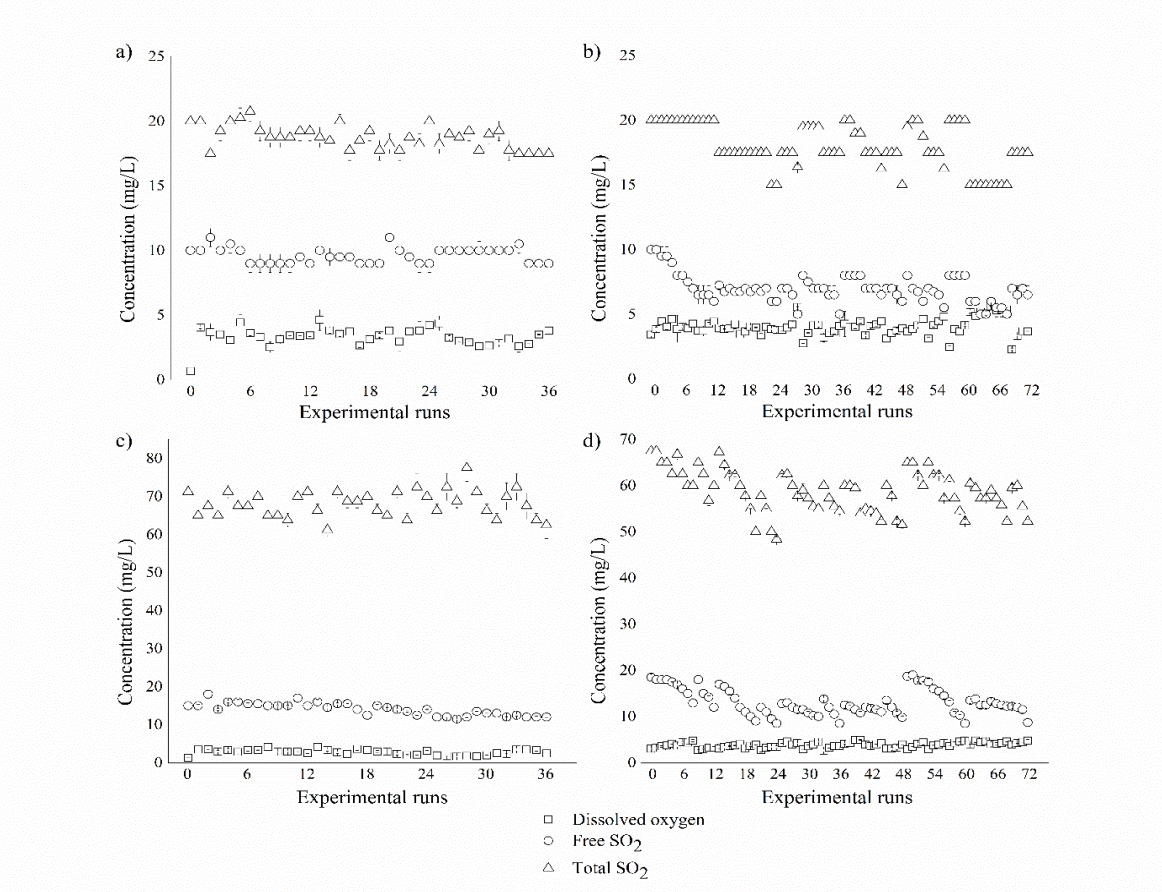

Figure 1. *Effect of High Power Ultrasound (HPU) on the concentrations of dissolved oxygen, total and free SO_2 in red and white wines. Red wine: a) ultrasonic probe, b) ultrasonic bath. White wine: c) ultrasonic probe, d) ultrasonic bath.*

Except of short-term effects of HHP and HPU treatments, the long-term effects of these techniques and antioxidants additions (SO_2 and GSH) on the sulfur dioxide and oxygen concentrations in red and white wines during 12 months of bottle aging are summarized in Figure 2 and Figure 3, respectively. At the beginning of storage, no major difference among untreated and HHP treated red and white wine samples with standard concentration of SO_2 was observed for all analyzed parameters, indicating that HHP did not affect the sulfur dioxide and dissolved oxygen concentrations in wines immediately after the treatment (Figure 2). Generally, independently of treatments applied, the oxygen can dissolve into the wine during bottling and later during aging process. Several authors have indicated the bottling process as a critical step for oxygen pickup (Dimkou et al., 2011; Skouroumounis et al., 2005; Vidal and Moutounet, 2006). For example, the filling of wine into the bottles can increase the concentration of dissolved oxygen by 0.5 to 2.0 mg/L (Peynaud, 1984). Furthermore, the oxygen that was trapped in the bottle headspace during the filling can also influence the final amount of dissolved oxygen in wine (Lopes et al., 2007). Also, one of the influencing factor during aging on wine composition, particularly sensory characteristics, is oxygen ingress through used closures (Caillé et al., 2010; Kwiatkowski et al., 2007; Wirth et al., 2012). In this study, the initial average concentrations of dissolved oxygen in wines prior to bottling were around 1.2-2.2 mg/L, while at the end of storage the concentrations were around 0.4 mg/L (Figure 2). The ranges of dissolved oxygen determined in this study are similar to that reported by other authors (Danilewicz, 2016; Dimkou et a., 2013; Fracassetti et al., 2013; Gambuti et al., 2017, Ling et al., 2019; Lopes et al., 2009; Waterhouse et al., 2016). Namely, dissolved oxygen in all wine samples started to decrease immediately after bottling and was consumed in the majority of the treatments in the first three months of aging. The concentration of free SO_2 in control and HHP treated wines also decreased during bottle aging, with a faster decrease in the first 3 months followed by a slower decrease after 6 and 12 months of aging. In comparison with the beginning of storage, control (unpressurized) red wine showed 81, 43 and 42% less of dissolved oxygen, free and total SO_2 after 12 months, respectively (Figure 2a-c). A similar trend can be also observed in HHP treated red wine samples during storage. In addition, slightly higher decrease of total SO_2 (52%) was found in HHP treated red wine with standard concentration of SO_2 during storage, while slightly lower decrease of 25% was noticed in wine samples with lower concentration of SO_2. Moreover, it can be seen that the concentration of dissolved oxygen was influenced by the concentration and type of antioxidants used. Additionally, both antioxidants used, SO_2 and GSH, can react with quinones, altering the oxygen uptake toward the products, resulting in an increased oxygen uptake (Danilewicz et al., 2008; Danilewicz and Wallbridge, 2010), explaining the lower concentration of oxygen in wines with a higher concentration of SO_2. Furthermore, it was already reported that HHP processing could lead to the generation of radicals and consequently alter the equilibrium of SO_2 reaction in wine during aging (Santos et al., 2016; Tao et al., 2012). As expected, regarding the changes in concentration of sulfur dioxide and dissolved oxygen in white wine samples (Figure 2d-f), a decrease of dissolved oxygen, free and total SO_2 was also detected in unpressurized and pressurized wines. After 12 months of aging, unpressurized white wine presented 74, 44 and 20% less of dissolved oxygen, free and total SO_2, respectively, when compared with the beginning of storage. Furthermore, the HHP treated white wines also showed a similar trend during storage, where slightly higher decrease of free SO_2 (52%) was determined in wine with standard concentration of SO_2. As earlier stated, a higher decrease of SO_2 concentration in HHP treated white wines during storage might be due to the possibility that free SO_2 reacts with high reactive oxygen species formed from phenolic compounds during HHP treatment (Santos et al., 2016). On the other hand, HHP treated white wine samples with lower concentration of SO_2 showed around 38 and 14% less of free and total SO_2 comparing with the beginning of storage. In relation to the oxygen decrease in wine samples during storage, the possible explanation could be unavailability of both antioxidants used. It is known that in this state accelerated uptake of oxygen occurs due to production of radicals by Fenton's reaction, which then react fast with oxygen (Gambuti et al., 2015). Moreover, the loss of dissolved oxygen and total SO_2 seems to be slightly faster in red wine than in white, independent from applied treatments. This is probably due to the fact that the rate of reaction of oxygen as well as SO_2 mainly depends on the concentration of phenolic compounds available for oxidation (Morozova, 2014; Danilewicz and Wallbridge, 2010). For very long time it was considered that SO_2 reacts direct with oxygen. However, this reaction is inhibited under the chemical conditions of wine. Specifically, the oxidation of SO_2 in wine is prevented by the presence of phenolic compounds (Danilewicz, 2007). Namely, sulfites in wine react with hydrogen peroxide, which is an oxidation product of phenolic compounds (Boulton et al., 2013; Danilewicz and Wallbridge, 2010; Waterhouse and Laurie, 2006)

The effects of HPU treatments (ultrasonic probe and ultrasonic bath) and antioxidants additions (SO_2 and GSH) on the concentration of sulfur dioxide and dissolved oxygen in red and white wines during 12 months of aging are presented in Figure 3. The results showed that at the beginning of storage, there were no great changes in analyzed parameters after applying HPU treatments comparing untreated and treated wine samples with standard concentration of SO_2. As it was already mentioned, the role of oxygen during aging of bottled wine is very important and it depends on numerous factors. During bottle aging, wine is exposed to relatively small amounts of oxygen, but even these concentrations are sufficient to impact the outcome of aging process (Ugliano, 2013). In this study, the average concentrations of dissolved oxygen in wines prior to bottling were around 3.3-4.0 mg/L, while at the end of storage the concentrations were in range from 0.2 to 0.5 mg/L, which is similar to those found in other studies (Danilewicz, 2016; Dimkou et a., 2013; Fracassetti et al., 2013; Gambuti et al., 2017, Ling et al., 2019; Lopes et al., 2009; Waterhouse et al., 2016). Immediately after bottling, a rapid decrease of dissolved oxygen can be observed in all treatments in the first three months of aging (Figure 3). Additionally, the concentration of free SO_2 also decreased to a large extent in the same period of aging, while after 6 and 12 months of aging a slower decrease was perceived. Namely, the direct reaction between oxygen and SO_2 is supremely slow in wine as medium (Waterhouse and Laurie, 2006), thus a decrease of SO_2 is related to oxygen through reaction of SO_2 with the products of wine oxidation, primarily hydrogen peroxide (Danilewicz et al., 2008). From the results of the ultrasonic probe treatment of red wine samples (Figure 3a-c), it can be seen that control (unsonicated) red wine presented 88, 61 and 24% less of dissolved oxygen, free and total SO_2, respectively, when compared with the beginning of storage. A slightly higher decrease of dissolved oxygen and free SO_2 (91 and 63%) was found in HPU treated red wine sample with standard concentration of SO_2, whereas slightly lower decrease of dissolved oxygen and free SO_2, approximately 85 and 38%, was noticed in HPU treated red wine samples with lower concentration of SO_2 after 12 months of aging. Furthermore, the results of the ultrasonic bath treatment of white wine samples (Figure 3d-f) showed that, when compared with the beginning of storage, control white wine presented 95, 44 and 20% less of dissolved oxygen, free and total SO_2, respectively. HPU treated white wine sample with standard concentration of SO_2 showed slightly higher decrease of free and total SO_2 (66 and 25%), while HPU treated white wine samples with lower concentration of SO_2 presented slightly lower decrease of dissolved oxygen and total SO_2, approximately 91 and 15% after 12 months of aging. The observed behaviors could be attributed to the degassing effect of ultrasound for which is known that accelerates removal of dissolved oxygen in liquids. Namely, dissolved oxygen can act as nuclei to form bubbles, which could float to the surface and be removed from the treated medium (Feng et al., 2011). Moreover, the ultrasound has the ability to induce free radicals, which are considered as important triggering factors to initiate chemical reactions in liquids. Additionally, it was confirmed that ultrasound triggers the generation of

1-hydroxyethyl free radical into wine, which is considered to be a main radical intermediate in natural oxidation of wine (Zhang et al., 2015). When comparing all variations of wine samples regardless of HPU treatment applied, it is clearly that the presence of higher concentration of SO_2 had a great effect on oxygen uptake. This observation was expected, since previous studies showed the same tendencies (Danilewicz et al., 2007; Danilewicz et al., 2008; Fracassetti et al., 2013). Also, the addition of GSH did not lead to an enlargement of the SO_2 consumption rate, indicating that unlike other antioxidants, GSH does not increase the production of hydrogen peroxide, which consumes SO_2 (Panero et al., 2015). Comparing both HHP and HPU techniques, the highest concentrations of free SO_2 as well as the lowest concentrations of dissolved oxygen were determined in samples treated by HHP, particularly after 12 months of storage. Finally, independent from applied techniques, better protective effect was obtained by addition of higher concentration of SO_2 than glutathione, since these samples were characterized by lower concentrations of dissolved oxygen.

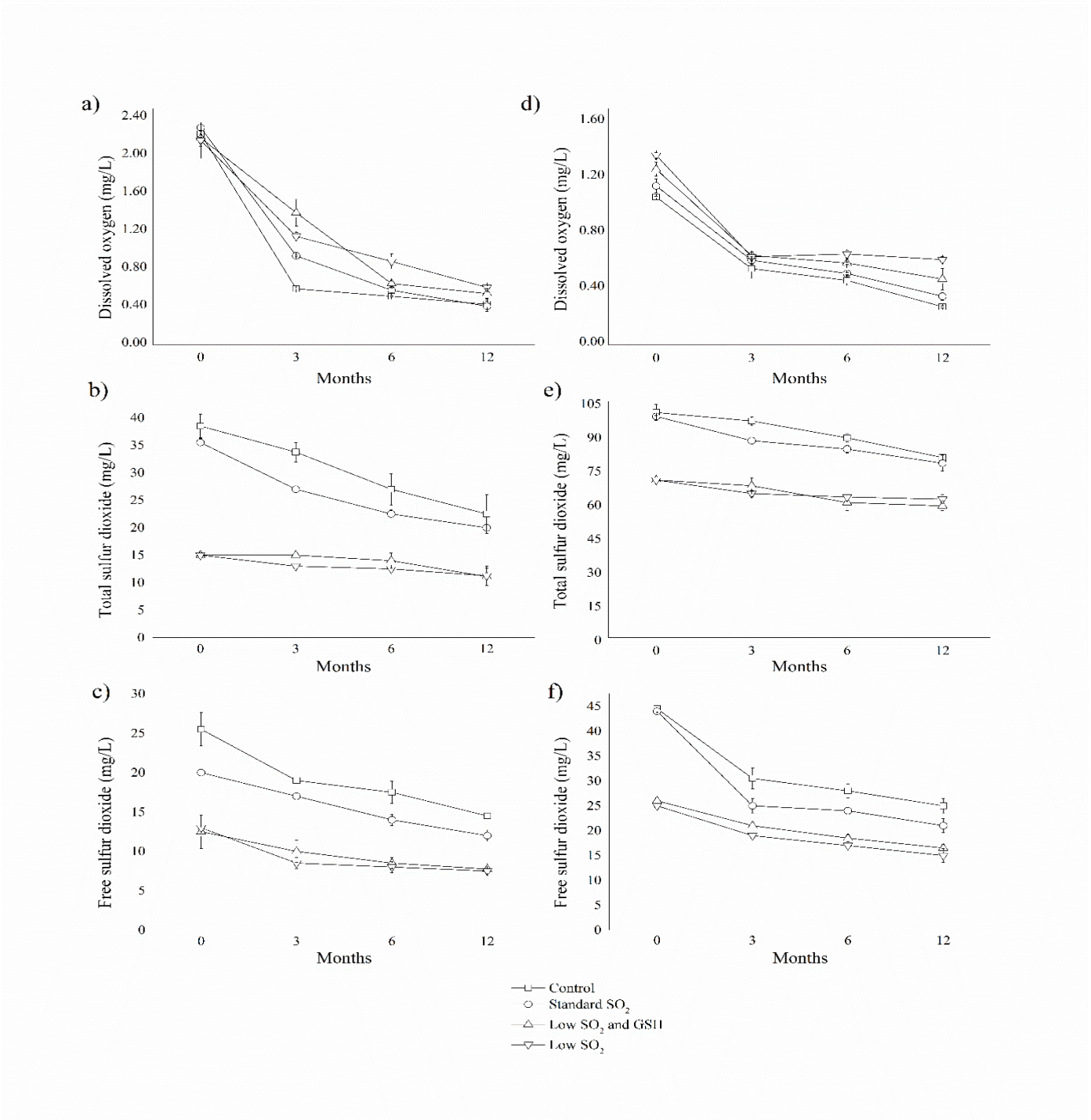

Figure 2. *Effects of High Hydrostatic Pressure (HHP) and antioxidants additions (SO_2 and GSH) on the concentrations of dissolved oxygen, total and free SO_2 in red and white wines during 12 months of aging. HHP treatment of red wine samples (a-c). HHP treatment of white wine samples (d-f).*

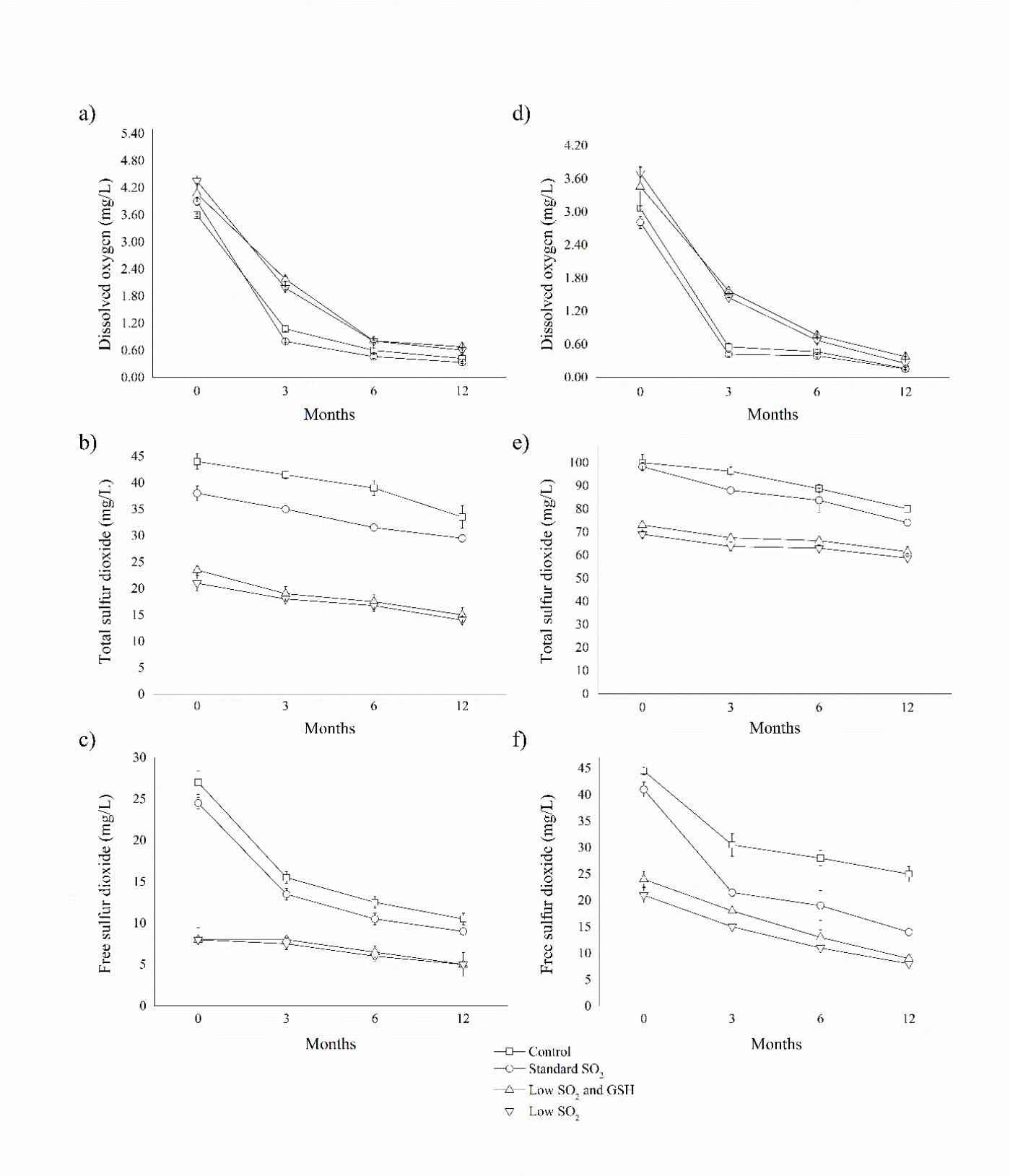

Figure 3. *Effects of High Power Ultrasound (HPU) and antioxidants additions (SO_2 and GSH) on the concentrations of dissolved oxygen, total and free SO_2 in red and white wines during 12 months of aging. Ultrasonic probe treatment of red wine samples (a-c). Ultrasonic bath treatment of white wine samples (d-f).*

Conclusions

In summary, this study showed that HHP did not affect both, free and total sulfur dioxide, immediately after the treatments, while there was no clear trend in its concentrations after application of HPU. Moreover, results showed that both, free and total SO_2, decreased during storage period of red and white wines. After 12 months of storage, regarding both applied techniques, slightly higher concentrations of free SO_2 were observed in samples treated by HHP. Dissolved oxygen concentration slightly increased immediately after the treatments, particularly after HPU processing. During aging, its concentrations decreased and were similar or slightly higher than of those determined in control, untreated samples. Among antioxidants used in all experiments performed, the addition of glutathione did not influence the oxygen and SO_2 consumption rate in HHP and HPU treated red and white wines. Namely, better protective effect was obtained by addition of sulfur dioxide, since these samples were characterized by lower concentrations of dissolved oxygen. Altogether, our results demonstrated the importance of measuring the concentration of sulfur dioxide and dissolved oxygen in wine after exposure to HHP and HPU treatments. Finally, this research provided important insights for possible application of HHP and HPU techniques and lower concentrations of SO_2 for production of high quality wines during storage. Nevertheless, it is important to investigate the effects of mentioned techniques on wine sensory and chemical characteristics (phenolic and aroma composition) in order to evaluate their efficiency in winemaking.

Acknowledgments

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Paper 4

Tomašević, M., **Lukić, K.**, Bosiljkov, T., Kelšin, K., Ćurko, N., Kovačević Ganić, K. (2017) Effect of high hydrostatic pressure on the volatile compounds in wine. *Works of the Faculty of Agriculture and Food Science* **62**(67 (2)), 505-516.

EFFECT OF HIGH HYDROSTATIC PRESSURE ON THE VOLATILE COMPOUNDS IN WINE*

UTJECAJ VISOKOG HIDROSTATSKOG TLAKA NA HLAPIVE SPOJEVE U VINU

Marina Tomašević[1], Katarina Lukić[1], Tomislav Bosiljkov[1], Karla Kelšin[1], Natka Ćurko[1], Karin Kovačević Ganić[1]

Original scientific paper

Summary

Application of high hydrostatic pressure (HHP) as innovative technology for food preservation and processing has increased substantially during the last decade. Recently, HHP has been identified as potential alternative process for microbial preservation of wine, as well as wine aging accelerator throughout modifying wine physicochemical and sensorial characteristics, primarily phenolic composition, color and astringency intensity. Due to the lack of information about its influence on aroma composition, the aim of this paper was to study the effect of HHP on volatile aroma compounds of young white and red wines (*Vitis vinifera* L. Graševina and Cabernet Sauvignon). Wines were pressurized at 200, 400 and 600 MPa for 5, 15 and 25 min and analyzed immediately after treatment. Volatile aroma compounds were identified and quantified by solid-phase microextraction coupled with gas chromatography-mass spectrometry (SPME-GC/MS). Applied treatments resulted in slight changes in concentrations of aroma compounds, primarily decrease of esters in both, white and red wine. But, in most cases the observed differences were not significant. Obtained results suggest that HHP could be potentially used as an alternative process to sulfur dioxide addition, primarily to inactivate bacteria and yeasts without causing quality changes.

Key words: *high hydrostatic pressure, aroma compounds, wine, GC/MS*

Sažetak

Primjena visokog hidrostatskog tlaka (HHP) kao inovativne tehnologije u konzerviranju i preradi hrane u zadnjem je desetljeću u značajnom porastu. U posljednje vrijeme HHP tehnologija prepoznata je kao potencijalna, alternativna metoda za mikrobiološko konzerviranje vina te također, kao metoda čijom bi se

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primjenom kroz modifikaciju fizikalno-kemijskih i senzorskih karakteristika, prvenstveno polifenolnog sastava, intenziteta boje i trpkoće ubrzao proces starenja vina. Uslijed nedostatka informacija o utjecaju navedene tehnologije na sastav arome, cilj ovog rada bio je istražiti utjecaj HHP tretmana na hlapive komponente mladih bijelih i crnih vina (*Vitis vinifera* L. Graševina and Cabernet Sauvignon). Uzorci vina tretirani su pri tlakovima od 200, 400 i 600 MPa u trajanju od 5, 15 i 25 minuta te analizirani odmah po završetku tretmana. Spojevi arome identificirani su i kvantificirani primjenom plinske kromatografije uz masenu detektciju (GC/MS) uz prethodnu mikroekstrakciju na čvrstoj fazi (SPME tehnika). Primijenjeni HHP tretmani rezultirali su blagim promjenama u koncentracijama spojeva arome, prvenstveno smanjenjem estera u uzorcima bijelog i crnog vina. U većini provedenih tretmana uočene razlike nisu bile značajne. Dobiveni rezultati impliciraju kako bi se HHP tehnika mogla koristiti kao alternativni postupak dodavanju sumporovog dioksida, prvenstveno u cilju inaktivacije bakterija i kvasaca, a da se pritom ne uzrokuju promjene u kvaliteti vina.

Ključne riječi: *visoki hidrostatski tlak, spojevi arome, vino, GC/MS*

INTRODUCTION

The application of high hydrostatic pressure (HHP) in wine technology has shown a great potential during last few years since it is known that wine is very sensitive to temperature increases and it cannot be treated by heat due to its negative influence on aroma, taste and color properties. At the same time, application of HHP, as non-thermal technology, does not result in a significant increase of temperature of wine, and thereby provide preservation of physicochemical properties and overall quality of treated wine. Previous research, regarding the application of HHP treatment on wine, are mainly focused on its influence on the inactivation of undesired microorganisms (Buzrul, 2012; Briones – Labarca *et al*, 2017). In addition to microbial inactivation, HHP has shown the effect of enhancing some properties without affecting important quality characteristics such as color, pH and turbidity (Briones – Labarca *et al*., 2017). Recent studies have shown that HHP can also be successfully applied as a technique for increasing the extraction of polyphenolic compounds from grapes and improving the overall quality of wine (Morata *et al*., 2015), as well as in accelerating the aging process of wine (Sun *et al*., 2016). It has also been found that the application of HHP does not significantly affect the basic physicochemical characteristics of wine, immediately after processing (Mok *et al*., 2006). Moreover, changes in physicochemical and sensory properties of wine are only visible in the case of extreme HHP treatment parameters (650 MPa for 1 and 2 hours) (Buzrul, 2012; Tao *et al*., 2012) and after a certain period of storage through stimulation of Maillard's reactions and polymerization reactions of polyphenolic compounds (Santos *et al*., 2013; Santos *et al*., 2015; Santos *et al*., 2016). Despite mentioned studies, most of the conducted researches regarding the application of HHP technique

on wine are related on its effect on the inactivation of undesired microorganisms. There is a lack of information about its influence on chemical changes in wine, primarily aroma and polyphenol compounds. As reported by Tao *et al.* (2012) the chemical reactions influenced by HHP are expected to develop during aging according to Le Chatelier's principle, where the volume reducing during HHP processing could change of equilibrium of chemical reactions (Norton and Sun, 2008). Given the above, HHP potentially could affect chemical reactions in wine and accelerate wine aging process. It is previously demonstrated that applied HHP treatment (350 MPa during 10 min) did not resulted in sensory different wines in comparison to non-treated ones (Mok *et al*., 2006). Similarly, Puig *et al*. (2003) found no changes in physicochemical properties after HHP treatment (500 MPa for 5 min). However, combination of HHP treatment along with higher temperature resulted in condensation reactions of anthocyanins (Corrales *et al*., 2008). To our best knowledge, there is only one research regarding the influence of HHP on volatile aroma compounds: Morata *et al.* (2012) investigated the influence of HHP treatment (100 MPa for 24 h) on wines contaminated with *Brettanomyces bruxelensis* yeast where only small differences were observed in concentration of higher alcohols and esters after applied HHP. Since HHP represents potent technique in a view of controlling microbial population in wine, and consequently could result in reduced sulfur dioxide additions during wine production, the aim of this paper was to evaluate the influence of HHP processing parameters on the volatile aroma compounds, as one of the most important quality parameter, in white and red wines, *Vitis vinifera* L. Graševina and Cabernet Sauvignon.

MATERIAL AND METHODS

Wine samples

The research was conducted on one young quality dry white wine, variety Graševina (Erdutski vinogradi, Erdut, Croatia) and one young quality dry red wine, variety Cabernet Sauvignon (Erdutski vinogradi, Erdut, Croatia); all vintage 2016. Physicochemical properties of Graševina were: 11.4 vol %, total acidity (as tartaric acid) 5.1 g/L, volatile acidity (as acetic acid) 0.31 g/L, pH 3.37, reducing sugars 2.8 g/L, total extract 20.2 g/L, malic acid 1.2 g/L, while those of Cabernet Sauvignon were: 13.1 vol %, total acidity (as tartaric acid) 5.3 g/L, volatile acidity 0.61 g/L, pH 3.46, reducing sugars 4.1 g/L, total extract 31.7 g/L, lactic acid 1.3 g/L.

Chemicals

Ethanol was HPLC grade and purchased from J.T. Baker (Deventer, Netherlands), sodium chloride p.a. was purchased from Carlo Erba (Val de Reuil, Spain), while the aroma reference standards were purchased from Sigma Aldrich (St. Louis, USA).

High hydrostatic pressure treatment

A high hydrostatic pressure system FPG7100 (Stansted Fluid Power, Harlow, UK) was used for pressurization. The 100 mL of wine was poured into plastic bottle, sealed and placed in the working vessel with maximum capacity of 2000 mL. To assess the possible effects of the HHP treatment experimental test included variations of pressures (200, 400 and 600 MPa) and processing time (5, 15 and 25 min). All the treatments were conducted at room temperature (25 °C) and in triplicate. Control sample represents the wine sample not exposed to the HHP treatment.

Volatile compounds analysis

Volatile compounds were extracted by solid-phase microextraction (SPME) and analyzed by gas chromatography coupled with mass spectrometry (GC/MS) using an Agilent Gas Chromatography 6890 series equipped with an Agilent 5973 Inert mass selective detector (Agilent Technologies, Santa Clara, USA) according to the method in detail described by Tomašević *et al.* (2017).

Data analysis

Significant differences between samples for each of the constituents was determined by one-way analysis of variance (ANOVA) using the Statistica V.10 software (StatSoft Inc., Tulsa, USA). Tukey's honestly significant difference (HSD) test ($p<0.05$) was used for comparison when samples differed significantly after ANOVA was performed. The principal component analysis (PCA) was performed on the correlation matrix using the attributes of aroma compounds analysis in order to examine any possible grouping of samples by different applied treatments.

RESULTS AND DISCUSSION

Different aroma compounds were identified and quantified in analyzed wines, where the esters represent the largest group, followed by higher alcohols, volatile fatty acids, terpene and aldehyde. Concentrations determined in control Graševina wine, as well as in HHP treated ones, are presented in Table 1. As can be seen in table, slight changes occurred after the HHP treatment. Primarily, most of the esters lightly decreased, as well as terpene linalool, volatile fatty acids and benzaldehyde. On the other hand, increase in concentration of amyl alcohol was observed in most of the treated wines, while concentration of 1-hexanol increased at higher pressure (600 MPa). Regarding treatment duration, longer treatment resulted in lower concentration of most analyzed compounds (esters, terpene and aldehyde) except the most of higher alcohols which were determined in slightly higher concentrations in treated wines.

Similar trend was observed after red wine Cabernet Sauvignon treatment: decrease of esters, terpene linalool and benzaldehyde and volatile fatty acids, while concentration of previously mentioned higher alcohols (amyl alcohol and 1-hexanol) slightly increased. Despite similarity with trend found in the case of white wine, after HHP treatment of Cabernet Sauvignon more pronounced changes occurred. For example, concentrations of ethyl hexanoate, ethyl octanoate and especially ethyl decanoate

significantly decreased after applied pressures. The most pronounced changes occurred after pressurization with pressure of 600 MPa. Also, as previously stated, longer treatment duration resulted in higher loss of observed volatiles. Generally, esters represent one of the most important wine aroma groups, contributing to the fresh and fruity characters of wines and they are very sensitive to either thermal treatments or freezing (Lambert *et al.*, 1999). Morata *et al.* (2012) investigated influence of HHP treatment (100 MPa during 24 h) on the inactivation of spoilage *Brettanomyces* yeast in red wine, and beside the antimicrobial effect, they examined the influence of this process on the concentrations of esters and found no significant differences in concentrations of ethyl acetate and ethyl lactate in treated wines, compared to control ones.

In the present literature data, contradictory results of HHP effect on volatile composition could be observed. For example, Briones-Labarca *et al.* (2017) found no change in several aroma compounds (aldehydes, ethyl acetate, propanol, *i*-butanol, butanol and *i*-amyl alcohol) after HHP treatment, while Santos *et al.* (2015) found quite significant changes in aroma composition of treated wine samples. Latter authors found that HHP treatment had a large impact on the volatile composition of both white and red wines. Authors investigated the long-term effect (9 months after treatment) of HHP treatments (425 and 500 MPa during 5 min) on the volatile compounds of red and white native Portugal grape wines and concluded that pressurized wines had higher concentrations of acetals, ketones, furans and aldehydes, compounds that are usually characteristic for wine aging aroma. Also, these authors suggest that HHP treatment accelerate Maillard reaction and oxidation of alcohols and fatty acids, producing the wines with characteristics of faster aging.

Projection of analyzed sensory variables and the distribution of control and HPU treated Graševina wines in the two-dimensional coordinate system defined by first two variables explaining 84.26 % of the total variance is shown in Figure 1 First variable (PC 1) showed strong negative correlation with the content of majority of the analyzed volatile compounds: ethyl acetate (-0.99), *i*-butyl acetate (-0.90), ethyl butyrate (-0.97), *i*-amyl acetate (-0.96), ethyl hexanoate (-0.90), hexyl acetate (-0.96), *cis*-3-hexenol (-0.76), ethyl octanoate (-0.89), benzaldehyde (-0.92), linalool (-0.88), ethyl decanoate (-0.93), 2-phenylethyl acetate (-0.72), diethyl succinate (-0.78), hexanoic acid (-0.88), octanoic acid (-0.73), decanoic acid (-0.89) and on the other side, was highly positively correlated with volatile *i*-amyl acohol (0.70), 1-hexanol (0.82) and 2-phenylethanol (0.95). Furthermore, the second principal component showed a slight negative correlation with most of the analyzed volatile compounds. Control Graševina wine sample (Control W) was placed on the left side of first factorial plane (in the third quadrant) and was displaced from rest of the treated wines due to higher concentrations of aroma compounds which negatively correlate with both first and second factorial plane. The distribution of HHP treated wine samples in the coordinate system indicate clear separation of treated wine samples according to the height of the applied pressure and the treatment duration. In accordance to the mentioned, wine sample pressurized by 200 MPa during 5 minutes was placed within third quadrant

and according to that, characterized by higher concentrations of acetate esters and fatty acids in comparison with other HHP treatments. Wine samples pressurized by 200 MPa during 15 and 25 minutes as well as the sample pressurised by 400 MPa during 5 minutes were positioned in second quadrant and characterized by higher concentrations of volatile compounds which correlate negatively with PC1 and positively with PC 2. Furthermore, samples pressurized by 400 and 600 MPa during 5, 15 and 25 minutes are positioned in first and fourth quadrant and are characterized by more significant content of higher alcohols (2-phenylethanol, amyl alcohol, 1-hexanol).

Table 1. Concentration of volatile compounds in control and HHP treated white wines Graševina

	Control	200 MPa			400 MPa			600 MPa		
		5 min	15 min	25 min	5 min	25 min	15 min	5 min	15 min	25 min
Esters (mg/L)										
ethyl acetate	26,90±0,48^{b}	26,29±0,52^{ab}	26,06±0,41^{ab}	26,03±0,21^{ab}	26,05±0,08^{ab}	25,62±0,26^{ab}	25,51±1,19^{a}	25,50±0,24^{a}	25,27±0,45^{a}	25,03±0,06^{a}
ethyl butyrate	0,74±0,04^{b}	0,72±0,01^{ab}	0,72±0,03^{ab}	0,72±0,04^{ab}	0,71±0,04^{ab}	0,70±0,01^{ab}	0,69±0,01^{ab}	0,68±0,03^{ab}	0,68±0,02^{ab}	0,67±0,01^{a}
ethyl hexanoate	0,89±0,12^{a}	0,88±0,07^{a}	0,87±0,06^{a}	0,86±0,03^{a}	0,86±0,03^{a}	0,85±0,03^{a}	0,84±0,02^{a}	0,78±0,03^{a}	0,78±0,00^{a}	0,75±0,06^{a}
ethyl octanoate	0,59±0,01^{b}	0,55±0,08^{ab}	0,51±0,05^{ab}	0,51±0,03^{ab}	0,50±0,04^{ab}	0,49±0,08^{ab}	0,47±0,06^{a}	0,48±0,06^{ab}	0,47±0,05^{ab}	0,48±0,01^{ab}
ethyl decanoate	0,27±0,01^{c}	0,26±0,01^{bc}	0,25±0,01^{abc}	0,24±0,01^{abc}	0,22±0,01^{abc}	0,21±0,01^{abc}	0,23±0,00^{abc}	0,20±0,06^{ab}	0,19±0,06^{a}	0,19±0,02^{a}
diethyl succinate	0,24±0,03^{c}	0,20±0,02^{ab}	0,19±0,01^{a}	0,18±0,01^{a}	0,18±0,02^{a}	0,19±0,01^{ab}	0,19±0,01^{a}	0,19±0,02^{a}	0,18±0,02^{a}	0,18±0,03^{a}
i-butyl acetate	0,11±0,00^{b}	0,11±0,01^{b}	0,11±0,01^{ab}	0,10±0,00^{ab}	0,11±0,01^{ab}	0,11±0,01^{ab}	0,10±0,01^{ab}	0,10±0,00^{ab}	0,10±0,01^{ab}	0,09±0,00^{a}
i-amyl acetate	3,61±0,02^{a}	3,58±0,05^{a}	3,56±0,01^{a}	3,57±0,04^{a}	3,55±0,01^{a}	3,53±0,05^{a}	3,50±0,18^{a}	3,52±0,04^{a}	3,50±0,06^{a}	3,50±0,01^{a}
hexyl acetate	0,41±0,03^{d}	0,36±0,01^{bc}	0,34±0,04^{ab}	0,33±0,02^{ab}	0,34±0,00^{ab}	0,33±0,01^{ab}	0,31±0,01^{a}	0,32±0,01^{ab}	0,31±0,01^{a}	0,30±0,01^{a}
2-phenylethyl acetate	0,28±0,01^{a}	0,27±0,02^{a}	0,26±0,01^{a}	0,25±0,03^{a}	0,27±0,01^{a}	0,24±0,01^{a}	0,25±0,01^{a}	0,25±0,02^{a}	0,26±0,02^{a}	0,24±0,01^{a}
Higher alcohols (mg/l)										
amyl alcohol	57,17±2,45^{a}	58,85±0,49^{ab}	59,63±2,56^{ab}	57,53±0,06^{a}	58,92±0,32^{ab}	59,04±0,76^{ab}	59,26±0,97^{ab}	57,91±0,53^{a}	60,39±3,13^{ab}	62,82±3,44^{b}
2-phenylethanol	4,39±0,60^{a}	4,36±0,06^{a}	4,57±0,04^{a}	4,51±0,05^{a}	4,62±0,56^{a}	4,67±0,17^{a}	4,65±0,67^{a}	4,71±0,60^{a}	4,84±0,01^{a}	4,95±0,04^{a}
1-hexanol	1,15±0,02^{a}	1,15±0,01^{a}	1,15±0,03^{a}	1,16±0,01^{a}	1,16±0,02^{a}	1,16±0,04^{a}	1,17±0,02^{a}	1,17±0,03^{ab}	1,20±0,04^{ab}	1,24±0,07^{b}
cis-3-hexenol	0,12±0,00^{a}	0,12±0,00^{a}	0,12±0,01^{a}	0,11±0,00^{a}	0,11±0,00^{a}	0,11±0,00^{a}	0,11±0,00^{a}	0,10±0,01^{a}	0,11±0,01^{a}	0,11±0,00^{a}
Fatty acids (mg/l)										
hexanoic acid	3,71±0,16^{a}	3,69±0,07^{a}	3,60±0,15^{a}	3,53±0,09^{a}	3,57±0,35^{a}	3,53±0,28^{a}	3,58±0,11^{a}	3,57±0,04^{a}	3,50±0,13^{a}	3,50±0,04^{a}
octanoic acid	10,90±0,72^{a}	10,73±0,36^{a}	10,70±0,05^{a}	10,69±0,09^{a}	10,79±1,27^{a}	10,72±0,33^{a}	10,71±0,30^{a}	10,62±0,09^{a}	10,70±0,02^{a}	10,67±0,12^{a}
decanoic acid	2,25±0,00^{a}	2,22±0,07^{a}	2,16±0,49^{a}	2,08±0,25^{a}	2,04±0,21^{a}	2,05±0,05^{a}	2,01±0,09^{a}	2,05±0,13^{a}	2,01±0,10^{a}	2,03±0,18^{a}
Terpenes (µg/l)										
linalool	4,85±0,07^{a}	4,79±0,09^{a}	4,78±0,03^{a}	4,75±0,20^{a}	4,78±0,07^{a}	4,82±0,72^{a}	4,72±0,02^{a}	4,70±0,06^{a}	4,66±0,04^{a}	4,64±0,05^{a}
Aldehydes (µg/l)										
benzaldehyde	56,17±1,01^{c}	54,31±1,33^{bc}	54,08±2,18^{bc}	54,78±2,38^{c}	53,90±3,92^{bc}	49,48±0,74^{ab}	48,41±1,61^{a}	47,62±1,85^{a}	46,49±3,99^{a}	45,54±1,36^{a}

Data presented as average value of three analytical repetitions (N=3) ± standard deviation. ANOVA to compare data; different letters indicate statistical differences between wines of all treatments at the same time (Tukey's test, <0.05).

Table 2. Concentration of volatile compound in control and HHP treated red wines Cabernet Sauvignon

	Control	200 MPa			400 MPa			600 MPa		
		5 min	15 min	25 min	5 min	15 min	25 min	5 min	15 min	25 min
Esters (mg/L)										
ethyl acetate	37,34±1,82^{b}	37,28±0,65^{b}	36,51±0,78^{ab}	35,79±1,68^{ab}	35,39±0,90^{ab}	34,29±2,27^{ab}	34,10±0,95^{ab}	33,43±0,80^{ab}	33,73±1,87^{ab}	32,45±1,90^{a}
ethyl butyrate	0,44±0,03^{a}	0,41±0,04^{a}	0,38±0,03^{a}	0,39±0,01^{a}	0,38±0,06^{a}	0,38±0,06^{a}	0,38±0,01^{a}	0,37±0,02^{a}	0,36±0,02^{a}	0,34±0,05^{a}
ethyl hexanoate	0,42±0,01^{c}	0,39±0,01^{c}	0,36±0,03^{bc}	0,31±0,04^{ab}	0,30±0,02^{ab}	0,28±0,02^{a}	0,27±0,01^{a}	0,27±0,04^{a}	0,26±0,01^{a}	0,26±0,01^{a}
ethyl octanoate	0,19±0,03^{d}	0,15±0,02^{cd}	0,12±0,03^{bc}	0,13±0,02^{abc}	0,11±0,02^{abc}	0,09±0,01^{ab}	0,08±0,01^{a}	0,09±0,00^{ab}	0,09±0,00^{ab}	0,08±0,01^{ab}
ethyl decanoate	0,09±0,02^{b}	0,07±0,05^{ab}	0,05±0,02^{ab}	0,04±0,02^{ab}	0,03±0,01^{a}	0,02±0,01^{a}	0,02±0,01^{a}	0,02±0,01^{a}	0,01±0,01^{a}	0,01±0,01^{a}
diethyl succinate	0,61±0,03^{a}	0,59±0,02^{a}	0,60±0,04^{a}	0,57±0,13^{a}	0,56±0,02^{a}	0,54±0,03^{a}	0,53±0,05^{a}	0,53±0,05^{a}	0,51±0,09^{a}	0,51±0,02^{a}
i-butyl acetate	0,07±0,01^{c}	0,06±0,00^{ba}	0,06±0,00^{bc}	0,06±0,00^{bc}	0,06±0,00^{bc}	0,06±0,00^{ac}	0,05±0,00^{ab}	0,04±0,00^{a}	0,06±0,00^{bc}	0,05±0,00^{ab}
i-amyl acetate	0,63±0,05^{d}	0,61±0,05^{d}	0,60±0,05^{d}	0,57±0,05^{d}	0,56±0,04^{cd}	0,54±0,04^{bcd}	0,47±0,01^{abc}	0,45±0,02^{a}	0,46±0,02^{ab}	0,44±0,02^{a}
hexyl acetate	0,02±0,01^{b}	0,01±0,00^{ab}	0,01±0,01^{ab}	0,00±0,01^{ab}	0,00±0,01^{ab}	0,00±0,01^{ab}	0,00±0,00^{a}	0,01±0,00^{ab}	0,01±0,00^{ab}	0,01±0,01^{ab}
2-phenylethyl acetate	0,04±0,01^{a}	0,04±0,01^{a}	0,04±0,00^{a}	0,05±0,02^{a}	0,04±0,00^{a}	0,04±0,01^{a}	0,04±0,00^{a}	0,04±0,01^{a}	0,03±0,01^{ab}	0,03±0,01^{b}
Higher alcohols (mg/L)										
amyl alcohol	150,24±4,89^{a}	150,12±5,55^{a}	150,61±6,91	153,97±0,31^{a}	158,53±1,17^{a}	160,38±4,83^{a}	159,01±4,25^{a}	160,53±4,48^{a}	159,19±4,02^{a}	161,87±5,09^{a}
2-phenylethanol	27,35±1,70^{bc}	27,48±0,69^{c}	26,45±1,75	25,18±0,03^{abc}	24,01±2,30^{abc}	23,37±1,79^{abc}	23,85±2,98^{abc}	22,19±0,20^{a}	22,77±0,41^{ab}	22,02±2,26^{a}
1-hexanol	1,11±0,07^{a}	1,13±0,02^{ab}	1,21±0,02	1,21±0,02^{ab}	1,20±0,04^{ab}	1,23±0,04^{b}	1,21±0,06^{ab}	1,20±0,02^{ab}	1,21±0,05^{ab}	1,24±0,04^{b}
Fatty acids (mg/L)										
hexanoic acid	0,87±0,05^{a}	0,87±0,01^{a}	0,85±0,13	0,84±0,03^{a}	0,82±0,08^{a}	0,84±0,02^{a}	0,83±0,05^{a}	0,80±0,13^{a}	0,75±0,02^{a}	0,73±0,02^{a}
octanoic acid	2,62±0,11^{a}	2,62±0,23^{a}	2,59±0,08	2,54±0,06^{a}	2,56±0,12^{a}	2,55±0,11^{a}	2,54±0,08^{a}	2,57±0,28^{a}	2,54±0,06^{a}	2,49±0,22^{a}
decanoic acid	0,39±0,04^{e}	0,36±0,02^{de}	0,34±0,02	0,32±0,06^{bcde}	0,29±0,03^{abcd}	0,28±0,02^{abc}	0,26±0,02^{ab}	0,26±0,01^{ab}	0,25±0,02^{a}	0,26±0,02^{ab}
Terpenes (µg/L)										
linalool	7,67±0,02^{a}	7,65±0,16^{a}	7,67±0,10	7,69±0,08^{a}	7,69±0,33^{a}	7,67±0,19^{a}	7,53±0,21^{a}	7,48±0,04^{a}	7,45±0,06^{a}	7,41±0,05^{a}
Aldehydes (µg/L)										
benzaldehyde	259,98±1,94^{a}	256,50±5,38^{a}	254,99±6,63	253,75±3,47^{a}	253,07±0,79^{a}	253,54±3,15^{a}	252,37±1,95^{a}	252,72±6,26^{a}	251,12±5,76^{a}	251,67±3,70^{a}

Data presented as average value of three analytical repetitions (N=3) ± standard deviation. ANOVA to compare data; different letters indicate statistical differences between wines of all treatments at the same time (Tukey's test, <0.05).

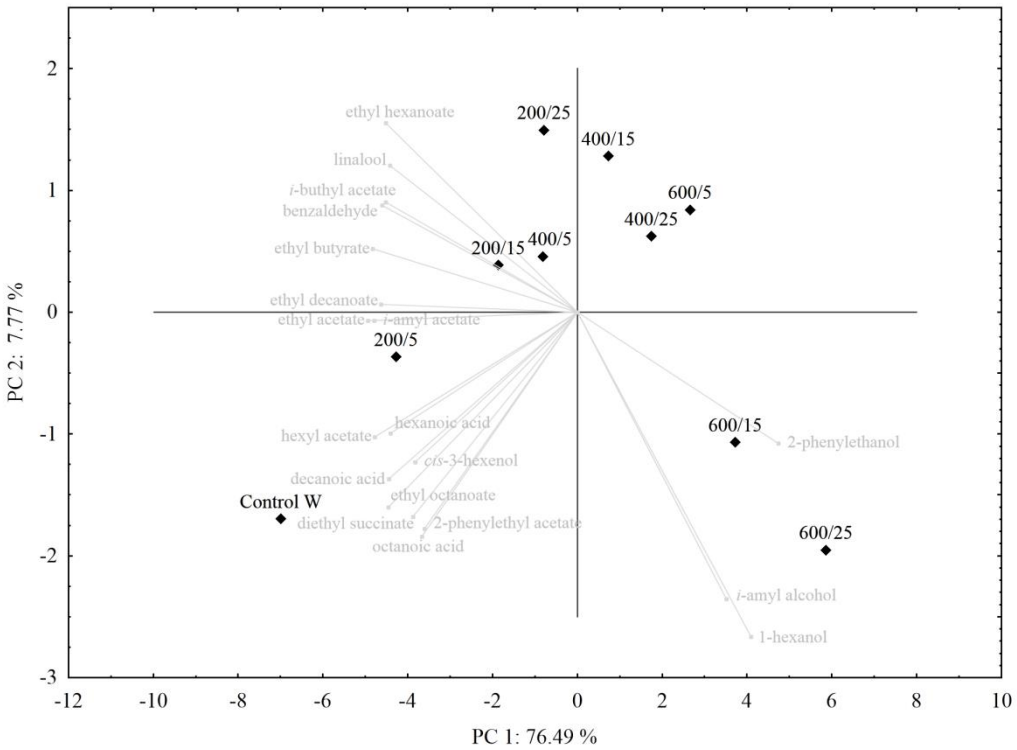

Fig. 1. Distribution of the white wine Graševina in two dimensional coordinate system defined by first two principal components (PC1 and PC2) according to applied HHP treatments

Projection of red wine Cabernet Sauvignon samples as well as analyzed aroma variables in the two-dimensional coordinate system defined by first two variables, explaining 86.3 %, is shown in Figure 2. First variable, that explain 73.37 % of the total variance (PC 1), showed strong negative correlations with the content of the ethyl acetate (-0.97), ethyl butyrate (-0.94), *i*-amyl acetate (-0.89), ethyl hexanoate (-0.98), *cis*-3-hexenol (-0.76), ethyl octanoate (-0.94), benzaldehyde (-0.92), linalool (-0.77), ethyl decanoate (-0.96), diethyl succinate (-0.89), hexanoic acid (-0.86), octanoic acid (-0.88), decanoic acid (-0.96) and 2-phenylethanol (-0.97). Moreover, PC 1 highly positively correlated with volatile amyl acohol (0.93) and 1-hexanol (0.85). PC 2 showed a negative correlation with 2-phenyelthyl acetate (-0.83) and linalool (-0.55) as well as positive correlation with hexyl acetate (0.87).

Separation of control and HHP treated red wine samples according to PCA analysis are presented in Figure 2. As it can be seen, control wine sample (Control R) of Cabernet Sauvignon and the wine samples pressurized by 200 MPa during 5, 15 and 25 minutes were placed on the left side of the first factorial plane and displaced from all treated wines due to higher concentrations of compounds which correlate negatively with first factorial plane. Red wine samples pressurized by 400 and 600

MPa were placed in fourth and first quadrant since they are characterized by higher concentration of volatile compound which correlate positively with the PC 1, primarily higher alcohols.

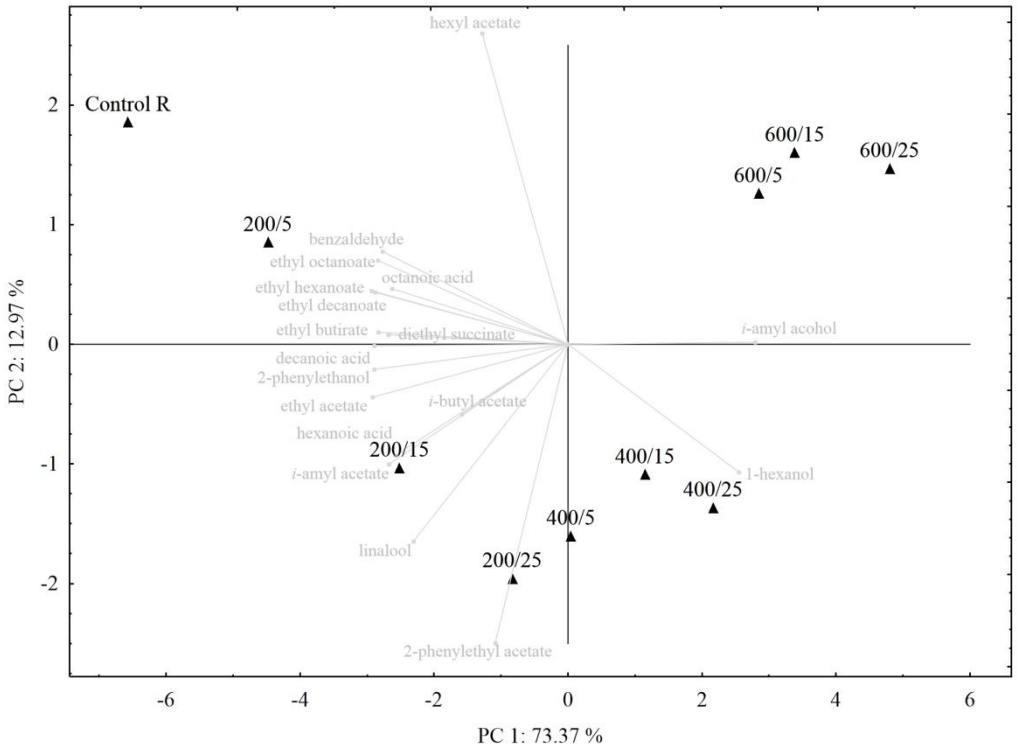

Fig. 2. Distribution of the red wine Cabernet Sauvignon in two dimensional coordinate system defined by first two principal components (PC1 and PC2) according to applied HHP treatments

CONCLUSION

High hydrostatic treatment influenced a slight change in concentrations of aroma compounds, primarily decrease of esters, volatile fatty acids and terpenes, while slight increase in concentration of higher alcohols was observed. Hence, this technique potentially could be very important in wine technology, especially in terms of wine production with lower sulfur dioxide additions. But, it is necessary to investigate long-term effect of this technique on overall quality of wine, including aroma and polyphenolic compounds, as well as sensory characteristics.

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Paper 5

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Phenolic and Aroma Changes of Red and White Wines during Aging Induced by High Hydrostatic Pressure

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Abstract: The aim of this study was to investigate use of high hydrostatic pressure (HHP) along with different antioxidants (glutathione and SO_2) as an alternative method for wine preservation and production of low-SO_2 wines. In the first phase of the study, low-SO_2, young red and white wines were pressurized at three pressure levels (200, 400 and 600 MPa) for 5, 15 and 25 min at room temperature, and analyzed immediately after treatments. Additionally, for the wine aging experiment, red and white wines with standard-SO_2, low-SO_2+glutathione and low-SO_2 content were treated with HHP treatment (200 MPa/5 min) and stored for 12 months in bottles. Color parameters, phenolic and aroma compounds were determined. The sensory evaluation was also conducted. HHP showed very slight, but statistically significant changes in the chemical composition of both red and white wine right after the treatment, and the main variations observed were related to the different pressures applied. Furthermore, during aging, most of the differences observed in chemical composition of pressurized wines, both red and white, were statistically significant, and greater in wines with a lower content of antioxidants. However, after 12 months of aging, some differences between unpressurized and pressurized samples with standard SO_2 content were lost, primarily in aroma compounds for red wine and in color and phenolics for white wine. Additionally, similar values were obtained for mentioned characteristics of red and white wines in pressurized samples with standard SO_2 and low SO_2+glutathione, indicating that HHP in combination with glutathione and lower doses of SO_2 might potentially preserve wine. The sensory analysis confirmed less pronounced changes in the sensory attributes of pressurized wines with higher concentration of antioxidants. Furthermore, the treatments applied had a slightly higher effect on the sensory properties of white wine.

Keywords: high hydrostatic pressure; wine; phenolics; aroma; aging; SO_2 content; glutathione

1. Introduction

High hydrostatic pressure (HHP) is one of the most researched, nonthermal techniques for preserving and modifying food products in the last decade. In general, the HHP treatment itself involves the subjection of food, with or without packaging, to high pressure in the range of 100 to 600 MPa [1]. This technique characterizes a minimal increase of temperature, as well as a small effect on low molecular weight compounds during processing [2]. The primary goal of its use is to achieve inactivation of undesirable microorganisms and enzymes with minimal effect on the sensory and nutritional characteristics of the treated product. Therefore, research related to the application of HHP in winemaking have mainly been focused on the microbial control of wine [3–7]. However, in order to achieve full HHP potential for wine industry application, the effect of HHP on the overall wine quality must not be disregarded. Previous studies have shown that HHP does not markedly affect the basic physicochemical properties of wine immediately after processing [3,8,9]. On the other hand, Buzrul [4] and Tao et al. [10] reported that HHP processing at extreme conditions (650 MPa for 1 and 2 h) resulted

in changes of physicochemical and sensory properties of wines. Additionally, some investigations revealed that HHP influenced the long-term physicochemical and sensory properties of wines through promotion of reactions associated with those observed during wine aging [9,11–13]. According to Santos et al. [9], HHP seems to be a more adequate processing technique for red wines than white wines, since its effect on color properties was only positive for red wines. It was found that HHP accelerates the wine aging process, since it promotes various chemical reactions, namely condensation and oxidation of phenolic compounds and Maillard reactions [9,12–15]. In addition, Tao et al. [10] reported that chemical reactions affected by HHP are assumed to be promoted during the aging process according to Le Chatelier's principle, which states that a decrease in volume induced by HHP could change the equilibrium of chemical reactions [16]. Altogether, this technique has a great potential in multiple fields, such as modifying wine composition, processing wines with low aging potential and reducing the sulfur dioxide additions during wine production.

In the past few years, there is a growing interest in multidisciplinary approaches, meaning the combination of microbial, physical and chemical treatments to elaborate high-quality low- or even free-SO_2 wines [17]. Namely, due to multiple actions of SO_2, antimicrobial and antioxidant, this additive is considered to be irreplaceable in wine production. However, in sensitive populations SO_2 can cause allergic reactions and thus adversely affect health [18], so its use tends to be reduced. In the present literature, most studies regarding HHP-treated wines were carried out in either free-SO_2 wines [11–13] or in wines with only one concentration of SO_2 [9,13,14,19,20]. Recently, Christofi et al. [21] performed a study where the HHP treatment was studied in combination with different SO_2 concentrations. However, there are no studies so far where the combination of HHP and different antioxidant treatments has been tested. The use of alternative physical and chemical treatments to SO_2 in wine production was reviewed not so long ago by several authors [17,22,23]. These have investigated a lot of antioxidant and antimicrobial substitutes between which one of them is reduced glutathione (GSH). The addition of GSH, which has the ability to indirectly inhibit wine browning [24], preserve and improve aroma [25] and donate an electron to reactive oxygen species [26,27], has particularly increased the attention of many researchers. Although effective, so far, studied physical and chemical techniques do not possess the multiple SO_2 action. Therefore, the aims of this paper were (i) to evaluate the effects of various HHP processing conditions on the phenolic and color composition of red and white wines right after the treatment and (ii) to investigate the potential use of HHP in winemaking along with the addition of antioxidants (glutathione and sulfur dioxide) during 12 months of aging.

2. Materials and Methods

2.1. Samples and Experimental Conditions

Red Cabernet Sauvignon and white Graševina wines with low SO_2 content were produced by Erdutski vinogradi (Erdut, Croatia) during the 2016 harvest. For both red and white wines, classical winemaking procedures were used. In the red winemaking process, the Cabernet Sauvignon grapes were destemmed and gently crushed after being harvested and were placed in stainless steel tanks. Additionally, enzymes (3 mL/100 kg of Lafase XL Extraction, Laffort, France) and bisulfite solution (20 mg/L of total SO_2) were added. Prior to the fermentation process, the must was inoculated with rehydrated yeast (20 g/hL of Zymaflore RX60®, Laffort, Bordeaux, France). The maceration/fermentation was carried out under 25 °C for 14 days. After 14 days of maceration, when alcoholic fermentation was finished, the wine was racked and pressed. Then, lactic acid bacteria (Lactoenos 450 PreAc®, Laffort, Bordeaux, France) were added to the wine for malolactic fermentation. After malolactic fermentation was over, wine was immediately decanted, microfiltered (0.2–0.4 μ) and sulfited at a concentration of 25 mg/L of free SO_2. The basic parameters of red wine at the start of our experiment were: 13.1% *v*/*v* alcohol, pH 3.46, total acidity 5.3 g/L (as tartaric acid), volatile acidity 0.61 g/L (as acetic acid), reducing sugar 4.1 g/L, lactic acid 1.3 g/L, malic acid 0.1 g/L and dry extract 31.7 g/L. In the white winemaking process, after the Graševina grapes were immediately destemmed and crushed, reductive pressing

with the addition of enzymes (2 mL/hL of Lafase XL Clarification, Laffort, France) and bisulfite solution (20 mg/L of total SO_2) was carried out. After must was clarified by cold settling, it was transferred in stainless steel tanks for fermentation. Then, it was inoculated with rehydrated yeast (20 g/hL of Zymaflore X16®, Laffort, France). The fermentation conditions were as follows: temperature under 16 °C and duration of 12 days. After alcoholic fermentation, the wine was decanted, stabilized with 60 g/hL of Microcol®Alpha (Laffort, France), microfiltered (0.2 µ) and sulfited at a concentration of 25 mg/L of free SO_2. The basic parameters of white wine at the start of our experiment were: 11.4% *v*/*v* alcohol, pH 3.37, total acidity 5.1 g/L (as tartaric acid), volatile acidity 0.31 g/L (as acetic acid), reducing sugar 2.8 g/L, lactic acid 0.3 g/L, malic acid 1.2 g/L and dry extract 20.2 g/L. These conventional wine analyses were carried out according to the official methods OIV-MA-AS312-01B, OIV-MA-AS313-15, OIV-MA-AS313-01, OIV-MA-AS313-02, OIV-MA-AS311-01A, OIV-MA-AS313-07, OIV-MA-AS313-10, and OIV-MA-AS2-03A of the International Organization of Vine and Wine [28]. Prior to pressurization, both red and white wines were first bottled in 100 mL plastic bottles and vacuum-sealed using plastic bags. The samples were further transferred to the pressure chamber of the high hydrostatic pressure system (Stansted Fluid Power FPG7100, Harlow, UK). Propylene glycol was used as the pressure-transmitting medium. Wine samples were pressurized during 5, 15 and 25 min at 200, 400 and 600 MPa. Nonthermal conditions were maintained during HHP processing with a maximum temperature ≤ 25 °C. All treatments were carried out in triplicate. The wines' color and phenolic composition were analyzed immediately after performed pressurization. Besides that, the analyses of physicochemical parameters such as dissolved oxygen, total and free SO_2 were also performed and have already presented in our previous work [29]. Namely, the dissolved oxygen was measured using a luminescent dissolved-oxygen sensor (NomaSense™ O_2 P6000, Nomacorc, Belgium), and the obtained values ranged from 1.2 to 1.9 mg/L in control wines and 1.9 to 2.6 mg/L in pressurized red and white wines, respectively. As already presented in our previous work, the total and free SO_2 were also determined by potentiometry using a sulfur dioxide measurement device (LDS Sulfilyser, Laboratories Dujardin-Salleron, Noizay, France), and the results showed 20 and 10 mg/L of total and free SO_2 in pressurized and control red wines, as well as 70 and 25 mg/L of total and free SO_2 in pressurized and control white wines, respectively [29]. Figure 1 shows a schematic diagram of experimental variables applied during HHP treatment of the wines.

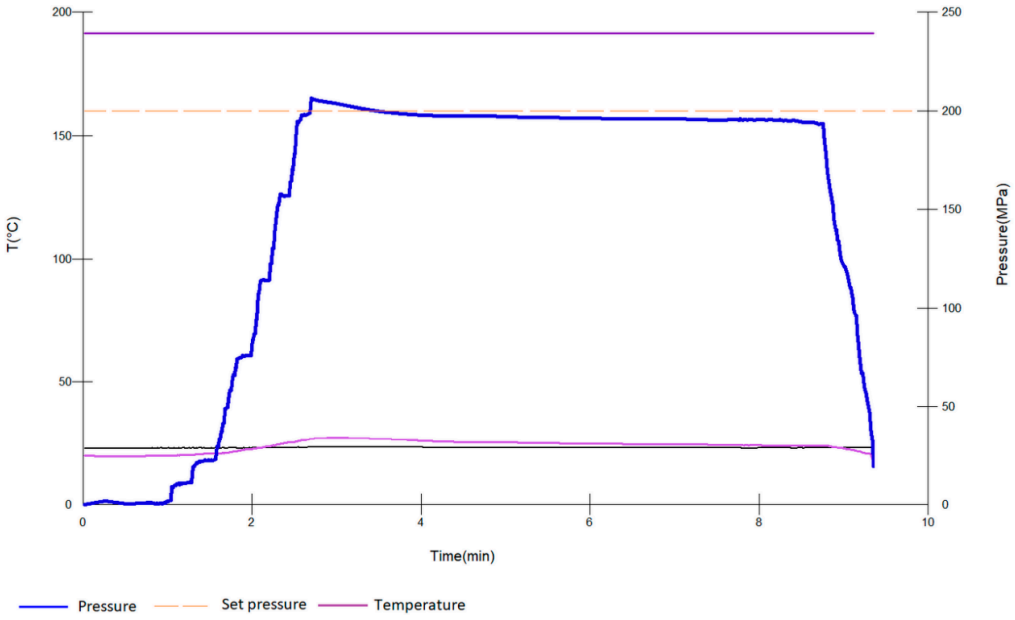

Figure 1. Example of the schematic diagram of the pressure and temperature during high hydrostatic pressure (HHP) processing of wines.

2.2. HHP Treatment and Antioxidants—Wine Bottle Aging

In order to investigate the effect of HHP treatment in combination with SO_2 and GSH additions on wine color, phenolic and aroma profile during 12 months of aging in bottles, the following red and white wines were used: standard SO_2 wine (25 mg/L free SO_2—red wine; 45 mg/L free SO_2—white wine), low SO_2+GSH wine (10 mg/L free SO_2+20 mg/L GSH—red wine; 25 mg/L free SO_2+20 mg/L GSH—white wine) and low SO_2 wine (10 mg/L free SO_2—red wine; 25 mg/L free SO_2—white wine). Control samples were the standard SO_2 wines not subjected to HHP treatment. An HHP of 200 MPa for 5 min was used, as this treatment resulted in similar or even slightly improved phenolic profile of pressurized wines compared to control (untreated) wines established in the first phase of the experiment as described in the Section 2.1. Additionally, it was reported that HHP in the range of 200–500 MPa can offer adequate inactivation rate of bacteria and yeasts in red and white wines, suggesting that it may be used to produce microbiologically stable wines [30]. All treatments were run in triplicate. After HHP processing, all wines (pressurized and unpressurized) were sealed in glass wine bottles and stored at 12 °C for 12 months. The chemical analyses (color, phenolic and aroma composition) were conducted on each wine after 0, 3, 6 and 12 months of aging in bottles.

As already presented in our previous work [29], the concentrations of dissolved oxygen, total and free SO_2 were also controlled at the point of bottling and during 12 months of aging. As previously mentioned in Section 2.1., we used the same analytical methods and the results showed that the initial levels of dissolved oxygen at bottling in red wine amounted up to 2.2 mg/L in both pressurized and control wines, while after 12 months the levels were in the range from 0.4 to 0.6 mg/L. In the case of white wine, the initial levels were around 1.1 and 1.4 mg/L in control and pressurized samples, while at the end of aging the levels amounted up to 0.3 and 0.6 mg/L in control and pressurized samples, respectively [29]. During aging, pressurized standard SO2 wines (red and white) showed similar or slightly lower levels of total and free SO_2 compared to untreated ones, amounting around 20 and 15 mg/L in the red wine and 80 and 25 mg/L in the white wine after 12 months. Also, the standard SO_2 wines were characterized by lower amounts of dissolved oxygen, whereas the addition of GSH had no significant effect on oxygen and SO_2 consumption rate in the red and white wines [29].

In exception, the volatile acidity (as acetic acid), known as important marker of microbiological spoilage, was monitored in this phase of experiment in order to assess the final quality of the wines. This parameter was analyzed according to the official OIV method [28]. After 12 months of aging, the data related to the volatile acidity showed the concentrations for red wine up to 0.69 g/L in control and in the range from 0.71 to 0.76 g/L in pressurized samples, and for white wine up to 0.37 g/L in control and from 0.43 to 0.47 g/L in pressurized samples, respectively. The obtained values were below the maximum allowable concentration of acetic acid in wines, which amounts to approximately around 1 g/L.

2.3. Chemical Analysis of Wine

Color properties (lightness, redness/greenness, yellowness/blueness, chroma, hue angle and total color difference) of the wine samples were determined using the CIELab system according to the OIV method [28]. Total phenolics (TP) were determined according to Singleton and Rossi [31], total anthocyanins (TA) according to Ribéreau-Gayon and Stonestreet [32] and total tannins (TT) according to Ribéreau-Gayon and Stonestreet [33].

Changes in phenolic composition of the red wine were monitored by high-performance liquid chromatography (HPLC). Analysis of free anthocyanins (FA) (delphinidin (Dph), cyanidin (Cy), petunidin (Pt), peonidin (Pn) and malvidin (Mv) -3-*O*-glucosides; peonidin- and malvidin-3-*O*-glucoside acetate (PnAc and MvAc); peonidin- and malvidin-3-*O*-glucoside *p*-coumarates (PnCm and MvCm)) was conducted according to the method described by Lorrain et al. [34]. The separation was performed on a Phenomenex Nucleosil C18 (250 mm × 4.6 mm, 5 µm) column and the mobile phases were water/formic acid (95:5, *v*/*v*) and acetonitrile/formic acid (95:5, *v*/*v*). The mobile phase gradient was: 0–25 min, 10–35% B linear; 25–26 min, 35–100% B linear; 26–28 min, 100% B isocratic; 28–29 min,

100–10% B linear, with re-equilibration of the column from 29–35 min under initial gradient conditions. The analysis conditions were: injection volume 20 µL, column temperature 40 °C, flow rate 1 mL/min and detection wavelength 520 nm. Results are expressed as the sum of free anthocyanins [35]. Analysis of individual flavanols (Fl) ((+)-catechin, (-)-epicatechin, procyanidins B1, B2, B3, B4 and C1) was conducted according to Ćurko et al. [36]. The separation was performed on a LiChrospher RP-18 (250 mm × 4 mm, 5 µm) column. The injected volume was also 20 µL. The mobile phase consisted of two solvents: water/formic acid (99:1, *v*/*v*) and acetonitrile/formic acid (99:1, *v*/*v*). The gradient conditions were: 0–11 min, 3–8% B linear; 11–16 min, 8% B isocratic; 16–20 min, 8–10% B linear; 20–27 min, 10% B isocratic; 27–32 min, 10–12% B linear; 32–34 min, 12–14% B linear; 34–45 min, 14–25% B linear; 45–46 min, 25–100% B linear; 46–50 min, 100% B isocratic, 50–51 min, 100–3% B linear, with re-equilibration of the column from 51–55 min under initial gradient conditions. The flow rate was 1 mL/min, column temperature 25 °C and detection wavelengths were 280 nm (excitation) and 320 nm (emission). Results are expressed as the sum of flavanols [35].

Changes in phenolic composition of the white wine were monitored by HPLC analysis of phenolic acids (Pa) (hydroxybenzoic (gallic, protocatechuic, vanillic and syringic) and hydroxycinnamic (caftaric, chlorogenic, caffeic, *p*-coumaric and ferulic)) and individual flavanols (Fl) ((+)-catechin, (-)-epicatechin, procyanidins B1 and B2) according to the method described by Monagas et al. [37].

For the phenolic acids and flavanols analysis, a column Phenomenex Gemini C18 (250 mm × 4.6 mm, 5 µm) was used. A modified gradient consisting of water/formic acid (98:2, *v*/*v*) and methanol was applied at flow rate of 1 mL/min as follows: 0 min, 2% B; 20 min, 32% B; 30 min, 40% B; 40–50 min, 50% B; 53 min, 2% B, with re-equilibration of the column from 53–55 min under initial gradient conditions. This simultaneous separation was conducted under following conditions: column temperature 25 °C, injection volume 20 µL and detection wavelengths 280 nm (hydroxybenzoic acids and flavanols) and 320 nm (hydroxycinnamic acids). Results are expressed as the sum of phenolic acids and sum of flavanols [38].

The aroma profile of the red and white wine samples was characterized by gas chromatographic analysis in detail described by Tomašević et al. [39]. Solid-phase microextraction (SPME) and gas chromatography-mass spectrometry (GC-MS) were used to extract and analyze free aroma compounds. For SPME extraction, 10 mL of wine sample, containing internal standard n-amyl alcohol (20 mg/L), was placed in the vial containing NaCl p.a. (2 g) and sealed with a crimp cap and silicone-PTFE septum. After the 100 µm PDMS fiber (Supelco, Bellefonte, USA) was exposed in the upper space of the vial at 40 °C for 30 min with constant shaking, it was immediately transferred to the GC injector for desorption at 250 °C for 5 min in splitless mode. Additionally, chromatographic analysis was performed on BP20 capillary (50 m × 220 µm × 0.25 µm) column (SGE Analytical Science, Victoria, Australia). The GC-MS working conditions were as follows: the detector interface temperature 250 °C, the electron ionization ion source at 70 eV and 280 °C, vector gas helium 5.0 and constant flow rate 1.2 mL/min. The temperature program for aroma analysis was: 40 °C, 5 min → 200 °C, 3 °C/min → 240 °C, 30 °C/min; 1 min, with the acquisition in scan mode. Due to a large number of identified and quantified aroma compounds, they were classified into four aroma groups: esters (*i*-butyl acetate, *i*-amyl acetate, ethyl acetate, 2-phenylethyl acetate, hexyl acetate, ethyl butyrate, ethyl hexanoate, ethyl octanoate, ethyl decanoate and diethyl succinate), higher alcohols (amyl alcohol, phenylethyl alcohol, 1-hexanol and *cis*-3-hexenol), fatty acids (hexanoic, octanoic and decanoic acid) and terpenes (α-terpineol and linalool). Results are expressed as the sum of esters, sum of higher alcohols, sum of fatty acids and sum of terpenes.

2.4. Sensory Evaluation

The wines were subjected to sensory evaluation by the nine-point hedonic scale method, with 25 judges. Generally, each sample (25–30 mL of wine) was presented in a coded, standard ISO 3591 tasting wineglass covered with a plastic Petri dish and served randomly. The judges were required to evaluate the treated wines with respect to the control (untreated). Additionally, all judges

were informed that the wines had undergone different treatments, but they did not have any details of the experimental design. The total effect of combined HHP and antioxidant treatments on color, odor and taste was evaluated with a verbal scale of 9 possible responses (1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like nor dislike, 6 = like slightly, 7 = like moderately, 8 = like very much, 9 = like extremely) [40]. The sensory analysis was performed on each wine after 0, 3, 6 and 12 months of bottle aging. Mean liking ratings and standard deviations were calculated.

2.5. Statistical Analysis

The statistical analysis was performed by one-way ANOVA in Statistica V.10 software (StatSoft, Tulsa, OK, USA). Tukey's and Duncan's tests were used as a comparison test when samples were significantly different after ANOVA ($p < 0.05$) for chemical and sensory analysis. The data were expressed as the mean value of three analytical repetitions with standard deviation.

3. Results and Discussion

3.1. Phenolic Profile and Color Properties of Red and White Wines after HHP Processing

The profiles for total and individual phenolic compounds, as well as color properties in both HHP-treated and untreated red and white wines are provided in Table 1; Table 2, respectively.

3.1.1. Red Wine

As can be seen in Table 1, slight changes occurred in the phenolic profile and color properties of the red wine after HHP processing. Generally, applied HHP treatments resulted in slightly lower content of TP and TA, except TT which remained constant. Also, the results concerning the evaluation of the sum of FA and sum of Fl showed that the content of individual anthocyanins and flavanols slightly decreased in all pressurized wines compared to control. These changes, although statistically significant ($p < 0.05$), remained relatively small at lower pressure levels. Almost no significant differences were observed between the sample pressurized at 200 MPa for 5 min and untreated (control) wine, respectively. Nevertheless, the main variations obtained can related to the differences in pressures applied, indicating that this parameter was a more discriminatory factor than the processing time. Namely, a decrease in both total and individual phenolics was most pronounced in samples treated with higher pressure (600 MPa). Moreover, when considering only the effect of pressurization time, longer treatments also resulted in a lower content of analyzed phenolic compounds. Taken all together, an HHP treatment of 600 MPa for 25 min resulted in the most significant decrease of all phenolics in the red wine when compared to the unpressurized sample. These results are in accordance with the findings of Tao et al. [41], who reported that pressurization at conditions of 250–650 MPa for 15–120 min mainly resulted in the decrease of phenolic compounds such as total phenolics, total anthocyanins, flavonols, tannins and tartaric esters. The same study also demonstrated the significant impact of both process parameters, pressure and time, on wine quality, where the first had the more influence than the latter. Chen et al. [42] also observed the decrease in the content of flavanols and the increase in phenolic acids of young red wine after HHP treatments (100–600 MPa/30 min, 500 MPa/5–60 min).

Table 1. Phenolic profile and color parameters of pressurized and unpressurized red wine samples.

Analysis	RW	High Hydrostatic Pressure Processing								
	Untreated	**200 MPa/5 min**	**200 MPa/15 min**	**200 MPa/25 min**	**400 MPa/5 min**	**400 MPa/15 min**	**400 MPa/25 min**	**600 MPa/5 min**	**600 MPa/15 min**	**600 MPa/25 min**
TP (mg/L)	2455.0 ± 3.2 ^{a}	2440.5 ± 4.5 ^{ab}	2436.4 ± 6.4 ^{bc}	2424.6 ± 5.1 ^{cd}	2417.7 ± 0.6 ^{d}	2396.4 ± 1.3 ^{e}	2358.2 ± 3.9 ^{f}	2366.8 ± 3.2 ^{f}	2364.6 ± 3.9 ^{f}	2337.3 ± 3.9 ^{g}
TA (mg/L)	333.1 ± 0.4 ^{a}	331.9 ± 2.9 ^{ab}	328.6 ± 0.7 ^{abc}	326.0 ± 2.1 ^{bc}	330.4 ± 0.4 ^{abc}	329.2 ± 0.4 ^{abc}	326.4 ± 0.9 ^{bc}	324.6 ± 2.2 ^{c}	315.7 ± 2.5 ^{d}	315.6 ± 1.6 ^{d}
TT (g/L)	2.94 ± 0.06 ^{a}	2.94 ± 0.06 ^{a}	2.92 ± 0.07 ^{a}	2.92 ± 0.06 ^{a}	2.94 ± 0.02 ^{a}	2.91 ± 0.11 ^{a}	2.91 ± 0.00 ^{a}	2.88 ± 0.05 ^{a}	2.86 ± 0.03 ^{a}	2.85 ± 0.05 ^{a}
FA (mg/L)										
Dph	18.42 ± 0.34 ^{a}	17.92 ± 0.09 ^{ab}	18.20 ± 0.15 ^{a}	17.31 ± 0.02 ^{c}	17.57 ± 0.15 ^{bc}	17.21 ± 0.06 ^{c}	17.62 ± 0.12 ^{bc}	15.82 ± 0.01 ^{d}	14.28 ± 0.17 ^{e}	14.44 ± 0.03 ^{e}
Cy	2.66 ± 0.09 ^{bc}	2.56 ± 0.03 ^{cd}	2.49 ± 0.00 ^{cde}	2.96 ± 0.03 ^{b}	2.68 ± 0.17 ^{bc}	2.31 ± 0.02 ^{def}	3.47 ± 0.05 ^{a}	2.25 ± 0.12 ^{ef}	2.13 ± 0.08 ^{f}	2.23 ± 0.04 ^{ef}
Pt	17.64 ± 0.39 ^{a}	17.65 ± 0.16 ^{a}	17.09 ± 0.02 ^{abc}	17.35 ± 0.06 ^{ab}	16.68 ± 0.15 ^{bc}	17.20 ± 0.12 ^{abc}	16.47 ± 0.17 ^{c}	14.35 ± 0.29 ^{d}	13.64 ± 0.17 ^{d}	13.85 ± 0.04 ^{d}
Pn	14.33 ± 0.34 ^{a}	13.39 ± 0.31 ^{b}	13.42 ± 0.11 ^{b}	12.15 ± 0.10 ^{c}	13.34 ± 0.37 ^{b}	12.40 ± 0.02 ^{c}	13.70 ± 0.31 ^{ab}	12.29 ± 0.10 ^{c}	11.90 ± 0.04 ^{c}	12.37 ± 0.02 ^{c}
Mv	92.36 ± 0.77 ^{a}	93.87 ± 0.38 ^{a}	85.15 ± 0.51 ^{b}	85.93 ± 0.13 ^{b}	85.88 ± 0.60 ^{b}	85.71 ± 1.01 ^{b}	84.15 ± 0.14 ^{bc}	82.20 ± 0.56 ^{c}	82.27 ± 0.12 ^{c}	82.19 ± 0.18 ^{c}
PnAc	4.76 ± 0.16 ^{a}	2.68 ± 0.22 ^{c}	4.55 ± 0.07 ^{a}	2.83 ± 0.07 ^{c}	4.42 ± 0.14 ^{a}	2.73 ± 0.13 ^{c}	4.66 ± 0.31 ^{a}	3.73 ± 0.12 ^{b}	3.26 ± 0.03 ^{b}	3.08 ± 0.06 ^{c}
MvAc	25.35 ± 0.74 ^{a}	24.73 ± 0.15 ^{ab}	23.75 ± 0.10 ^{bc}	22.67 ± 0.08 ^{c}	23.50 ± 0.74 ^{bc}	23.58 ± 0.31 ^{bc}	22.31 ± 0.08 ^{c}	22.34 ± 0.33 ^{c}	18.44 ± 0.21 ^{c}	15.11 ± 0.07 ^{d}
PnCm	2.26 ± 0.06 ^{a}	2.27 ± 0.15 ^{a}	2.18 ± 0.01 ^{a}	2.27 ± 0.00 ^{a}	2.09 ± 0.09 ^{ab}	2.29 ± 0.01 ^{a}	2.27 ± 0.14 ^{a}	2.15 ± 0.02 ^{a}	1.49 ± 0.05 ^{c}	1.85 ± 0.03 ^{b}
MvCm	8.81 ± 0.28 ^{ab}	8.96 ± 0.16 ^{a}	8.46 ± 0.05 ^{abc}	7.96 ± 0.05 ^{cd}	8.12 ± 0.25 ^{cd}	7.78 ± 0.30 ^{d}	8.86 ± 0.01 ^{ab}	8.23 ± 0.13 ^{bcd}	6.74 ± 0.04 ^{e}	6.74 ± 0.13 ^{e}
∑ FA	186.6 ± 1.6 ^{a}	184.0 ± 0.1 ^{a}	175.3 ± 1.0 ^{b}	171.4 ± 0.4 ^{c}	174.3 ± 1.1 ^{bc}	171.2 ± 0.6 ^{c}	173.5 ± 0.5 ^{bc}	163.4 ± 1.0 ^{d}	154.2 ± 0.3 ^{e}	151.9 ± 0.1 ^{e}
Fl (mg/L)										
Pro B1	33.65 ± 0.14 ^{a}	33.78 ± 0.56 ^{a}	33.35 ± 0.01 ^{a}	32.02 ± 0.08 ^{ab}	32.96 ± 0.22 ^{a}	32.60 ± 0.62 ^{ab}	31.98 ± 1.11 ^{ab}	32.74 ± 0.75 ^{ab}	32.06 ± 0.52 ^{ab}	30.77 ± 0.47 ^{b}
Cat	52.89 ± 0.55 ^{a}	52.57 ± 0.30 ^{a}	51.47 ± 0.73 ^{ab}	51.16 ± 0.05 ^{ab}	51.52 ± 0.43 ^{ab}	51.02 ± 0.65 ^{ab}	50.45 ± 0.14 ^{ab}	50.81 ± 1.75 ^{ab}	50.34 ± 0.59 ^{ab}	49.51 ± 0.92 ^{b}
Pro B2	35.84 ± 0.39 ^{a}	35.78 ± 0.51 ^{a}	35.59 ± 2.49 ^{a}	33.60 ± 1.37 ^{ab}	35.30 ± 2.09 ^{a}	33.79 ± 2.23 ^{ab}	32.47 ± 1.23 ^{ab}	32.27 ± 0.92 ^{ab}	28.95 ± 0.04 ^{b}	28.91 ± 0.55 ^{b}
Epicat	51.43 ± 1.30 ^{a}	47.98 ± 2.14 ^{ab}	46.17 ± 2.30 ^{b}	45.65 ± 1.18 ^{b}	46.04 ± 0.62 ^{b}	45.90 ± 0.53 ^{b}	43.59 ± 0.12 ^{b}	45.79 ± 0.29 ^{b}	45.69 ± 0.97 ^{b}	43.46 ± 1.12 ^{b}
Pro B3	4.41 ± 0.18 ^{a}	4.37 ± 0.06 ^{a}	4.28 ± 0.00 ^{ab}	4.18 ± 0.15 ^{ab}	4.22 ± 0.07 ^{ab}	4.11 ± 0.06 ^{ab}	4.07 ± 0.08 ^{ab}	4.07 ± 0.02 ^{ab}	4.06 ± 0.05 ^{ab}	3.95 ± 0.02 ^{b}
Pro B4	10.30 ± 0.49 ^{a}	10.06 ± 0.07 ^{a}	9.85 ± 0.51 ^{a}	9.49 ± 0.42 ^{ab}	9.30 ± 0.22 ^{ab}	8.50 ± 0.26 ^{ab}	7.62 ± 0.56 ^{ab}	8.45 ± 0.83 ^{ab}	7.63 ± 1.78 ^{ab}	7.02 ± 0.34 ^{b}
Pro C1	12.47 ± 0.31 ^{a}	11.55 ± 0.85 ^{ab}	10.51 ± 0.49 ^{bc}	10.03 ± 0.06 ^{bc}	10.47 ± 0.03 ^{bc}	9.81 ± 0.18 ^{bc}	9.70 ± 0.75 ^{bc}	9.62 ± 0.56 ^{c}	9.46 ± 0.46 ^{c}	8.94 ± 0.31 ^{c}
∑ Fl	201.0 ± 1.1 ^{a}	196.1 ± 0.5 ^{ab}	191.2 ± 6.5 ^{abc}	186.1 ± 0.2 ^{bcd}	189.8 ± 2.3 ^{bc}	185.7 ± 4.0 ^{bcd}	179.9 ± 0.4 ^{cde}	183.8 ± 3.4 ^{cd}	178.2 ± 2.3 ^{de}	172.6 ± 1.0 ^{e}
Color										
L^*	14.6 ± 0.2 ^{e}	14.6 ± 0.1 ^{de}	15.0 ± 0.1 ^{bc}	14.8 ± 0.1 ^{cde}	15.0 ± 0.0 ^{bc}	15.1 ± 0.1 ^{bc}	15.2 ± 0.0 ^{b}	16.4 ± 0.1 ^{a}	16.5 ± 0.2 ^{a}	16.3 ± 0.1 ^{a}
a^*	45.8 ± 0.1 ^{e}	46.0 ± 0.1 ^{cde}	46.2 ± 0.1 ^{cde}	45.9 ± 0.1 ^{de}	46.3 ± 0.0 ^{bcd}	46.4 ± 0.1 ^{bc}	46.1 ± 0.3 ^{b}	47.9 ± 0.0 ^{a}	47.9 ± 0.2 ^{a}	47.7 ± 0.2 ^{a}
b^*	24.8 ± 0.1 ^{d}	25.4 ± 0.2 ^{bc}	25.3 ± 0.1 ^{bcd}	25.0 ± 0.1 ^{cd}	25.5 ± 0.0 ^{bc}	25.6 ± 0.1 ^{b}	25.3 ± 0.1 ^{bcd}	27.7 ± 0.1 ^{a}	27.7 ± 0.2 ^{a}	27.4 ± 0.2 ^{a}
C^*	52.2 ± 0.1 ^{e}	52.4 ± 0.2 ^{cde}	52.7 ± 0.1 ^{cde}	52.3 ± 0.1 ^{de}	52.8 ± 0.0 ^{bcd}	53.0 ± 0.1 ^{bc}	53.4 ± 0.0 ^{b}	55.3 ± 0.1 ^{a}	55.3 ± 0.3 ^{a}	55.0 ± 0.3 ^{a}
H^*	0.5 ± 0.0 ^{d}	0.5 ± 0.0 ^{cd}	0.5 ± 0.0 ^{cd}	0.5 ± 0.0 ^{bc}	0.5 ± 0.0 ^{cd}	0.5 ± 0.0 ^{cd}	0.5 ± 0.0 ^{bc}	0.5 ± 0.0 ^{a}	0.5 ± 0.00 ^{a}	0.5 ± 0.0 ^{ab}
ΔE^*	-	0.6	0.7	0.4	1.0	1.2	0.9	4.0	4.1	3.7

$^{a–g}$ Different letters in the same row show significant difference ($p < 0.05$) among the samples. RW: red wine; TP: total phenolics; TA: total anthocyanins; TT: total tannins; FA: free anthocyanins (Dph: delphinidin-3-*O*-glucoside; Cy: cyanidin-3-*O*-glucoside; Pt: petunidin-3-*O*-glucoside; Pn: peonidin-3-*O*-glucoside; Mv: malvidin-3-*O*-glucoside; PnAc: peonidin-3-*O*-glucoside acetate; MvAc: malvidin-3-*O*-glucoside acetate; PnCm: peonidin-3-*O*-glucoside *p*-coumarate; MvCm: malvidin-3-*O*-glucoside *p*-coumarate); Fl: flavanols; Pro: procyanidin; Cat: (+)-catechin; Epicat: (−)-epicatechin.

Table 2. Phenolic profile and color parameters of pressurized and unpressurized white wine samples.

Analysis	WW	High Hydrostatic Pressure Processing								
	Untreated	**200 MPa/5 min**	**200 MPa/15 min**	**200 MPa/25 min**	**400 MPa/5 min**	**400 MPa/15 min**	**400 MPa/25 min**	**600 MPa/5 min**	**600 MPa/15 min**	**600 MPa/25 min**
TP (mg/L)	261.7 ± 0.3 ^{a}	259.1 ± 0.5 ^{abc}	258.7 ± 1.1 ^{abcd}	256.5 ± 0.5 ^{cd}	256.1 ± 0.3 ^{d}	258.8 ± 0.8 ^{abcd}	256.1 ± 0.5 ^{cd}	259.7 ± 0.1 ^{ab}	257.1 ± 1.1 ^{bcd}	256.6 ± 1.4 ^{cd}
Pa (mg/L)										
Gal	2.56 ± 0.01 ^{de}	2.66 ± 0.02 ^{a}	2.63 ± 0.00 ^{ab}	2.62 ± 0.02 ^{abc}	2.64 ± 0.01 ^{a}	2.61 ± 0.01 ^{abcd}	2.57 ± 0.01 ^{cde}	2.58 ± 0.02 ^{bcde}	2.56 ± 0.01 ^{de}	2.55 ± 0.01 ^{e}
Protocat	5.67 ± 0.02 ^{bc}	5.91 ± 0.11 ^{a}	5.89 ± 0.02 ^{a}	5.77 ± 0.03 ^{ab}	5.75 ± 0.09 ^{ab}	5.72 ± 0.01 ^{ab}	5.64 ± 0.02 ^{bc}	5.63 ± 0.03 ^{bc}	5.61 ± 0.00 ^{bc}	5.48 ± 0.00 ^{c}
Van	0.78 ± 0.06 ^{a}	0.72 ± 0.10 ^{a}	0.52 ± 0.04 ^{bc}	0.51 ± 0.05 ^{c}	0.70 ± 0.06 ^{ab}	0.51 ± 0.01 ^{bc}	0.49 ± 0.00 ^{c}	0.41 ± 0.03 ^{c}	0.40 ± 0.00 ^{c}	0.38 ± 0.00 ^{c}
Syr	0.25 ± 0.02 ^{a}	0.25 ± 0.03 ^{a}	0.22 ± 0.03 ^{ab}	0.19 ± 0.00 ^{ab}	0.23 ± 0.01 ^{ab}	0.20 ± 0.00 ^{ab}	0.18 ± 0.03 ^{ab}	0.19 ± 0.00 ^{ab}	0.18 ± 0.01 ^{ab}	0.17 ± 0.01 ^{b}
Caft	30.61 ± 0.25 ^{a}	30.47 ± 0.02 ^{a}	29.69 ± 0.26 ^{bcd}	29.25 ± 0.01 ^{ef}	30.38 ± 0.02 ^{ab}	29.47 ± 0.12 ^{cde}	28.88 ± 0.03 ^{ef}	30.21 ± 0.01 ^{abc}	28.76 ± 0.40 ^{ef}	28.59 ± 0.25 ^{f}
Chlo	2.40 ± 0.00 ^{b}	2.44 ± 0.00 ^{a}	2.39 ± 0.02 ^{b}	2.37 ± 0.01 ^{b}	2.39 ± 0.02 ^{b}	2.36 ± 0.00 ^{b}	2.30 ± 0.00 ^{c}	2.37 ± 0.00 ^{b}	2.28 ± 0.02 ^{c}	2.26 ± 0.01 ^{c}
Caf	2.30 ± 0.01 ^{b}	2.41 ± 0.01 ^{a}	2.27 ± 0.01 ^{bc}	2.26 ± 0.01 ^{bc}	2.28 ± 0.01 ^{bc}	2.25 ± 0.02 ^{bc}	2.24 ± 0.02 ^{cd}	2.24 ± 0.00 ^{cd}	2.20 ± 0.02 ^{d}	2.20 ± 0.00 ^{d}
p-Coum	1.43 ± 0.01 ^{bc}	1.49 ± 0.01 ^{a}	1.47 ± 0.01 ^{ab}	1.45 ± 0.01 ^{abc}	1.49 ± 0.01 ^{a}	1.46 ± 0.01 ^{abc}	1.43 ± 0.01 ^{bc}	1.45 ± 0.02 ^{abc}	1.44 ± 0.01 ^{bc}	1.42 ± 0.02 ^{c}
Fer	0.57 ± 0.01 ^{a}	0.58 ± 0.03 ^{a}	0.56 ± 0.00 ^{a}	0.56 ± 0.00 ^{a}	0.56 ± 0.00 ^{a}	0.56 ± 0.01 ^{a}	0.55 ± 0.00 ^{a}	0.56 ± 0.01 ^{a}	0.56 ± 0.00 ^{a}	0.55 ± 0.01 ^{a}
∑ Pa	46.6 ± 0.3 ^{a}	46.9 ± 0.1 ^{a}	45.6 ± 0.3 ^{b}	45.0 ± 0.1 ^{bc}	46.4 ± 0.1 ^{a}	45.1 ± 0.2 ^{b}	44.3 ± 0.0 ^{cd}	45.7 ± 0.1 ^{b}	44.0 ± 0.3 ^{d}	43.6 ± 0.2 ^{d}
Fl (mg/L)										
Pro B1	11.47 ± 0.01 ^{a}	11.30 ± 0.00 ^{ab}	11.18 ± 0.01 ^{abc}	11.14 ± 0.06 ^{abc}	11.29 ± 0.01 ^{ab}	11.03 ± 0.01 ^{bc}	10.84 ± 0.19 ^{cd}	10.81 ± 0.10 ^{cd}	10.64 ± 0.17 ^{d}	10.55 ± 0.13 ^{d}
ProB2	2.66 ± 0.12 ^{a}	2.57 ± 0.16 ^{a}	2.28 ± 0.09 ^{ab}	2.00 ± 0.15 ^{bc}	1.83 ± 0.02 ^{c}	1.76 ± 0.14 ^{c}	1.67 ± 0.09 ^{c}	1.77 ± 0.09 ^{c}	1.67 ± 0.08 ^{c}	1.58 ± 0.02 ^{c}
Cat	6.83 ± 0.04 ^{a}	6.02 ± 0.21 ^{b}	4.41 ± 0.22 ^{c}	3.40 ± 0.05 ^{de}	3.70 ± 0.05 ^{d}	3.21 ± 0.05 ^{ef}	3.12 ± 0.06 ^{ef}	2.97 ± 0.02 ^{f}	2.95 ± 0.06 ^{f}	2.92 ± 0.04 ^{f}
Epicat	10.53 ± 0.11 ^{a}	10.24 ± 0.03 ^{ab}	9.89 ± 0.14 ^{bc}	9.50 ± 0.01 ^{c}	9.67 ± 0.05 ^{c}	8.88 ± 0.01 ^{d}	8.77 ± 0.22 ^{d}	8.68 ± 0.29 ^{d}	8.55 ± 0.03 ^{d}	7.83 ± 0.03 ^{e}
∑ Fl	31.5 ± 0.0 ^{a}	30.1 ± 0.4 ^{b}	27.8 ± 0.0 ^{c}	26.1 ± 0.1 ^{d}	26.5 ± 0.1 ^{d}	24.9 ± 0.2 ^{e}	24.4 ± 0.1 ^{ef}	24.2 ± 0.1 ^{ef}	23.8 ± 0.1 ^{f}	22.9 ± 0.2 ^{g}
Color										
L^*	101.8 ± 0.0 ^{a}	101.2 ± 0.6 ^{a}	101.1 ± 0.0 ^{a}	100.1 ± 0.0 ^{b}	100.0 ± 0.0 ^{b}	99.9 ± 0.1 ^{b}	100.0 ± 0.0 ^{b}	98.2 ± 0.1 ^{c}	98.1 ± 0.0 ^{c}	98.0 ± 0.0 ^{c}
a^*	−0.2 ± 0.0 ^{c}	−0.2 ± 0.1 ^{bc}	−0.1 ± 0.0 ^{bc}	−0.0 ± 0.0 ^{ab}	0.0 ± 0.0 ^{a}	0.0 ± 0.0 ^{a}	0.0 ± 0.0 ^{a}	−0.6 ± 0.1 ^{d}	−0.6 ± 0.0 ^{d}	−0.5 ± 0.1 ^{d}
b^*	−0.8 ± 0.0 ^{c}	−0.5 ± 0.3 ^{bc}	−0.5 ± 0.0 ^{b}	−0.0 ± 0.0 ^{a}	0.0 ± 0.0 ^{a}	0.1 ± 0.1 ^{a}	−0.0 ± 0.0 ^{a}	−0.6 ± 0.0 ^{bc}	−0.6 ± 0.0 ^{bc}	−0.6 ± 0.0 ^{bc}
C^*	0.9 ± 0.0 ^{a}	0.5 ± 0.3 ^{ab}	0.5 ± 0.0 ^{bc}	0.0 ± 0.0 ^{d}	0.0 ± 0.0 ^{d}	0.1 ± 0.0 ^{cd}	0.1 ± 0.0 ^{d}	0.6 ± 0.0 ^{ab}	0.6 ± 0.0 ^{ab}	0.6 ± 0.0 ^{ab}
H^*	1.3 ± 0.0 ^{a}	1.3 ± 0.0 ^{a}	1.3 ± 0.0 ^{a}	1.0 ± 0.2 ^{a}	0.1 ± 0.7 ^{ab}	1.0 ± 0.4 ^{a}	−0.8 ± 0.9 ^{ab}	−1.5 ± 0.0 ^{b}	−1.5 ± 0.0 ^{b}	−1.5 ± 0.0 ^{b}
ΔE^*	-	0.7	0.9	1.9	2.0	2.2	2.0	3.7	3.7	3.9

$^{a–g}$ Different letters in the same row show significant difference ($p < 0.05$) among the samples. WW: white wine; TP: total phenolics; Pa: phenolic acids; Gal: gallic acid; Protocat: protocatechuic acid; Van: vanillic acid; Syr: syringic acid; Caft: caftaric acid; Chlo: chlorogenic acid; Caf: caffeic acid; *p*-Coum: *p*-coumaric acid; Fer: ferulic acid; Fl: flavanols; Pro: procyanidin; Cat: (+)-catechin; Epicat: (−)-epicatechin.

Furthermore, pressurized red wine samples showed different values ($p < 0.05$) of the CIELab parameters, when compared with the unpressurized ones (Table 1), respectively. A slight increase in parameters L*, a*, b*, C* and H* was observed after HHP treatments, indicating a slight change in the wine color, shifting from red-purple to more orange-red and lighter. The observed changes were particularly pronounced after applying higher pressure (600 MPa) and longer time of processing (25 min). Similarly, Sun et al. [43] reported significant changes of color properties (chroma and hue values) of young red wine after HHP treatments (100–600 MPa/30 min, 500 MPa/5–60 min). Furthermore, the total color differences (ΔE*) between pressurized and unpressurized samples were calculated in order to determine whether the observed changes in the chromatic properties of the red wine were visually relevant. Generally, the values above 3 reflect differences which are noticeable and clearly perceived by the observer in the case of the red wine [44].The results demonstrated that ΔE* values were even lower than 1 or around 1 CIELab unit after HHP treatments at 200 and 400 MPa during 5, 15 and 25 min. However, all treatments at 600 MPa resulted in ΔE* values around 4 CIELab units, which is clearly higher than limit value of 3 CIELab units considered for perceiving the differences by the human eye in red wine [44].

3.1.2. White Wine

The results regarding the phenolic and color changes of white wine after HHP treatments also showed significant differences ($p < 0.05$) between pressurized and unpressurized samples (Table 2), respectively. As it can be observed, the pressurized wines were characterized by slightly lower content of TP, sum of Pa and sum of Fl. In addition, most of individual phenolic acids (vanillic, syringic, caftaric, chlorogenic and caffeic acid) and flavanols showed decreasing trend after applying higher HHP process conditions (pressure and time). But, on the other hand, slightly higher content of gallic, protocatechuic and *p*-coumaric acid was found in HHP-treated samples at lower pressures of 200 and 400 MPa compared to control wine, while after applying pressure of 600 MPa differences diminished. Particularly, the pressurized sample at 200 MPa for 5 min compared to control wine was significantly higher in content of previously mentioned phenolic acids and additionally in chlorogenic and caffeic acid. On the other hand, the content of ferulic acid remain unchanged in all wines. This increasing trend in the content of corresponding phenolic acids could be explained by the possibility of pressure to promote the decomposition of some compounds [42]. Overall, as already observed in the case of red wine, the lowest content of analyzed phenolic compounds in the white wine was also determined after treatment at 600 MPa for 25 min. Similar results were reported by Briones-Labarca et al. [19], whose study showed that the total phenolic and flavonoid contents of young white wine were not severely reduced by HHP treatments (300–500 MPa/5–15 min). Moreover, Santos et al. [12] found that HHP treatments (425 and 500 MPa for 5 min) had no effect on the total phenolics and antioxidant activity of white wine immediately after processing.

As regards the white wine color after HHP treatments, most of the pressurized samples were characterized by slightly lower values of L* and C* and higher values of a* and b* ($p < 0.05$) compared to control wine, respectively. The observed changes indicate that the wine color shifts from pale and practically colorless to a more yellow color. However, all samples treated at 600 MPa showed oppositely significantly lower values of parameters a* and H*, while values of b* and C* were very close to ones of control. In all other cases, there was no significant difference in parameter H* when comparing with unpressurized sample. Moreover, the pressurized sample at 200 MPa for 5 min and the unpressurized one did not differ drastically in their color parameters compared to all other samples. Additionally, the total color difference values (ΔE*) indicated that the pressurized wines at the highest pressure level (600 MPa) visually differed from the control sample, since the values were higher than 3 CIELab units (3.7 and 3.9, respectively). On the other hand, all other HHP treatments led to wines more like the untreated one. Namely, established ΔE* values were in the 0.7–2.0 CIELab unit range, which cannot be clearly detected by the human eye. These results agree with those reported by

Briones-Labarca et al. [19], who observed slight changes in the chromatic properties of white wine after applying HHP, but also stating that these changes were not visually perceived.

In addition, our own earlier work demonstrated that, in general, these HHP processing conditions resulted in slight aroma changes, primarily decrease of volatiles like esters, fatty acids and terpenes, and increase of higher alcohols in both red and white wine [45]. Also, it was found that these changes were more pronounced in red wine, and particularly after pressurization with higher pressure of 600 MPa and longer processing time of 25 min. As regards to the above-mentioned, with properly selected treatment conditions not causing major quality changes, HHP could be very promising in wine technology to complement the protective action of SO_2, enabling to reduce its content in wines.

3.2. Phenolic and Aroma Changes of Red Wine during 12 Months of Aging Induced by HHP and Antioxidant Treatments

3.2.1. Phenolic Profile and Color Properties

Figure 2 presents the evolution of total phenolics (TP), total anthocyanins (TA), total tannins (TT), sum of free anthocyanins (FA), sum of flavanols (Fl) and color properties (L*, a*, b*, C* and H*) of HHP-treated and untreated red wine samples during 12 months of aging in relation to their antioxidants (SO_2 and GSH) content. In general, there is a decreasing trend in the content of analyzed phenolics with time, independently of applied treatments. The phenolic changes during aging of wine are mainly due to their potential chemical oxidation, polymerization, condensation and precipitation [46,47]. As can be seen, the significant differences ($p < 0.05$) were observed between phenolic composition of pressurized and unpressurized wines along the aging period, respectively (Figure 2a–e). At the beginning of aging, pressurized samples contained lower concentrations of TP, TA and Fl when compared with control (untreated) wine. However, the HHP effect on the content of FA and TT was not observed immediately after pressurization. These differences slightly varied between pressurized and unpressurized samples during first 6 months, but after 12 months of aging significant differences can be clearly seen in TP, TA, FA and Fl, while there were no major changes in TT content. These agrees with the findings of other studies [9,11,20,21,48] which demonstrate that HHP treatment results in a decrease of phenolic compounds, primarily anthocyanins and flavanols. HHP-induced changes can be related to the reduction in volume during HHP processing, which could impact the chemical equilibrium of a reaction [30]. These results support the hypothesis that pressurization reduces the content of anthocyanins and flavanols due to enhancement of numerous chemical reactions involving phenolic compound such as condensation, polymerization and oxidation [21]. In addition, the lowest content of analyzed phenolic compounds among pressurized samples was observed in the sample with low SO_2 content. Obviously, the HHP treatment in combination with higher content of SO_2 can slow down the chemical reactions rate, which are otherwise accelerated in the treated samples with higher concentrations of antioxidants. However, light effect of GSH on phenolic composition was evident at the beginning of aging and up to a period of 6 months, but after 12 months no differences were found between low-SO_2+GSH and low-SO_2 wines (except in FA). Therefore, the different trends observed among treated samples are not just a consequence of potential acceleration of chemical reactions by HHP, but also, they are the result of different SO_2 content in wines. Namely, the SO_2 actions in wine primarily refer to the reduction of polymerization reactions rate of phenolic compounds and the protection from oxidation [9,11,21]. However, pressurized standard-SO_2 and low-SO_2+GSH samples showed similar content of TP and TT after 12 months of aging. As far as we are aware, this is the first time that the HHP treatment was investigated in combination with the addition of different amounts of antioxidants, SO_2 and GSH. Nevertheless, few earlier studies have focused on the joint effects of HHP and SO_2. For instance, the study by Santos et al. [11] compared the pressurized and unpressurized wine samples containing 0 and 40 mg/L of SO_2. Recently, the study of Christofi et al. [21] involved pressurized and unpressurized red wine samples containing 0, 30, 60 and 100 mg/L of SO_2. The same authors found that a combination of HHP treatment (350 MPa/10 min) and 60 mg/L SO_2 may slow down the rate of chemical reactions, which take place much faster in pressurized samples.

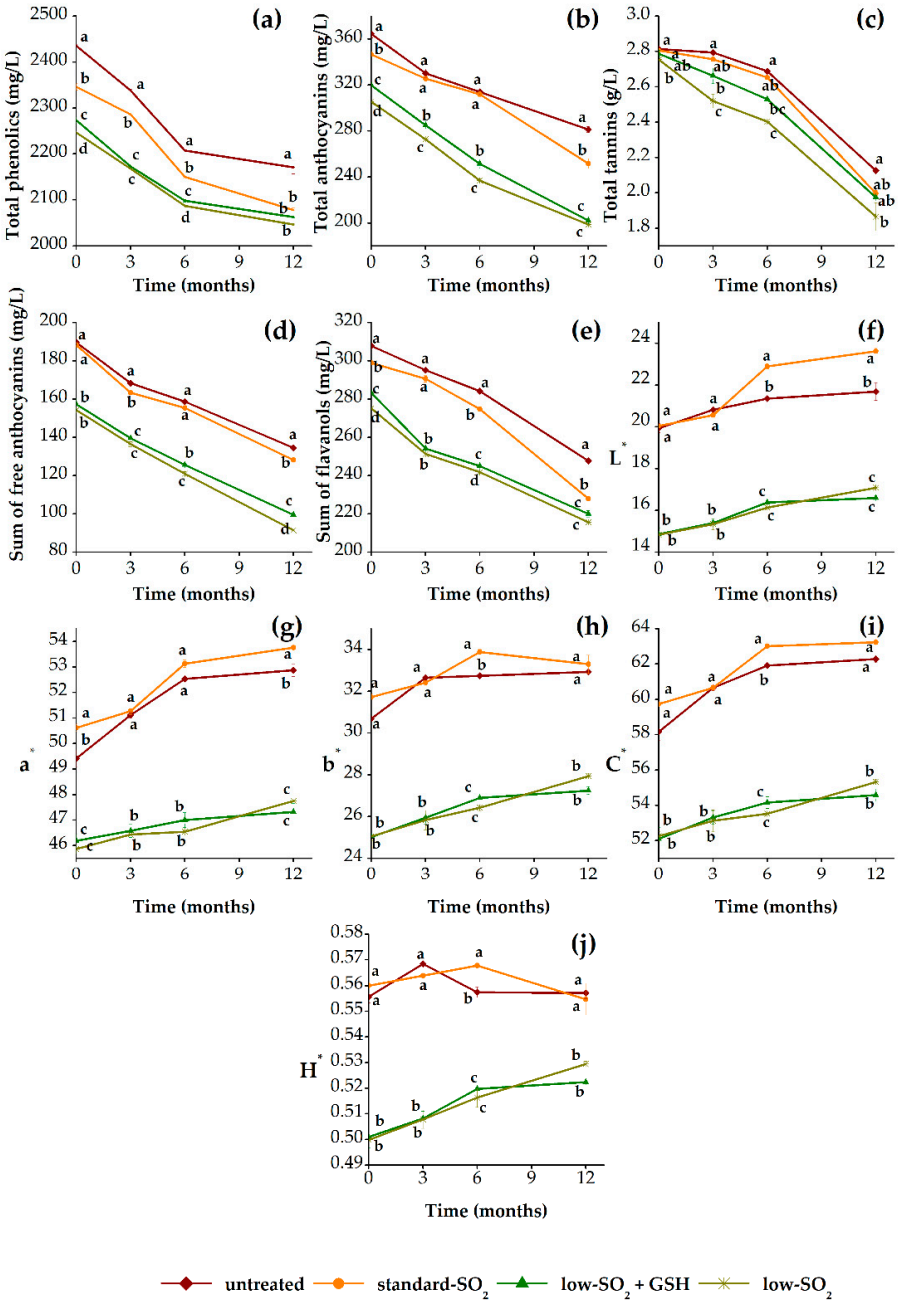

Figure 2. Phenolic (**a**–**e**) and color (**f**–**j**) changes of pressurized (standard SO_2, low SO_2+GSH and low SO_2) and unpressurized (untreated) red wine samples during 12 months of aging in bottles. GSH: glutathione.

Since the phenolic composition and color of wine are closely related, it is also important to highlight the results regarding color properties (Figure 2f–j). In accordance with the findings of other research [9,11,21,48], an increase of all color parameters, namely L*, a*, b*, C* and H*, with time was observed in all wine samples. It is known that anthocyanins provide the initial color of red wine, while as wine ages, its color significantly changes due to the decrease of free anthocyanins and formation of polymeric pigments [30,49]. This increment of corresponding color parameters indicates that the color becomes more lighter and orange-red-like in the aged wines [9,15]. Furthermore, no significant differences were observed in color parameters among pressurized and unpressurized standard-SO_2 wines up to the period of 3 months of aging. However, after 12 months of aging, significant differences ($p < 0.05$) in parameters L^* and a^* were found, whereas parameters b^*, C^* and H^* remained unchanged.

Moreover, the pressurized standard-SO_2 sample presented higher values of CIELab parameters when compared with control wine after 12 months of aging, respectively. Further, there was obvious difference among HHP-treated samples concerning the effect of antioxidants, primarily SO_2, while no GSH effect was noticed. The samples containing lower content of SO_2 as well as GSH presented the same trends and values of color parameters during observed period of aging. These wines had much lower values of CIELab parameters compared to samples with standard SO_2 content (both pressurized and unpressurized). A study by van Wyk et al. [20] also found that HHP treatment (400 MPa/5 s) resulted in decreased color density and increased brownish color in SO_2 free red wine. Moreover, the total color difference (ΔE^*) was calculated to express the overall color difference between treated samples and control. In the early stages after pressurization and after 12 months of aging, ΔE^* values for the pressurized standard-SO_2 wine were lower than 3 CIELab units, increasing along the aging time from 0.4 to 2.2 (data not shown). These results suggest that the difference in color of the pressurized standard-SO_2 sample in relation to the unpressurized (control) wine was not perceived by the human eye. This seems to be due to protective effect of SO_2, which can protect wine from excessive oxidation of phenolic compounds and consequently avoid the undesirable modifications [50]. On the other hand, for the rest of pressurized samples (low SO_2+GSH and low SO_2) ΔE^* values were around 8 at the beginning of aging, when compared to control. Moreover, at the end of 12 months of aging, ΔE^* values increased to around 9 and 10, respectively. These results indicate that the color changes are mainly due to a combination of HHP treatment with different content of SO_2 in presented wines.

3.2.2. Aroma Profile

Figure 3 shows the evolution of sum of esters, sum of higher alcohols, sum of fatty acids and sum of terpenes of HHP-treated and untreated red wine samples during 12 months of aging in relation to their content of antioxidants (SO_2 and GSH). Generally, the content of esters and higher alcohols increased, while a decrease in the content of fatty acids and terpenes was observed in all wines during aging period of 12 months (Figure 3a–d). However, observed slight increase of esters is due to increase in the content of two individual aroma compounds, namely ethyl acetate and diethyl succinate, while other quantified compounds included in sum of esters actually decreased. Esters are reported to decrease during aging due to chemical reactions of hydrolysis or oxidation. The same evolution pattern follows terpenes, which also decrease during aging [51]. Altogether, in this way, the wines are known to lose some of their fruity and floral aromas. Furthermore, higher alcohols are reported to be mainly stable during aging, but some increases have been observed, which are explained through hydrolysis of the corresponding esters [52] or a certain microbial activity occurred in wines [39]. On the other hand, the stability of fatty acids is not uniform, as some compounds could increase while others decrease or remain stable during aging [51]. As can be seen from Figure 3, HHP-treated samples contained, in general, slightly lower content of aroma compounds when compared with the untreated sample. This can be due to an increase of interactions among aroma and phenolic compounds in wine during aging induced by HHP [13]. Immediately after pressurization, no differences were found in the content of esters and higher alcohols among unpressurized and pressurized samples, whereas the significant differences ($p < 0.05$) were observed for the content of fatty acids and terpenes, respectively. Up to a period of 3 months of aging, the differences were significant for almost all aroma groups (except terpenes), while after 6 months they were noticeable in the case of esters and fatty acids. Additionally, after 12 months of aging, significant differences were determined only for the content of fatty acids, indicating that HHP treatment influenced this group of aroma compounds. Although the aroma is an important factor in defining the quality of wine, in the present literature there is only one study that specifically determined the aroma composition of HHP-treated red wine along the storage period [13], while all other studies were primarily oriented toward the effect of HHP on wine sensory attributes [9,11,20,21]. Namely, Santos et al. [13] demonstrated that there were minor differences in aroma composition of pressurized wines after 2 months of storage, while after 9 months quite remarkable changes occurred, indicating a significant impact of HHP on aroma composition of

SO_2 free red and white wines. Also, the same authors found that treated samples contained higher content of aldehydes, ketones, acetals and furans, and suggested that HHP treatment accelerates oxidation of higher alcohols and fatty acids and Maillard reactions, lastly giving the aroma profile of aged wines. Further, regarding the effect of SO_2 content, it can be clearly seen that among treated samples those with low SO_2 content were characterized by lower content of all aroma compounds, respectively. Probably, the well-known antioxidant activity of SO_2 resulted in its inhibitory action of slowing down their loss during aging. It was already presented that the presence or absence of SO_2 had a great impact on the evolution of esters and higher alcohols and to lesser extent fatty acids during wine aging in the bottle [53]. Aside from that, not of lesser importance is the effect of GSH, which had a significant impact on fatty acids and much less impact on higher alcohols and terpenes, while no effect was observed for the group of esters during aging period. As regards to the role of GSH in protecting wine aroma compounds, it was shown that this reduced form of glutathione can react as a strong nucleophile with quinones [54]. Additionally, after 12 months of aging, the pressurized wines (standard SO_2 and low SO_2+GSH) showed very close values in the most of aroma compounds, except esters, indicating that HHP can be applied with lower content of SO_2 without causing major changes in aroma composition.

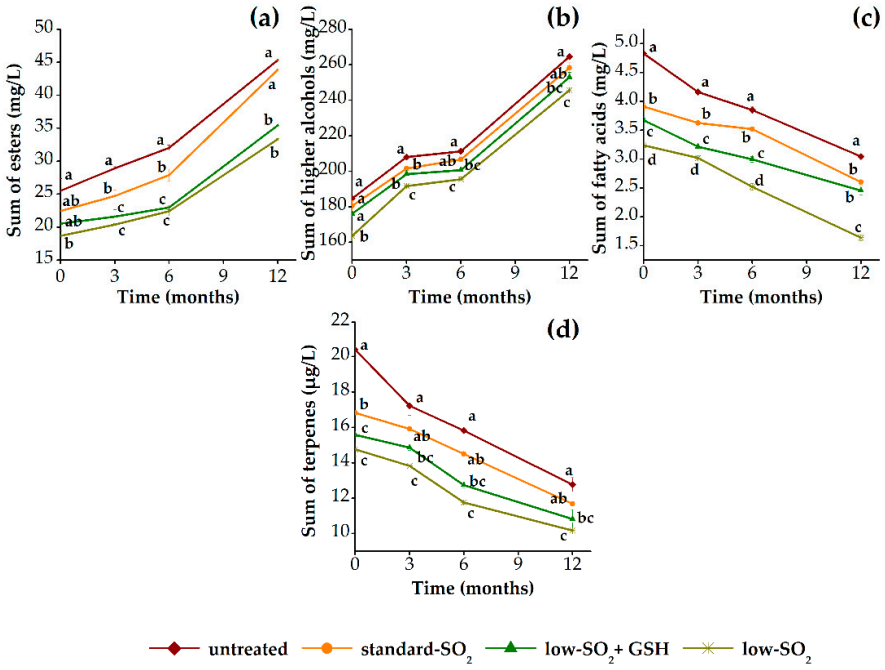

Figure 3. Aroma changes of pressurized (standard SO_2, low SO_2+GSH and low SO_2) and unpressurized (untreated) red wine samples during 12 months of aging in bottles: (**a**) sum of esters; (**b**) sum of higher alcohols; (**c**) sum of fatty acids; (**d**) sum of terpenes. GSH: glutathione.

3.3. Phenolic and Aroma Changes of White Wine during 12 Months of Aging Induced by HHP and Antioxidant Treatments

3.3.1. Phenolic Profile and Color Properties

Figure 4 presents the evolution of total phenolics (TP), sum of phenolic acids (Pa), sum of flavanols (Fl) and color properties (L*, a*, b*, C* and H*) of HHP-treated and untreated white wine samples during 12 months of aging in relation to their antioxidant (SO_2 and GSH) content. As it can be observed, the content of TP and Fl decreased, while the content of Pa increased with aging time in all presented samples (Figure 4a–c). Generally, during aging of white wine, browning and oxidation reactions take place. The most important phenolic compounds involved in these reactions are hydroxycinnamic esters and flavanols, which content consequently decreases with time [55]. On the

other hand, this reduction of hydroxycinnamates due to hydrolysis reactions is mainly responsible for the increment of certain free phenolic acids [56]. Additionally, this increase can be related to their participation in reactions with glutathione [57]. Moreover, the pressurized samples were characterized by slightly lower content of analyzed phenolics compared to control wine. In general, at the beginning of aging, no significant differences were observed in the content of TP and Pa (except Fl) between pressurized and unpressurized standard-SO_2 wines, respectively. However, after 3 months and up to a period of 6 months of aging, significant differences ($p < 0.05$) were observed among pressurized and unpressurized standard-SO_2 wines in overall phenolic composition. At the end of the aging period of 12 months, HHP treatment significantly influenced the content of TP, whereas no significant differences in Pa and Fl content compared to control were found. Santos et al. [12] also observed the decrease of the total phenolic content as well as antioxidant activity in pressurized white wine samples after 12 months of storage. It is suggested that the generation of highly reactive radicals during HHP processing and enhancement of oxidation and polymerization reactions of phenolic compounds are responsible for reduction in their content [9,12,19,42]. Concerning the effect of antioxidant treatments (SO_2 and GSH), the pressurized low SO_2 wine showed the lowest content of analyzed phenolics. Namely, higher content of SO_2 seems to obstruct the loss of these compounds during aging due to the reasons described earlier. Although, after 6 months of aging, GSH effect was evident in TP, Pa and Fl, respectively, after 12 months, it was only noticed for Pa. Since all phenolic compounds are susceptible to oxidation changes, GSH could react with the quinonic form of the hydroxycinnamic acids through an electrophilic addition, triggering the regeneration of free forms [58]. Additionally, the pressurized wines, standard SO_2 and low SO_2+GSH, presented very similar values in Pa content at the end of aging.

Considering the color properties of the white wine, in general, there was a decreasing trend in parameters L* and H*, while parameters a*, b* and C* increased with time in all wine samples (Figure 4d–h). During aging, oxidative processes involving phenolics would surely result in a change of color, from pale yellow to more yellow-brown. Other authors also reported similar changes in the chromatic data during aging of white wine [12,56,59], where oxidation of phenolics, especially flavanols (catechins and procyanidins) to quinones, which than polymerize to form yellow-brown products, are mainly responsible for these color changes. In addition, no significant differences were found in the most of the CIELab parameters, except lightness (L*), immediately after HHP treatment and during 12 months of aging between pressurized and unpressurized standard-SO_2 wines. Furthermore, among pressurized wines, at the beginning of aging and after 12 months, there was only significant difference in parameter L*, whereas the values of parameters a*, b*, C* and H* did not differ significantly. However, there were some apparent differences in parameters L*, b* and C* between pressurized wines with standard SO_2 and those with low SO_2/low SO_2+GSH content after 3 and 6 months of aging. A previous study by Santos et al. [12] showed that HHP-treated white wine without SO_2 had more brownish color and lower phenolic content than untreated wines with 0 and 40 mg/L of SO_2 after 12 months of bottle aging, indicating that HHP probably accelerates the Maillard reaction in white wine. Additionally, the results of calculated total color difference (ΔE*) confirmed that the observed changes in the color parameters between pressurized and unpressurized samples were not visually relevant. Although, there is no specified limit value for determining that the color differences in white wine are observable by the human eye in the literature, all obtained values were far below 3 CIELab units (data not shown), otherwise considered as a relevant value in the case of red wine. During 12 months of aging, the pressurized standard-SO_2 sample presented ΔE* values in the CIELab unit range from 0.3 to 1.1 in comparison to control. As emphasized earlier, SO_2 is very important in preventing the oxidative color changes, particularly in white wines as they have less of other antioxidants such as phenolic compounds than the red wines. Furthermore, compared to control, pressurized low-SO_2+GSH and low-SO_2 wines showed slightly higher values of ΔE* ranging from 1.0 to 1.6 and from 1.2 to 2.6. Moreover, the addition of GSH in our case did not significantly affect the overall color of white wine, although it was reported that glutathione in the presence of small amounts of SO_2 has the ability to delay the oxidative color changes and the formation of xanthylium cation

pigments [60,61]. Therefore, combination of HHP treatment with the addition of antioxidants (SO_2 and GSH) did not remarkably influence the color properties of white wine, except lightness, as stated above.

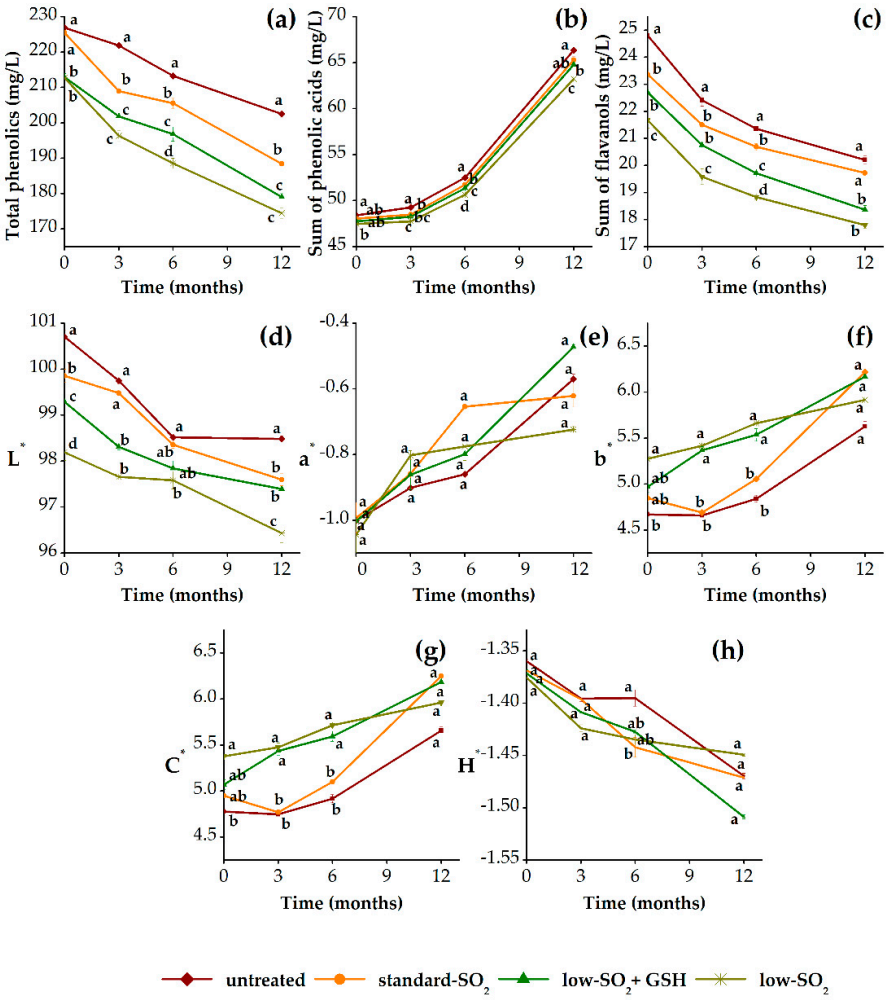

Figure 4. Phenolic (**a**–**c**) and color (**d**–**h**) changes of pressurized (standard SO_2, low SO_2+GSH and low SO_2) and unpressurized (untreated) white wine samples during 12 months of aging in bottles. GSH: glutathione.

3.3.2. Aroma Profile

Figure 5 shows the evolution of sum of esters, sum of higher alcohols, sum of fatty acids and sum of terpenes of HHP-treated and untreated white wine samples during 12 months of aging in relation to their content of antioxidants (SO_2 and GSH). The results showed that the content of esters, fatty acids and terpenes decreased, while the content of higher alcohols increased in all wines during 12 months of aging. These aroma changes are known to naturally occur during the wine aging process, as already described in the case of red wine. Namely, the transformation of aroma compounds leads to a loss of characteristic aromas of young wines and gradual formation of more complex aroma composition typical for aged wines [62]. In addition, significant difference ($p < 0.05$) between pressurized and unpressurized wines regarding their content of analyzed groups of aroma compounds were found at the beginning of aging and after 12 months (Figure 5a–d). Namely, the pressurized samples presented lower content of esters, higher alcohols, fatty acids and terpenes compared to control wine, respectively. Currently, there are only two studies that have investigated the effect of HHP and how it changes aroma composition as well as sensory properties of white wine during bottle aging [12,13]. As already described for the red wines, Santos et al. [13] found that the pressurized white wines were also

characterized mainly by aldehydes, furans, acetals and ketones. The same authors explained that the higher content of ketones in pressurized wine is due to occurrence of oxidation of fatty acids with pressure. This observation explains the decrease in the content of fatty acids of HHP-treated wines discussed previously. Furthermore, both mentioned studies suggested that HHP treatment can accelerate the formation of wine aging aroma due to enhancement of Maillard reaction and fatty acid and alcohol oxidation. In relation to antioxidant treatments (SO_2 and GSH), the significant differences were observed among pressurized wines in the content of aroma compounds during 12 months of aging. Particularly, the higher content of SO_2 resulted in wines with higher content of all aroma groups, whereas no unique effect was found regarding the addition of GSH during the observed period of aging. The effects of SO_2 on oxidation and aging of wine are well established [63–65]. Regarding wine aroma, it has been reported that SO_2 protects several groups of aroma compounds, such as esters, higher alcohols and fatty acids, during aging of wine [66,67]. However, after 12 months, the GSH effect was noticeable on the content of esters and terpenes, while practically no effect was determined in the case of higher alcohols and fatty acids. The GSH, with its thiol group, can react as a strong nucleophile with quinones, and in this way protect important aroma compounds such as esters, terpenes and thiols [27]. Moreover, the addition of GSH in white wine production has been demonstrated to limit the accumulation of acetaldehyde and to preserve the aroma complexity and freshness after 12 months of bottle aging [68]. From these results it follows that from all HHP treatments performed, the combined HHP and standard-SO_2 treatment reduced the rate of chemical reactions, such as hydrolysis or oxidation, to the greatest extent which seemed to happen faster in treated samples.

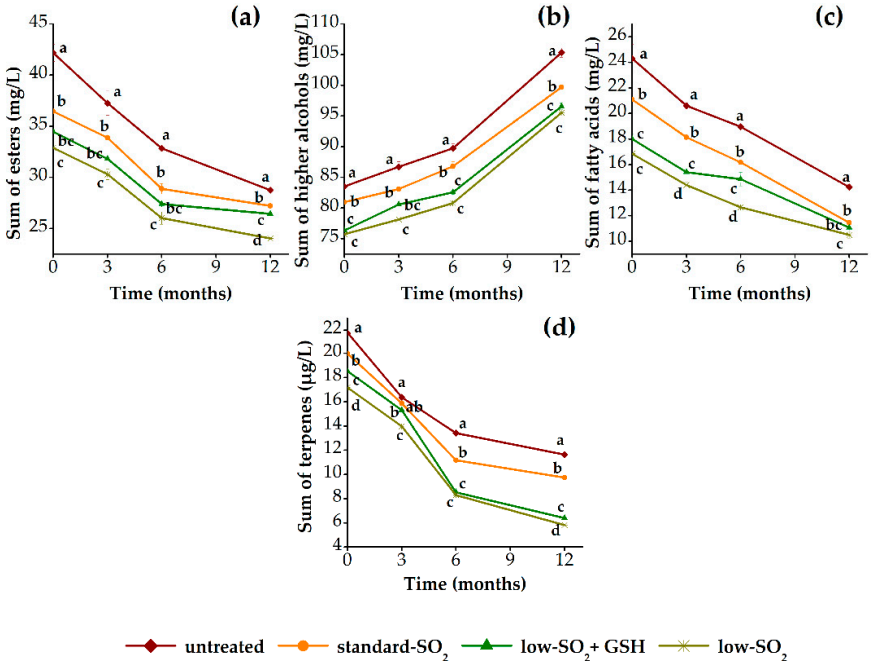

Figure 5. Aroma changes of pressurized (standard SO_2, low SO_2+GSH and low SO_2) and unpressurized (untreated) white wine samples during 12 months of aging in bottles: (**a**) sum of esters; (**b**) sum of higher alcohols; (**c**) sum of fatty acids; (**d**) sum of terpenes. GSH: glutathione.

3.4. Sensory Changes of Red and White Wines during 12 Months of Aging Induced by HHP and Antioxidant Treatments

The sensory properties of wines were analyzed by the nine-point hedonic scale method to assess the organoleptic characteristics in terms of color, odor and taste. The influence of HHP treatment along with antioxidants addition (SO_2 and GSH) on the wines' sensory attributes with the results represented

as the average scores of the panelists are shown in Table 3, for red and white wine, respectively. At the very beginning, the results showed that there were no significant differences among pressurized red wine samples for each of the attributes scored. On the other hand, in the case of white wine, there were significant differences ($p < 0.05$) between samples with higher and lower concentration of SO_2 in terms of color and odor. After 3 months of aging, significant differences were found among standard- and low-SO_2 white wines for each of the attributes, while in red wines the occurred differences were much less pronounced. A similar trend to that was observed after 6 months of aging. When sensory analysis was performed 12 months after bottling, very similar scores were given to standard SO_2 and low SO_2+GSH samples of both red and white wine, respectively (Table 3). In general, the lowest scores were assigned to both red and white wines with low SO_2 content for each of the sensory attributes. Moreover, when comparing red and white wine, it can be seen that red wine samples had slightly higher ratings in all three analyzed attributes. Overall, after 12 months of bottle aging, both the treated red and white wines were evaluated with fairly good scores (7 = like moderately and 6 = like slightly). Generally, the degradation rate of aroma of red wines is slower compared to white wines due to a higher content of phenolic compounds, which have antioxidant properties. According to Fuhrman et al. [69], the limited antioxidant character of white wines makes them more susceptible to oxidation in contrast to red wines, which was probably the reason why combined HHP and antioxidant treatments affected the white wine sensory attributes slightly more than those of the red wine. Moreover, it seems that the changes in phenolic and aromatic composition induced by both HHP and antioxidant treatments, can modify the sensory quality of wines. However, the relationship between chemical composition and sensory attributes is not always easy to evaluate, due to the complexity of wine's chemical composition and its numerous interacting components [70].

Table 3. The average scores for sensory attributes (color, odor and taste) of pressurized red and white wines.

Time (months)	Red Wine	Color	Odor	Taste
0	standard SO_2	8.7 ± 0.5 ^{a}	8.6 ± 0.5 ^{a}	8.5 ± 0.5 ^{a}
	low SO_2+GSH	8.6 ± 0.5 ^{a}	8.5 ± 0.5 ^{a}	8.4 ± 0.5 ^{a}
	low SO_2	8.3 ± 0.5 ^{a}	8.3 ± 0.5 ^{a}	8.4 ± 0.5 ^{a}
3	standard SO_2	8.1 ± 0.3 ^{a}	8.0 ± 0.5 ^{a}	7.9 ± 0.3 ^{a}
	low SO_2+GSH	7.8 ± 0.3 ^{ab}	7.7 ± 0.5 ^{ab}	7.5 ± 0.3 ^{a}
	low SO_2	7.4 ± 0.5 ^{b}	7.4 ± 0.3 ^{b}	7.2 ± 0.5 ^{a}
6	standard SO_2	7.8 ± 0.5 ^{a}	7.7 ± 0.5 ^{a}	7.6 ± 0.5 ^{a}
	low SO_2+GSH	7.6 ± 0.4 ^{ab}	7.4 ± 0.3 ^{ab}	7.3 ± 0.3 ^{ab}
	low SO_2	7.2 ± 0.3 ^{b}	7.0 ± 0.3 ^{b}	6.9 ± 0.3 ^{b}
12	standard SO_2	7.4 ± 0.5 ^{a}	7.2 ± 0.4 ^{a}	6.8 ± 0.3 ^{a}
	low SO_2+GSH	7.1 ± 0.3 ^{a}	6.9 ± 0.3 ^{ab}	6.6 ± 0.4 ^{ab}
	low SO_2	6.6 ± 0.5 ^{b}	6.5 ± 0.5 ^{b}	6.1 ± 0.4 ^{b}
Time (months)	**White Wine**	**Color**	**Odor**	**Taste**
0	standard SO_2	8.5 ± 0.5 ^{a}	8.3 ± 0.5 ^{a}	8.2 ± 0.3 ^{a}
	low SO_2+GSH	8.0 ± 0.4 ^{b}	7.8 ± 0.4 ^{ab}	7.7 ± 0.4 ^{a}
	low SO_2	7.9 ± 0.3 ^{b}	7.7 ± 0.5 ^{b}	7.6 ± 0.5 ^{a}
3	standard SO_2	7.5 ± 0.5 ^{a}	7.3 ± 0.5 ^{a}	7.2 ± 0.4 ^{a}
	low SO_2+GSH	6.9 ± 0.3 ^{b}	6.7 ± 0.5 ^{b}	6.6 ± 0.5 ^{b}
	low SO_2	6.8 ± 0.4 ^{b}	6.6 ± 0.3 ^{b}	6.3 ± 0.5 ^{b}
6	standard SO_2	7.3 ± 0.3 ^{a}	7.2 ± 0.4 ^{a}	7.0 ± 0.3 ^{a}
	low SO_2+GSH	6.7 ± 0.4 ^{b}	6.6 ± 0.5 ^{b}	6.5 ± 0.5 ^{ab}
	low SO_2	6.6 ± 0.5 ^{b}	6.5 ± 0.5 ^{b}	6.2 ± 0.4 ^{b}
12	standard SO_2	6.9 ± 0.4 ^{a}	6.6 ± 0.3 ^{a}	6.5 ± 0.5 ^{a}
	low SO_2+GSH	6.4 ± 0.5 ^{ab}	6.0 ± 0.4 ^{ab}	5.8 ± 0.4 ^{b}
	low SO_2	6.1 ± 0.3 ^{b}	5.8 ± 0.3 ^{b}	5.8 ± 0.4 ^{b}

^{a,b} The samples with different letters are statistically significant ($p < 0.05$).

4. Conclusions

In this work, we first have investigated the influence of HHP treatments on red and white wines' chemical composition. Besides that, the combination of HHP treatment and the addition of antioxidants (glutathione and sulfur dioxide) was examined on wine phenolic and aroma composition, as well as sensory properties during 12 months of bottle aging. The results of this study showed that slight changes occurred in the phenolic composition and color properties of red and white wines immediately after HHP treatments. In pressurized red wine these changes manifested as a decrease of both total and individual phenolic compounds, while all color parameters increased. Additionally, applied treatments resulted in the decrease of phenolic contents in white wine, with exception in the increase of some free phenolic acids. Regarding applied HHP conditions, higher pressures as well as longer processing times resulted in more noticeable changes of analyzed compounds, where the pressure was more responsible for main variations in data. After 12 months of aging, the HHP-treated red wines were characterized by lower content of TP, TA, FA and Fl, without major changes in the content of TT. On the other hand, HHP treatment after 12 months of aging did not influence most of the color parameters (except L* and a*) and aroma compounds (except fatty acids) of the red wine. Concerning the white wine, HHP treatment did not affect most of the phenolics (except TP) and color properties (except L*) after 12 months of aging, but it showed impact on the aroma compounds. Moreover, the higher content of antioxidants (SO_2 and GSH) resulted in HHP-treated red and white wines with a higher content of analyzed phenolic and aroma compounds possibly due to the decreased rates of condensation and oxidation reactions. Namely, no significant differences were observed among pressurized standard-SO_2 and low-SO_2+GSH red wines in concentrations of aroma compounds, primarily fatty acids, while for the white wines this was mostly evident in the color properties. Finally, the sensory analysis also showed that the wine samples were distinguished primarily by different amounts of antioxidants used. Additionally, the effect of combined HHP and antioxidant treatments was slightly more pronounced in the white wine. Therefore, HHP should be considered as a potential alternative for complementing the antioxidant and antimicrobial actions of SO_2. Thus, the aspect of multidisciplinary approaches such as the combination of physical and chemical treatments even with SO_2 may help to reduce SO_2 use during the wine production.

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Paper 6

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The impact of high voltage electrical discharge plasma on the chromatic characteristics and phenolic composition of red and white wines

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ABSTRACT

The cold plasma is an emerging electrotechnology for the improvement of food safety without loss of physicochemical or sensory properties. The purpose of this study was to evaluate the effects of plasma treatments on the chromatic characteristics and phenolic composition of red and white wines. The red wine Cabernet Sauvignon and white wine Graševina were treated with high voltage electrical discharge plasma considering the variations in frequency (60, 90 and 120 Hz) and processing time (3, 5 and 10 min). Total phenolics, total anthocyanins, total tannins and chromatic characteristics were analyzed by spectrophotometry while free anthocyanins, phenolic acids and flavan-3-ols by the HPLC-UV/Vis. Obtained results illustrated that plasma treatments have influenced the stability of phenolic compounds in wines without major changes in color parameters. Also, among two different processing parameters, the duration time was the most significant factor inducing changes on wines.

Industrial relevance: High voltage electrical discharge plasma has been shown to affect the stability of wine phenols without any significant change in the color. An increase in the concentration of certain phenolic compounds in white wine suggest that this technique could be used in the wine industry as an alternative technique for enhancing the oxidative stability of wine and consequently the wine quality during the aging process.

1. Introduction

The cold plasma, as new processing technology, has been already widely investigated in terms of microbial inactivation and food safety improvement (Misra & Jo, 2017; Moreau, Orange, & Feuilloley, 2008; Shi et al., 2011; Vukušić et al., 2016; Ziuzina, Patil, Cullen, Keener, & Bourke, 2013). Recently, the focus has begun to shift towards the use of cold plasma for food properties modification (Segat, Misra, Cullen, & Innocente, 2015; Zhu, 2017), enzyme inactivation (Pankaj, Misra, & Cullen, 2013; Surowsky, Fischer, Schlueter, & Knorr, 2013; Tappi et al., 2016) and bioactivity enhancement (Elez Garofulić et al., 2015; Herceg et al., 2016). Generally, the plasma is described as partially or completely ionized gas with characteristic electrical, chemical and physical properties, which can be generated by many methods such as electrical discharges (corona, spark, glow, arc, microwave discharge, plasma jets and radio frequency plasma) and shocks (electrically, magnetically and chemically driven) (Petitpas et al., 2007). The most important physical effects of electrical discharges are a high electric field, intense UV radiation and overpressure shock waves (Zhang, Chen, & Li, 2009), while major chemical effect is manifested through the generation of various reactive species, namely hydrogen peroxide, hydroxyl, oxygen and hydrogen radicals (Locke, Sato, Sunka, Hoffmann, & Chang, 2006). Recent studies showed that the types of cold plasma systems and their applications are numeorous, including the variety of methods used to generate cold plasma, size of the reactor, distance between the electrodes, working gas and sample type (Almeida et al., 2015; Grzegorzewski, Ehlbeck, Schlüter, Kroh, & Rohn, 2011; Misra & Jo, 2017; Shi et al., 2011; Surowsky, Fröhling, Gottschalk, Schlüter, & Knorr, 2014). In addition, each of these processing parameters can significantly affect the final outcome and therefore it is difficult to make general conclusions on plasma efficiency. Furthermore, the cold plasma is not yet entirely employed in the food industry primarily due to the largely unexplored effects on different food components. Therefore, it is important to understand the basic interactions of bioactive compounds with plasma-generated reactive species, in order to avoid nutritional degradation and any other undesired effects of plasma applications. Recently, the wine industry has focused on the possible application of innovative electrotechnologies in different stages of winemaking

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process, primarily in terms of wine microbial stabilization, but also in improving oxidative stability of wine and consequently the wine quality. In the case of application of cold plasma in wine production, to the best of our knowledge, no studies have investigated the influence of this technology on the quality characteristics of wine, such as phenolic composition and color.

Generally, the composition of wine is very complex and continuosly changes during aging. Phenols are a large and complex group of compounds that significantly affect the quality of the wine and play important role in distinguishing red and white wines (Ribéreau-Gayon, Glories, Maujean, & Dubourdier, 2000). These compounds are important in determining the color of wine as well as taste and flavor (Toshihiko, 2007). Furthermore, phenolic compounds in wines are primary substrates for oxidation (Oliveira, Ferreira, De Freitas, & Silva, 2011). They are known as natural antioxidants, which protect cells against the damaging effects of free radicals (Lopéz-Vélez, Martínez-Martínez, & Valle-Ribes, 2003). Due to the disadvantages of the standard aging technologies, such as long time needed and high costs, innovative aging technologies have been developed. The available literature reports about the wine quality improvement using physical methods, such as ultrasound (Ferraretto & Celotti, 2016; Martín & Sun, 2013), electric fields (Zeng, Yu, Zhang, & Chen, 2008) and high hydrostatic pressure (Chen et al., 2012; Santos et al., 2016). Considering plasma as the new electrotechnology, some studies have shown that its application on fruit juices resulted in numerous physical and chemical changes of phenolic compounds, with retention or even improvement of overall quality. Application of high voltage electrical discharge plasmas on fruit juices (apple juice and Marasca sour cherry nectar) and its influence on the physicochemical and organoleptic properties were described in a recent dissertation (Vukušić, 2016). Furthermore, an increase in total phenolic (Herceg et al., 2016) and anthocyanin content (Bursać Kovačević, Putnik et al., 2016) in pomegranate juice after argon plasma treatment has been reported, as well as the increase in anthocyanin and phenolic acid contents in sour cherry Marasca juice (Elez Garofulić et al., 2015). Also, the plasma treatment is mentioned in the context of improvement the extraction of phenolic compounds in pomegranate juice (Bursać Kovačević, Putnik et al., 2016).

Based on previously stated facts and possibilities of cold plasma, this technique has a great potential as an alternative to the current available aging technologies used in wine industry. But, firstly, the influence of plasma processing parameters on the overall quality of wine should be examined in more detail. Therefore, the aim of this study is to investigate the impact of high voltage electrical discharge plasma treatments on the chromatic characterictics and phenolic compounds of red and white wines.

2. Material and methods

2.1. Wine

The wines used for the present study were young red wine Cabernet Sauvignon (*Vitis vinifera* L.) and white wine Graševina (*Vitis vinifera* L.), harvest 2016, obtained from winery Erdutski vinogradi d.o.o., Erdut, Croatia. The physicochemical characteristics of the treated wines are presented in Table 1.

2.2. Chemicals

Sodium carbonate anhydrous (99%) and formic acid (98–100%) were purchased from T.T.T. (Sveta Nedjelja, Croatia). Folin-Ciocalteu reagent was obtained from Kemika (Zagreb, Croatia), sodium bisulfite from Acros Organics (Geel, Belgium), hydrochloric acid (37%) from Carlo Erba (Val del Reuil, France) and ethanol (96%) from Gram-Mol (Zagreb, Croatia). Malvidin-3-*O*-glucoside-chloride (≥ 95%), gallic acid (97.5–102.5%), protocatechuic acid (≥ 97%), *p*-hydroxybenzoic acid (≥ 99%), vanillic acid (≥ 97%), syringic acid (≥ 95%), caftaric acid (≥ 98%), chlorogenic acid (≥ 95%), caffeic acid (≥ 95%), *p*-coumaric acid (≥ 98%), ferulic acid (≥ 99%), (+)-catechin (≥ 99%), (−)-epicatechin (≥ 98%), procyanidin B1 (≥ 90%) and procyanidin B2 (≥ 90%) were obtained from Sigma-Aldrich (St. Louis, USA). HPLC-grade methanol and acetonitrile were purchased from J.T. Baker (Deventer, Netherlands).

Table 1
Oenological parameters of the red (Cabernet Sauvignon) and white (Graševina) wines.

Parameters	Cabernet Sauvignon	Graševina
Alcohol level (vol%)	13.1	11.4
Total acidity (g/L, expressed as tartaric acid)	5.30	5.10
Volatile acidity (g/L, expressed as acetic acid)	0.61	0.31
Reducing sugars (g/L)	4.10	2.80
pH	3.46	3.37
Malic acid (g/L)	0.10	1.20
Lactic acid (g/L)	1.30	0.30

2.3. The plasma treatments of wine

The plasma treatments were conducted in a 1000 mL glass vessel with a point to point electrode configuration in a so called hybrid reactor with discharges in and above the liquid (Fig. 1). The plasma was generated by high-voltage (HV) pulsed power supply (Spellman, UK), by charging a load capacitor of 1.13 nF to up to 30 kV and then discharging the stored charge into the plasma reactor via a rotating spark gap (Fig. 1a). The voltage in the plasma reactor was measured and recorded using a Tektronix P6015A high voltage probe connected to a Hantek DS05202BM oscilloscope (data not shown). The experiments were performed at positive polarity and argon (purity 99.99%; Messer Croatia, Zagreb, Croatia) was bubbled trough steinless steel needle (Micrlolance TM 3.81 cm) at the gas flow of 4 L/min. The influences of two main factors, namely applied frequency and duration of plasma treatment on wine quality parameters were taken into account. 300 mL of wine was treated with plasma running at the combination of following processing parameters: frequency at 60, 90 and 120 Hz and treatment duration of 3, 5 and 10 min. The temperature of samples before and after the plasma treatment was monitored using a InfraRed Thermometer PCE-777 (PCE Instruments, Germany). Before treatment all samples were at the room temperatures of 21 ± 1 °C, while after the plasma exposure temperature rised up to 6 °C, depending on the duration time and applied frequency. After treatments, wine samples were subjected to chemical analysis.

2.4. Color measurement

The chromatic characteristics measurements were carried out using the CIELab space (Method OIV-MA-AS2-11, 2006). The spectra were registered directly on the wine, using a 10 mm optical path glass cell and a Specord 50 Plus AnalytikJena spectrophotometer (Jena, Germany) set to measure in the visible spectra (λ = 380–770 nm) at constant intervals (Δλ = 5 nm) and integrated using the software WinASPECT PLUS (Jena, Germany). Color was expressed as CIE coordinates of L^* (lightness), a^* (redness/greenness) and b^* (yellowness/blueness) with illuminant D_{65} and observer 10° standardisation. From the CIELab space, other parameters were also defined, such as chroma (C^*) and hue angle (H^*). Three replicate measurements were performed and the results are showed as average measure with standard deviation.

2.5. Phenolic composition by spectrophotometric methods

The determinations of the main phenolic families were perfomed by a Specord 50 Plus spectrophotometer (AnalytikJena, Jena, Germany), with all the analyses conducted in triplicate.

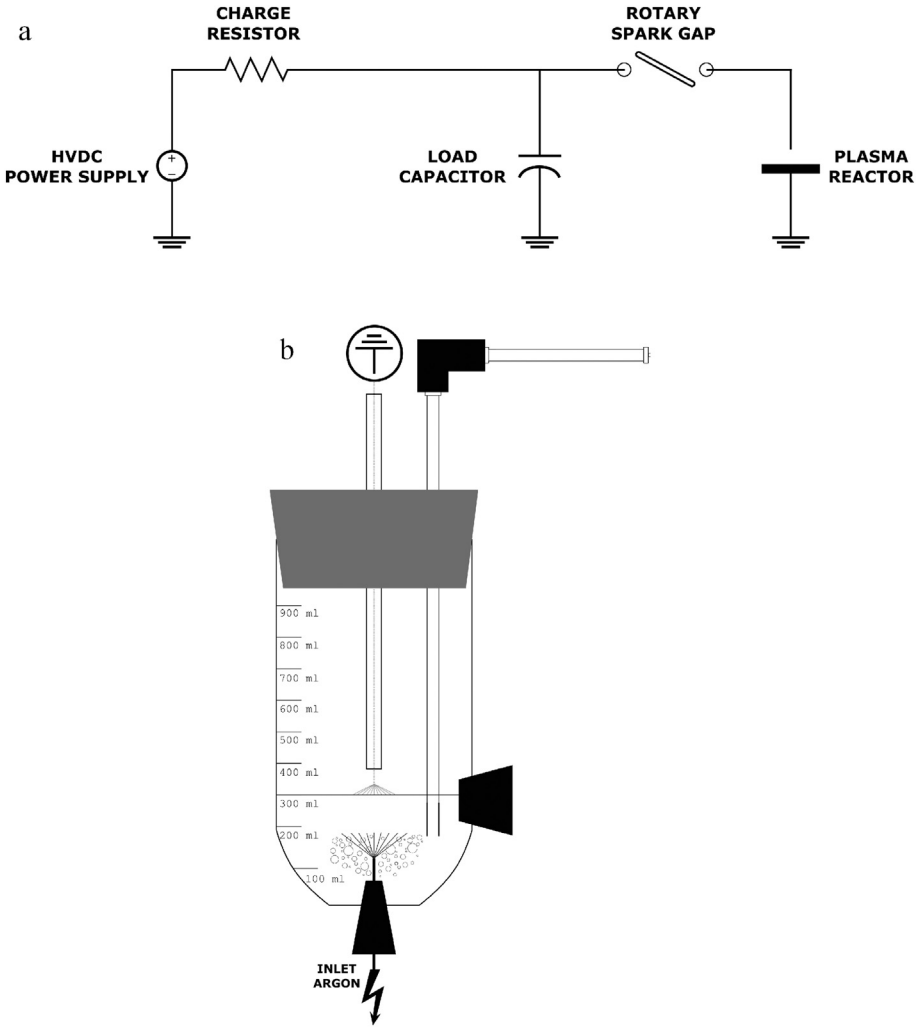

Fig. 1. Shematic description of the experimental setup. (a) Electrical circuit; (b) Plasma reactor.

The total phenolics (TP) content was determined by the Folin-Ciocalteu method (Singleton & Rossi, 1965). The red wine was diluted 1:9 in a distilled water, while white wine was analyzed without dilution. 250 μL of diluted sample, 1.25 mL of Folin-Ciocalteu reagent (diluted 1:2) and 15 mL of water was taken in a 25 mL volumetric flask and shaken well. After 30 s, 3.75 mL of 20% sodium carbonate was added to the mixture. The volume was made up to 25 mL with distilled water and incubated for 2 h at room temperature. A set of standard solutions of gallic acid (100, 200, 400, 600 and 800 mg/L) were prepared in the same manner as described earlier. The absorbance for samples and standard solutions were measured at a wavelength of 765 nm against the blind probe prepared in the same way as samples, except instead of sample was used distilled water. The results of phenols were expressed as mg/L of gallic acid equivalents (mg GAE/L).

The total anthocyanins (TA) content was determined using the SO_2 bleaching method (Ribéreau-Gayon & Stonestreet, 1965). In a 25 mL volumetric flask, 1 mL of wine, 1 mL of 0.1% HCl in ethanol and 20 mL of 2% aqueous HCl was added. 10 mL of this solution was transfered into two flasks, 4 mL of water was added to the first flask (sample A) and 4 mL of 15% $NaHSO_3$ to second (sample B). After 15 min, the absorbance was measured in both prepared samples at 520 nm against the distilled water as a blind probe. Total anthocyanins (mg/L) were calculated by the following Eq. (1):

$$TA\ (mg/L) = 875\ (Abs_{520\ nm}A_{sample} - Abs_{520\ nm}B_{sample}) \quad (1)$$

The total tannins (TT) content was estimated according to Ribéreau-Gayon and Stonestreet (1966). This method is based on the Bate–Smith reaction, in which the proanthocyanidins in acid medium release anthocyanidins by heating. The wines were diluted to 1:50. In two hydrolysis tubes, 2 mL of diluted sample, 1 mL of distilled water and 3 mL of concentrated HCl was taken and the mixture was homogenized. One tube was heated for 30 min in boiled water and cooled for 5 min with ice (sample A), while the other one was maintained at room temperature (sample B). To each tube, 500 μL of 96% ethanol was added. The absorbance was measured at 550 nm against the distilled water as a blind probe. Total tannins (g/L) were calculated by the following Eq. (2):

$$TT\ (g/L) = 19.33 \times (Abs_{550\ nm}A_{sample} - Abs_{550\ nm}B_{sample}) \quad (2)$$

2.6. HPLC analysis of phenolic compounds

The samples were filtered through a 0.45 μm pore size CA Syringe filter before injection. Analysis was performed on a Agilent Technologies 1200 Series HPLC system (Santa Clara, USA) consisting of an autosampler (HiP-ALS G1367B), a binary pump (Bin Pump SL G1312B), a diode array detector (DAD SL G1315C) coupled to a Agilent Chemstation data analysis software. The separation of phenolic acids and flavan-3-ols in white wine was performed on a Phenomenex Gemini C18 (4.6 mm × 250 mm. 5 μm) column, while the separation of the free anthocyanins in red wine was performed on a Phenomenex Nucleosil C18 (4.6 mm × 250 mm. 5 μm) column. All analyses were conducted in triplicate and the results were expressed as mean values in milligrams per liter of wine.

For phenolic acids and flavan-3-ols analysis the mobile phase consisted of two solvents: solvent A, water/formic acid (98:2; v/v) and solvent B, methanol, according to the method of Komes, Ulrich, Kovačević Ganić, and Lovrić (2007) with a slight modification of the mobile phase (solvent A) and gradient modification. The elution profile was as follows: 0 min, 2% B; 20 min, 32% B; 30 min, 40% B; 40 min, 50% B; 50 min, 50% B; 53 min, 2% B; 55 min, 2% B. Phenolic compounds were eluted under the following conditions: 1 mL/min flow rate, column temperature 25 °C, injection volume 20 μL and detection at 280 nm (hydroxybenzoic acids and flavan-3-ols) and 320 nm (hydroxycinnamic acids). Identification of phenolic compounds was carried out by comparison to the retention times of authentic standards, which were also used as the calibrating standards for identified phenolic acids and flavan-3-ols.

For the free anthocyanins analysis, water/formic acid (95:5. v/v) (solvent A) and acetonitrile/formic acid (95:5. v/v) (solvent B) were applied at a flow rate of 1 mL/min as follows: 0 min, 10% B; 25 min, 35% B; 26 min, 100% B; 28 min, 100% B; 29 min, 10% B; 35 min, 10% B. The injection volume was 20 μL and the column temperature was 40 °C (Lorrain, Chira, & Teissedre, 2011). The detection was conducted at 520 nm. Nine major free anthocyanins (delphinidin-3-*O*-glucoside, cyanidin-3-*O*-glucoside, petunidin-3-*O*-glucoside, peonidin-3-*O*-glucoside, malvidin-3-*O*-glucoside, peonidin-3-*O*-glucoside acetat, malvidin-3-*O*-glucoside acetat, peonidin-3-*O*-glucoside *p*-coumarat, malvidin-3-*O*-glucoside *p*-coumarat) were identified by comparison to the retention time of the most abundant anthocyanin in wine malvidin-3-*O*-glucoside-chloride, which was also used as the calibrating standard for all identified free anthocyanins.

2.7. Statistical analysis

The statistical analysis was carried out using statistical software (Statistica, Vers. 10.0, StatSoft Inc., USA). The multivariate analysis of variance (MANOVA) was used to quantify the influence of each experimental factor, frequency and treatment duration, on wine quality parameters, namely color and phenolic composition. The significant differences between mean values were determined by Tukey's HSD test ($p < 0.05$). Furthermore, a principal component analysis (PCA) was applied on the correlation matrix using the attributes of color and phenolic composition analysis to provide an overview of the characterization of plasma treated wines by different treatment conditions.

3. Results and discussion

3.1. Effect of plasma treatments on the chromatic characteristics of red and white wines

The effects of applied plasma treatments, depending on variations in frequencies and durations of treatment, on the color and phenolic composition are presented in Tables 2 (Cabernet Sauvignon) and 3 (Graševina). When compared to untreated red wine, obtained results of chromatic characteristics, expressed in CIE Lab units, indicate that with increasing treatment duration and frequency the L* (lightness) value slightly decreased from 26.28 to 24.51. The same effect can be observed with a*(redness), b*(yellowness) and C* (chroma) values, while value of H* (hue angle) parameter remain constant. These results indicate that the chromatic characteristics of red wine changed slightly, but these changes could not be visually observed. As far as white wine, the obtained values of color parameters were quite similar. The L* value ranged from 98.72 to 98.12, while the values of a* and H* ranged from −0.88 to −0.35 and −1.42 to −1.50. The values of b* and C* practically remain unchanged too, since they didn't show significant difference between plasma treated and untreated white wine samples. The analyzed white wine is expected to position in the green-yellow region of the chromatic space, which is in accordance with experimental data. Finally, despite some color parameters that showed statistical difference, the characteristic color of the wines was kept in the expected range for both wines, indicating that the plasma treatment did not negatively affected the product color. Even though the literature gives a little information about the effects of plasma treatments on food color, Bursać Kovačević, Putnik et al. (2016) demonstrated that plasma treatment resulted in an increase of anthocyanins in pomegranate juice due to chemical reactions induced by cold atmospheric gas phase plasma which lead to disruption of cell membrane, while the change of color did not vary with variation of sample volume and treatment duration, but it decreased with increased gas flow.

3.2. Effect of plasma treatments on phenolic composition of red and white wines

In relation to the phenolic composition, multivariate analysis of variance (MANOVA) showed that there was a significant influence ($p < 0.05$) of treatment duration, as well as of frequency on the total phenolics (TP) in both red and white wines. Similar trend can also be observed for the total anthocyanins (TA) and total tannins (TT) in red wine. Observed reduction of phenolic compounds, including TP, TA and TT, could be due to the possible degradation of these compounds by the plasma mechanisms. The plasma has been demonstrated to produce shock waves, cavitation, light emissions and free radicals. These physical processes and plasma-generated reactive species have been shown to degrade many organic compounds such us phenols (Locke et al., 2006). These results can be also related with an increase in temperature after the plasma exposure, especially because of the fact that stability of anthocyanins depends on various processing and storage conditions, such as temperature, process duration, oxidation status, light exposure and others (He et al., 2012). According to Vukušić (2016) it was also observed an increase in temperature of fruit juices after longer plasma exposures and higher frequencies, while after the shortest treatment the

Table 2
Influence of treatment duration and applied frequency on the chromatic characteristics, total phenolics, total anthocyanins and total tannins in red wine Cabernet Sauvignon.

Source of variation	L*	a*	b*	C*	H*	TP	TA	TT
Treatment duration (min)								
Control	26.28 ± 0.03^{d}	57.20 ± 0.03^{d}	38.13 ± 0.01^{c}	68.74 ± 0.02^{d}	0.59 ± 0.00^{a}	1816.06 ± 4.58^{d}	424.61 ± 1.10^{c}	2.20 ± 0.05^{b}
3	25.21 ± 0.09^{c}	56.07 ± 0.13^{c}	37.58 ± 0.09^{b}	67.50 ± 0.15^{c}	0.59 ± 0.00^{a}	1773.94 ± 26.50^{c}	409.21 ± 12.38^{c}	1.99 ± 0.10^{b}
5	24.93 ± 0.10^{b}	55.86 ± 0.12^{b}	37.50 ± 0.12^{ab}	67.28 ± 0.17^{b}	0.59 ± 0.00^{a}	1695.05 ± 10.49^{b}	383.31 ± 20.50^{b}	1.85 ± 0.07^{a}
10	24.51 ± 0.08^{a}	55.59 ± 0.05^{a}	37.43 ± 0.05^{a}	67.02 ± 0.07^{a}	0.59 ± 0.01^{a}	1606.57 ± 35.27^{a}	358.39 ± 37.90^{a}	1.75 ± 0.15^{a}
Frequency (Hz)								
Control	26.28 ± 0.03^{b}	57.20 ± 0.03^{b}	38.13 ± 0.01^{b}	68.74 ± 0.02^{b}	0.59 ± 0.00^{a}	1816.06 ± 4.58^{c}	424.61 ± 1.10^{c}	2.20 ± 0.05^{b}
60	24.87 ± 0.24^{a}	55.81 ± 0.14^{a}	37.50 ± 0.03^{a}	67.24 ± 0.13^{a}	0.59 ± 0.00^{a}	1722.42 ± 68.56^{b}	410.54 ± 10.93^{c}	1.87 ± 0.12^{a}
90	24.91 ± 0.31^{a}	55.85 ± 0.20^{a}	37.50 ± 0.09^{a}	67.27 ± 0.21^{a}	0.59 ± 0.00^{a}	1682.42 ± 74.74^{a}	383.66 ± 21.63^{b}	1.86 ± 0.12^{a}
120	24.87 ± 0.40^{a}	55.86 ± 0.32^{a}	37.52 ± 0.17^{a}	67.29 ± 0.35^{a}	0.59 ± 0.01^{a}	1670.71 ± 76.75^{a}	356.71 ± 35.28^{a}	1.86 ± 0.20^{a}

Data presented as average value of three analytical repetitions with standard deviation. MANOVA to compare data; different letters indicate statistical differences between wines of all treatments at the same time (Tukey's test, < 0.05). Abbreviations: TP - total phenolics (mg GAE/L); TA - total anthocyanins (mg/L); TT - total tannins (g/L).

Table 3
Influence of treatment duration and applied frequency on the chromatic characteristics and total phenolics in white wine Graševina.

Source of variation	L*	a*	b*	C*	H*	TP
Treatment duration (min)						
Control	98.72 ± 0.03^{c}	− 0.88 ± 0.01^{a}	5.83 ± 0.01^{a}	5.90 ± 0.01^{a}	− 1.42 ± 0.00^{b}	208.82 ± 0.90^{c}
3	98.65 ± 0.03^{bc}	− 0.87 ± 0.02^{a}	5.90 ± 0.08^{a}	5.96 ± 0.08^{a}	− 1.42 ± 0.01^{b}	207.58 ± 1.09^{bc}
5	98.62 ± 0.06^{b}	− 0.84 ± 0.02^{b}	5.86 ± 0.03^{a}	5.92 ± 0.02^{a}	− 1.43 ± 0.00^{b}	206.83 ± 1.21^{b}
10	98.12 ± 0.05^{a}	− 0.35 ± 0.04^{c}	5.91 ± 0.03^{a}	5.92 ± 0.03^{a}	− 1.50 ± 0.03^{a}	204.94 ± 0.43^{a}
Frequency (Hz)						
Control	98.72 ± 0.03^{b}	− 0.88 ± 0.01^{a}	5.83 ± 0.01^{a}	5.90 ± 0.01^{a}	− 1.42 ± 0.00^{b}	208.82 ± 0.90^{b}
60	98.47 ± 0.26^{a}	− 0.68 ± 0.24^{bc}	5.88 ± 0.03^{a}	5.92 ± 0.02^{a}	− 1.45 ± 0.04^{ab}	206.79 ± 1.46^{a}
90	98.45 ± 0.22^{a}	− 0.71 ± 0.24^{b}	5.89 ± 0.04^{a}	5.94 ± 0.05^{a}	− 1.45 ± 0.04^{a}	206.52 ± 1.73^{a}
120	98.47 ± 0.29^{a}	− 0.67 ± 0.27^{c}	5.90 ± 0.08^{a}	5.95 ± 0.08^{a}	− 1.46 ± 0.04^{a}	206.04 ± 1.25^{a}

Data presented as average value of three analytical repetitions with standard deviation. MANOVA to compare data; different letters indicate statistical differences between wines of all treatments at the same time (Tukey's test, < 0.05). Abbreviations: TP - total phenolics (mg GAE/L).

temperature was stabile. Also, by increasing the frequency a large number of discharges occur leading to the generation of numerous radicals. In addition, by creating a strong photoionization effect the part of energy transfers to the surrounding medium and warms it (Vukušić, 2016). To date, most of the studies have been mainly focused on microorganisms inactivation effects of plasma (Shi et al., 2011; Surowsky et al., 2014; Vukušić et al., 2016; Ziuzina et al., 2013), and there are only few researches about the plasma impact on food components (Bursać Kovačević, Gajdoš Kljusurić et al., 2016; Bursać Kovačević, Putnik et al., 2016; Elez Garofulić et al., 2015; Grzegorzewski et al., 2011). Also, considering the use of various plasma sources and process parameters in numerous researches, it is difficult to compare plasma mechanisms and its final effects. Since the plasma is oxidative method and phenols as main constituents of both red and white wines are primary substrates for oxidation (Du Toit, Marais, Pretorius, & Du Toit, 2006; Oliveira et al., 2011), the correlation between the lower concentrations and longer treatment duration was observed (Tables 2–3). The concentrations of TP in wines varied from 1606.57 to 1773.94 mg GAE/L in red wine and from 204.94 to 207.58 mg GAE/L in white wine depending on the duration of plasma treatment. It can be observed that the treatment duration represents factor which highly affected TP of both analyzed wines, since the lowest concentrations were found in samples treated by the longest treatment duration of 10 min. When observing the influence of frequency, it can be concluded that wines treated at higher frequency had lower content of TP. Regarding the TA content in red wine, similar trends can be observed. Depending on the treatment duration, their concentrations varied from 358.39 to 409.21 mg/L, while range of 356.71 to 410.54 mg/L was determined with respect to the applied frequency. Since the *p*-values for the applied factors (treatment duration and frequency) were lower than the critical value of 0.05, it can be concluded that the both factors significantly influenced the content of TP and TA in wines after plasma treatment. Furthermore, only the treatment duration factor had significant effect on the TT content in red wine, respectively. After applied plasma treatment, the TT amount varied from 1.75 to 1.99 g/L. The highest concentration was observed in sample that was treated for the shortest time, while the sample with lowest TT content was subjected to the treatment duration of 10 min, suggesting importance of treatment duration on this phenolic compound. Additionally, the fact that anthocyanins and tannins directly influence the quality characteristics of wine, the changes in their quantities will have a crucial effect on wine color. However, the effect of the plasma treatments on phenolic compounds did not alter significantly the color of the treated wines.

Furthermore, individual phenolic compounds were determined using the HPLC UV/Vis. Nine different free anthocyanins were identified and quantified in red wine Cabernet Sauvignon (Table 4) while ten different phenolic acids and four flavan-3-ols were identified and quantified in white wine Graševina (Table 5). As it can be seen in Table 4, the composition of all identified free anthocyanins slightly decreased with the increase of treatment duration and frequency, indicating that both factors affected their concentrations in treated red wine. However, compared to the frequency, treatment duration was more important factor that strongly affected the content of individual anthocyanins during applied plasma treatment. The wines treated for the longest time (10 min) showed lower content of delphinidin-3-*O*-glucoside (18% lower), cyanidin-3-*O*-glucoside (16% lower), petunidin-3-*O*-glucoside (14% lower), peonidin-3-*O*-glucoside (10% lower), malvidin-3-*O*-glucoside (10% lower), peonidin-3-*O*-glucoside acetat (5% lower), malvidin-3-*O*-glucoside acetat (7% lower), peonidin-3-*O*-glucoside *p*-coumarat (16% lower) and malvidin-3-*O*-glucoside *p*-coumarat (13% lower) when compared to the control wine. The obtained results are also in accordance with report from Bursać Kovačević, Gajdoš Kljusurić et al. (2016) where cold plasma treatment caused degradation of anthocyanins, explaining that their low stability is due to oxidation phenomena or by matrix interactions during exposure to plasma-generated reactive radicals. Also, these results are coherent with the above mentioned reduction in TA content. Among the identified two main groups of phenolic acids (hydroxybenzoic and hydroxycinnamic acids) and flavan-3-ols in control white wine, caftaric acid had the highest concentration (17.52 mg/L), followed by procyanidin B1 (11.63 mg/L), (−)-epicatechin (7.30 mg/L) and protocatechuic acid (5.31 mg/L) (Table 5). Other phenolic compounds including gallic acid, chlorogenic acid, caffeic acid, procyanidin B2, (+)-catechin, *p*-coumaric acid, ferulic acid, *p*-hydroxybenzoic acid, vanillic acid and syringic acid were found in concentrations lower than 5 mg/L. When compared to the control wine, obtained results indicate slightly increased concentrations of almost all phenolic acids in plasma treated white wine with more significant effect of treatment duration than applied frequency. The values obtained for flavan-3-ols, namely (+)-catechin and (−)-epicatechin also slightly increased after applied plasma treatment. A different effect was observed for the content of procyanidins B1 and B2, with the lowest concentrations determined in wine treated for the longest time of 10 min (8.05 mg/L and 1.25 mg/L). As previously stated, the influence of treatment duration was more effective than the one of frequency. Similar findings were obtained in studies with cloudy pomegranate juice (Herceg et al., 2016) and chokeberry juice (Bursać Kovačević, Gajdoš Kljusurić et al., 2016) where cold plasma treatment positively influenced the content of phenolic acids, namely hydroxycinnamic acids due to their better stability and therefore less reactivity with plasma-generated radicals. Elez Garofulić et al. (2015) also evaluated the effect of plasma treatment on anthocyanins and phenolic acids content in sour cherry Marasca juice, explaining that short exposure of plasma treatment dissociates the agglomerates or particles leading to increase in phenolic compounds. The observed differences in plasma affecting the phenols in red and white wines (Tables 4–5) could be attributed to the nature and quantity of phenols in a given wines. The differences in chemical composition, namely the concentration of specific phenolic groups, the presence of individual

Table 4
Influence of treatment duration and applied frequency on the concentrations of individual anthocyanins in red wine Cabernet Sauvignon.

Source of variation	Dph	Cy	Pt	Pn	Mv	PnAc	MvAc	PnCm	MvCm
Treatment duration (min)									
Control	17.04 ± 0.15^{b}	1.68 ± 0.02^{b}	17.09 ± 0.19^{b}	12.31 ± 0.21^{b}	97.08 ± 1.73^{b}	4.45 ± 0.04^{b}	20.73 ± 0.31^{b}	2.95 ± 0.07^{b}	9.62 ± 0.24^{b}
3	16.74 ± 0.21^{b}	1.63 ± 0.06^{b}	16.99 ± 0.25^{b}	12.18 ± 0.13^{b}	96.19 ± 0.72^{b}	4.41 ± 0.07^{b}	20.55 ± 0.20^{b}	2.91 ± 0.04^{b}	9.51 ± 0.11^{b}
5	16.33 ± 0.37^{b}	1.56 ± 0.13^{b}	16.64 ± 0.25^{b}	11.93 ± 0.20^{b}	94.90 ± 1.23^{b}	4.35 ± 0.05^{b}	20.11 ± 0.31^{b}	2.83 ± 0.06^{b}	9.34 ± 0.15^{b}
10	14.01 ± 0.77^{a}	1.41 ± 0.09^{a}	14.73 ± 0.67^{a}	11.10 ± 0.48^{a}	87.76 ± 3.29^{a}	4.21 ± 0.14^{a}	19.25 ± 0.63^{a}	2.49 ± 0.13^{a}	8.35 ± 0.38^{a}
Frequency (Hz)									
Control	17.04 ± 0.15^{b}	1.68 ± 0.02^{b}	17.09 ± 0.19^{b}	12.31 ± 0.21^{c}	97.08 ± 1.73^{b}	4.45 ± 0.04^{b}	20.73 ± 0.31^{b}	2.95 ± 0.07^{b}	9.62 ± 0.24^{b}
60	15.92 ± 1.32^{a}	1.57 ± 0.11^{ab}	16.31 ± 1.11^{a}	11.88 ± 0.60^{bc}	93.78 ± 4.56^{ab}	4.37 ± 0.07^{b}	20.14 ± 0.80^{ab}	2.78 ± 0.21^{a}	9.18 ± 0.58^{a}
90	15.78 ± 1.19^{a}	1.54 ± 0.13^{ab}	16.16 ± 0.99^{a}	11.81 ± 0.43^{ab}	93.35 ± 3.59^{a}	4.34 ± 0.09^{ab}	20.07 ± 0.58^{ab}	2.75 ± 0.18^{a}	9.10 ± 0.52^{a}
120	15.38 ± 1.52^{a}	1.49 ± 0.15^{a}	15.89 ± 1.27^{a}	11.52 ± 0.61^{a}	91.72 ± 4.79^{a}	4.25 ± 0.18^{a}	19.70 ± 0.64^{a}	2.69 ± 0.23^{a}	8.91 ± 0.63^{a}

Data presented as average value of three analytical repetitions with standard deviation. MANOVA to compare data; different letters indicate statistical differences between wines of all treatments at the same time (Tukey's test, < 0.05). Abbreviations: Dph – delphinidin-3-*O*-glucoside; Cy - cyanidin-3-*O*-glucoside; Pt - petunidin-3-*O*-glucoside; Pn - peonidin-3-*O*-glucoside; Mv - malvidin-3-*O*-glucoside; PnAc - peonidin-3-*O*-glucoside acetat; MvAc - malvidin-3-*O*-glucoside acetat; PnCm - peonidin-3-*O*-glucoside *p*-cumarat; MvCm - malvidin-3-*O*-glucoside *p*-cumarat. Results are expressed as mg of malvidin-3-*O*-glucoside equiv./L.

phenolic compounds and/or synergistic interactions between them are likely to contribute the free radical scavenging capacity of wines (De Beer, Joubert, Gelderblom, & Manley, 2003). Due to their structural differences these compounds have various antioxidative properties, which manifest in the interaction with plasma reactive species (Grzegorzewski, 2011). The complex nature of plasma chemistry, wine chemical composition and its numerous interacting components require further investigation to understand the mechanisms influencing the phenolic compounds.

3.3. Principal component analysis

Moreover, in order to evaluate the influence of plasma treatments on the color and phenolic composition of the red and white wines, the principal component analysis (PCA) was conducted and the results are represented in Fig. 2a and b. In Fig. 2a, the first variable (PC1), explaining 79.17% of the total variance was strongly negatively correlated with all variables except hue angle (H*). The second variable (PC2), explaining 11.00% of the total variance, showed a moderately strong positive correlation only with L*(0.43), a*(0.56), b*(0.63) and C*(0.57) variables. In view of presented PCA analysis it can be concluded that the color parameters have not been found to correlate with phenolic content of red wine. Firstly, it can be seen that the treated red wine samples are clearly displaced from untreated wine sample (control). This deviation from the control sample with respect to plasma treated wine samples clearly indicates the fact that plasma results in a decrease in phenolic content. Secondly, PC1 led to the separation of wine samples into three different groups according to the treatment duration. The wines treated for 3 min are grouped on the negative side of PC1, those treated for 5 min are mainly distributed in the central part on PC1 while those treated for 10 min on the positive side of PC1. More specifically, the wine samples positioned in the first quadrant are characterized by lowest values of all measured variables, which meant that the values of these parameters generally decreased as the plasma treatment duration increased.

In Fig. 2b, PC1 accounted for 75.28% of the variation and PC2 explained an additional 11.36%. The first variable was strongly negatively correlated with following phenolic acids: protocatechuic acid (− 0.94), *p*-hydroxybenzoic acid (− 0.95), vanillic acid (− 0.92), syringic acid (− 0.96), caftaric acid (− 0.97), chlorogenic acid (− 0.96), caffeic acid (− 0.91), *p*-coumaric acid (− 0.75) as well as with two flavan-3-ols, precisely (+)-catechin (− 0.95) and (−)-epicatechin (− 0.95) and color variable a* (− 0.90), while highly positively correlated with total phenolics (0.98), gallic acid (0.96), procyanidins B1 (0.93) and B2 (0.92) and with two color variables, L* (0.91) and H* (0.91). The best explained variances in PC2 were described by the

Table 5
Influence of treatment duration and applied frequency on the concentrations of individual phenolic acids and flavan-3-ols in white wine Graševina.

Source of variation		Treatment duration (min)			Frequency (Hz)		
	Control	3	5	10	60	90	120
Hydroxybenzoic acids (mg/L)							
Gallic acid	4.85 ± 0.00^{b}	4.83 ± 0.01^{b}	4.82 ± 0.01^{b}	4.75 ± 0.05^{a}	4.82 ± 0.05^{ab}	4.80 ± 0.04^{ab}	4.78 ± 0.05^{a}
Protocatechuic acid	5.31 ± 0.01^{a}	5.43 ± 0.09^{b}	5.54 ± 0.03^{c}	5.63 ± 0.05^{d}	5.49 ± 0.13^{b}	5.55 ± 0.09^{bc}	5.56 ± 0.07^{c}
p-Hydroxybenzoic acid	0.27 ± 0.00^{a}	0.29 ± 0.05^{a}	0.33 ± 0.05^{ab}	0.36 ± 0.02^{b}	0.31 ± 0.05^{a}	0.32 ± 0.05^{a}	0.34 ± 0.06^{a}
Vanillic acid	0.30 ± 0.01^{a}	0.39 ± 0.04^{b}	0.43 ± 0.05^{bc}	0.47 ± 0.03^{c}	0.41 ± 0.06^{b}	0.43 ± 0.04^{b}	0.45 ± 0.05^{b}
Syringic acid	0.06 ± 0.01^{a}	0.07 ± 0.01^{a}	0.08 ± 0.02^{a}	0.10 ± 0.01^{b}	0.07 ± 0.02^{a}	0.08 ± 0.02^{a}	0.08 ± 0.02^{a}
Hydroxycinnamic acids (mg/L)							
Caftaric acid	17.52 ± 0.01^{a}	17.57 ± 0.04^{ab}	17.61 ± 0.02^{bc}	17.66 ± 0.08^{c}	17.59 ± 0.05^{ab}	17.62 ± 0.06^{b}	17.64 ± 0.07^{b}
Chlorogenic acid	4.46 ± 0.01^{a}	4.67 ± 0.07^{b}	4.71 ± 0.03^{b}	5.38 ± 0.09^{c}	4.86 ± 0.32^{b}	4.93 ± 0.35^{bc}	4.97 ± 0.37^{c}
Caffeic acid	3.65 ± 0.00^{a}	3.67 ± 0.01^{ab}	3.68 ± 0.02^{bc}	3.70 ± 0.01^{c}	3.67 ± 0.02^{b}	3.68 ± 0.01^{bc}	3.69 ± 0.01^{c}
p-Coumaric acid	0.59 ± 0.01^{a}	0.60 ± 0.01^{a}	0.61 ± 0.01^{a}	0.68 ± 0.08^{b}	0.61 ± 0.02^{a}	0.62 ± 0.01^{a}	0.67 ± 0.09^{b}
Ferulic acid	0.39 ± 0.00^{a}	0.39 ± 0.01^{a}	0.39 ± 0.00^{a}	0.39 ± 0.01^{a}	0.39 ± 0.01^{a}	0.39 ± 0.01^{a}	0.39 ± 0.01^{a}
Flavan-3-ols (mg/L)							
Procyanidin B1	11.63 ± 0.07^{c}	11.52 ± 0.25^{c}	10.80 ± 0.74^{b}	8.05 ± 0.10^{a}	10.32 ± 1.74^{b}	10.28 ± 1.63^{b}	9.77 ± 1.52^{a}
Procyanidin B2	1.56 ± 0.22^{b}	1.45 ± 0.13^{b}	1.31 ± 0.02^{a}	1.25 ± 0.10^{a}	1.39 ± 0.16^{ab}	1.33 ± 0.08^{a}	1.30 ± 0.11^{a}
(+)-Catechin	1.63 ± 0.18^{a}	1.83 ± 0.14^{a}	2.30 ± 0.17^{b}	2.62 ± 0.20^{c}	2.16 ± 0.36^{b}	2.24 ± 0.40^{b}	2.36 ± 0.35^{b}
(−)-Epicatechin	7.30 ± 0.40^{a}	7.52 ± 0.42^{a}	7.82 ± 0.33^{ab}	8.02 ± 0.15^{b}	7.69 ± 0.45^{a}	7.77 ± 0.29^{a}	7.90 ± 0.37^{a}

Data presented as average value of three analytical repetitions with standard deviation. MANOVA to compare data; different letters indicate statistical differences between wines of all treatments at the same time (Tukey's test, < 0.05).

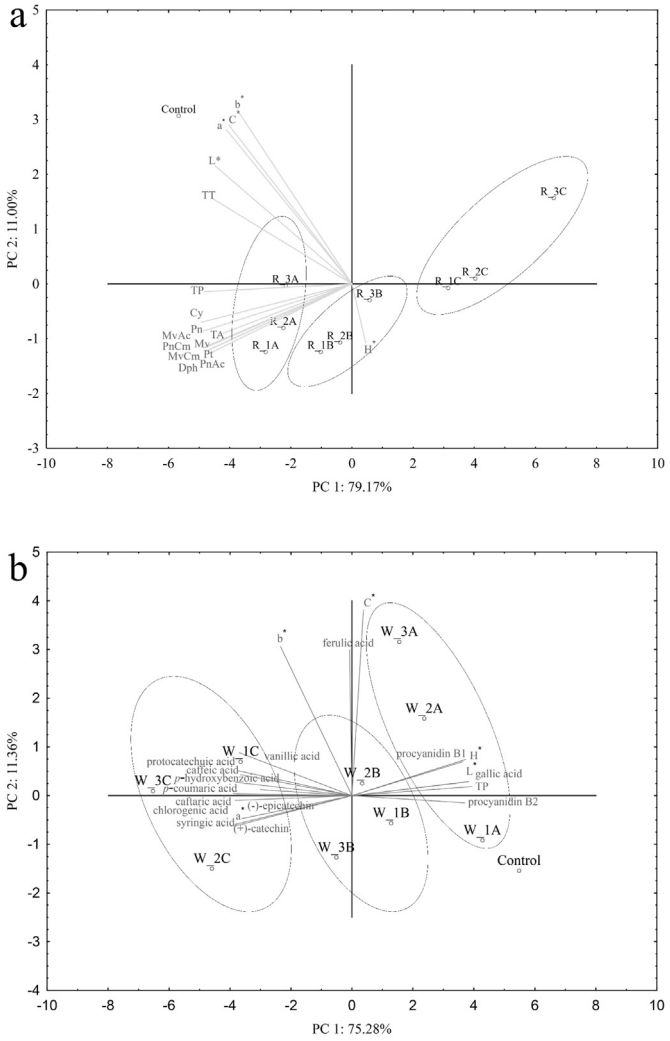

Fig. 2. Distribution of the wine samples in two dimensional coordinate systems defined by first two principal components (PC1 and PC2) according to the applied plasma treatments. (a) R: red wine; (b) W: white wine; Wines treated at 60 (1), 90 (2) and 120 (3) Hz for 3 (A), 5 (B) and 10 (C) min.

attribute of color variable C*, which had the highest loading (0.95) and by variable b* (0.76) and ferulic acid (0.75). Generally, the influence of plasma on white wine quality parameters varied according to the applied frequency and treatment duration. Again the separation of wine samples into several groups is apparent. The control sample is placed on the positive side of PC1, together with wines treated for the shortest time of 3 min. Although, the samples treated for 5 min are mainly distributed in the central part on PC1, they are still on the positive side except the sample treated at 120 Hz for 5 min. All of them are characterized by higher values of lightness, chroma, hue angle, total phenolics, gallic acid, procyanidins B1 and B2. In addition, the wine samples treated for 10 min are located on the negative side of PC1 and are characterized by higher concentrations of most compounds along with higher values of redness and yellowness. Again, as can be seen, the largest part of variation in the analyzed data set was due to the variation of processing time factor during plasma treatment.

Based on all discussions above, it could be concluded that the treatment duration plays an important role in modifying the quality characteristics of wine during exposure to plasma. The principal component analysis (PCA) of the wine quality properties confirmed that plasma treatments have modified the characteristics of both, red and white wines and resulted in grouping of wines according to the applied treatment conditions, also suggesting that duration of plasma exposure had the greatest influence on analyzed wine quality parameters. On the other hand, it can be seen from Tables 2-5 that the values of some wine quality parameters also varied, although to a lesser extent, according to the applied frequency, implying that the influence of frequency on wine quality characteristics also can not be absolutely ignored. Hence, the influences of plasma treatment duration and frequency should be taken into account when using to wine processing.

4. Conclusions

This study showed that plasma treatments have influenced the stability of phenolic compounds without altering significantly the color of the wines. In relation to untreated wines, high voltage electrical discharge plasma treatments mostly resulted in slight changes of chromatic characteristics and in reduction of phenolic compounds in both red and white wines, including total phenolics, total anthocyanins, total tannins and certain free anthocyanins, while the concentrations of the most individual phenolic acids and flavan-3-ols in white wine slightly increased. Both factors, the duration of the plasma treatment and applied frequency, had significant effect ($p < 0.05$) on the analyzed wine quality parameters. The treatment duration was more important factor than frequency, since it contributed to a larger part of the total variation in the whole data set in comparison to frequency factor. The result of an increase in phenolic concentration of white wine implies that this physical method could be used in improving the oxidative stability of wine and, consequently, the wine quality during the aging process. From current point of view it could be concluded that there are many challenges involving possible plasma application in winemaking, such as the wine types suitable for aging, the optimum processing parameters, compounds affected and the reaction mechanisms. In summary, these findings are the first step towards enhancing our understanding of plasma impact on wine quality properties and they give helpful insight in further optimization and evaluation steps of plasma processing conditions in winemaking. Nevertheless, further researches are required on a wider range of plasma processing parameters and quality properties of wine.

Acknowledgments

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Paper 7

Lukić, K., Tomašević, M., Vukušić, T., Kelšin, K., Gracin, L., Kovačević Ganić, K. (2017) Influence of high voltage electrical discharge plasma treatment on the physicochemical characteristics of wine. *Works of the Faculty of Agriculture and Food Science* **62**(67 (2)), 517-524.

INFLUENCE OF HIGH VOLTAGE ELECTRICAL DISCHARGE PLASMA TREATMENT ON THE PHYSICOCHEMICAL CHARACTERISTICS OF WINE*

UTJECAJ VISOKONAPONSKOG ELEKTRIČNOG PRAŽNJENJA-HLADNE PLAZME NA FIZIKALNO-KEMIJSKE KARAKTERISITKE VINA

Katarina Lukić[1], Marina Tomašević[1], Tomislava Vukušić[1], Karla Kelšin[1], Leo Gracin[1], Karin Kovačević Ganić[1]

Original scientific paper

Rezime

Cilj ovoga istraživanja bio je ispitati utjecaj visokonaponskog električnog pražnjenja-hladne plazme na fizikalno-kemijske karakteristike vina, zbog potencijalne primjene ove tehnologije u proizvodnji vina. Istražen je utjecaj procesnih parametara hladne plazme, frekvencije (60, 90, 120 Hz) i trajanja tretmana (3, 5, 10 min) pri pozitivnom polaritetu na koncentraciju otopljenog kisika, koncentracije slobodnog i ukupnog sumporovog dioksida (SO_2), te električnu provodljivost u bijelom i crnom vinu. Neposredno nakon tretmana provedene su analize, gdje je koncentracija otopljenog kisika određena pomoću uređaja za mjerenje kisika, koncentracija slobodnog i ukupnog SO_2 potenciometrijskom titracijom, dok je konduktometrom izmjerena električna provodljivost. Dobiveni rezultati pokazali su da primjenom tretmana hladnom plazmom dolazi do smanjenja koncentracije otopljenog kisika i ukupnog SO_2 u usporedbi sa kontrolnim vinima. S druge strane, električna provodljivost se povećala nakon primijenjenog tretmana, dok se koncentracija slobodnog SO_2 ili smanjila ili povećala. Također, rezultati su pokazali da frekvencija i trajanje tretmana značajno utječu na fizikalno-kemijske karakteristike vina.

Ključne riječi: *hladna plazma, vino, fizikalno-kemijski parametri*

Summary

The aim of proposed research was to study the influence of high voltage electrical plasma discharges on the physicochemical characteristics of wines, due to the potential use of this technique in winemaking. The effects of plasma discharge frequency (60, 90, 120 Hz) and treatment duration (3, 5, 10 min) with positive electrode polarity on the changes in concentrations of dissolved oxygen, free and total

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sulfur dioxide (SO_2) and electrical conductivity in white and red wines were investigated. The analyses were done immediately after treatment, where the dissolved oxygen was measured by oxygen-meter, free and total SO_2 by potentiometric titration while conductometer was used for electrical conductivity measurements. The results showed that applied treatments influenced the decrease in concentration of dissolved oxygen and total SO_2 in comparison to control wines. On the other hand, electrical conductivity increased after applied treatment, while concentration of free SO_2 was either decreased or increased. The results also showed that physicochemical characteristics of wines were significantly affected by frequency as well as processing time.

Key words: *high voltage electrical discharge plasma, wine, physicochemical characteristics*

INTRODUCTION

The new innovative technologies, such as high hydrostatic pressure, pulsed electric fields, high voltage arc discharge and non-thermal plasma, are today of great interest in food industry. Among these novel technologies, the application of plasma technology to wine has not been investigated so far. Most of the studies have been carried out on inactivation effect of various plasma treatments on microorganisms (Ziuzina *et al*., 2013; Shi *et al*., 2011; Vukušić *et al*., 2016; Misra and Jo, 2017). Recently, the focus has begun to shift towards impact of plasma on food constituents (Grzegorzewski *et al*., 2011; Misra *et al*., 2015; Bursać Kovačević *et al*., 2016; Elez Garofulić *et al*., 2015; Ramazzina *et al*., 2016), which is still insufficiently explained. Because of the lack of knowledge on the primary modes of action and on the effects on sensory and nutritional properties of the products, the use of plasma technology for food processing has not been yet allowed (Niemira, 2012). The plasma is a non-thermal technology, which is described as partially or completely ionized gas with electrical, chemical and physical properties (Petitpas *et al*., 2007). The plasma can be produced by many methods such as electric discharges (corona, spark, glow, arc, microwave discharge, plasma jets and radio frequency plasma) and shocks (electrically, magnetically and chemically driven) (Petitpas *et al*., 2007). The primary effects of electrical discharges are the UV radiation and the generation of reactive chemical species by the plasma ionization process (Niemira, 2012).The inactivation efficiency of plasma is associated with large number of variables, primarily with employed plasma sources and process parameters as well as with the characteristics of treated product (Misra and Jo, 2017). Apart from the nutritional and sensory quality, the physicochemical parameters are often employed for judging the quality of products. Basic physicochemical parameters such as pH, sulfur dioxide (SO_2) and others are generally used to define and to express wine quality (García Martín and Sun, 2013). In wine, oxygen can influence the composition and quality of wine drastically due to its involvement in various reactions (Du Toit *et al*., 2006).

The measurements of dissolved oxygen in wine are significant because the contact between wine and oxygen is a critical point during the wine production (Castellari *et al*., 2004). Another important physicochemical parameter of wine is the electrical conductivity because of its good correlation with pH and assimilable nitrogen during fermentation (Colombié *et al*., 2008). Electrical conductivity is defined as the ability of a solution to conduct electric current (Colombié *et al*., 2008). Thus, the aim of this research was to study the influence of the plasma treatment, as possible alternative technique to reduce the addition of SO_2 in wine, on the changes of previously mentioned physicochemical parameters (dissolved oxygen, electrical conductivity and total/free SO_2 concentrations).

MATERIAL AND METHODS

Material

The white wine Graševina (*Vitis Vinifera L.)* and red wine Cabernet Sauvignon (*Vitis Vinifera L.)* used in this study were acquired from the winery Erdutski vinogradi d.o.o., harvest 2016, in Erdut, Croatia. Physicochemical properties of Graševina were: 11.4 vol %, total acidity (as tartaric acid) 5.1 g/L, volatile acidity (as acetic acid) 0.31 g/L, reducing sugars 2.8 g/L, pH 3.37, malic acid 1.2 g/L, lactic acid 0.3 g/L, while those of Cabernet Sauvignon were: 13.1 vol %, total acidity (as tartaric acid) 5.3 g/L, volatile acidity 0.61 g/L, reducing sugars 4.1 g/L, pH 3.46, malic acid 0.1 g/L, lactic acid 1.3 g/L.

Chemicals

Sulfuric acid 1/3 (941), sodium hydroxide 2N (908), sulfuric acid 1/10 (932) and iodide/iodate N/64 (921) were purchased from LDS Laboratoires Dujardin-Salleron (Noizay, France).

Methods

The plasma treatments of wine

The plasma treatments were conducted in a 1000 mL glass vessel with a point to point electrode configuration in a so-called hybrid reactor with discharges in and above the liquid. The plasma was generated by high-voltage (HV) pulsed power supply (Spellman, UK) by charging a load capacitor of 1.13 nF to up to 30 kV and then discharging the stored charge into the plasma reactor via a rotating spark gap. The voltage in the plasma reactor was measured and recorded using a Tektronix P6015A high voltage probe connected to a Hantek DS05202BM oscilloscope (data not shown). The experiments were performed at positive polarity and argon (purity 99.99%; Messer Croatia, Zagreb, Croatia) was bubbled trough stainless steel needle (Micrlolance TM 3.81 cm) at the gas flow of 4 L/min. 300 mL of wine was treated with plasma running at the combination of following processing parameters: frequency at 60, 90 and 120 Hz and treatment duration of 3, 5 and 10 min. The temperature and pH value of samples before and after the plasma treatment were

monitored (data not shown). Before treatment, all samples were at the room temperatures of 21 ± 1 °C, while after the plasma exposure temperature raised up to 6 °C, depending on the treatment duration and applied frequency. The pH value of wines maintained relatively constant ranging from 3.1 to 3.4. After treatments, wine samples were subjected to physicochemical analysis.

Physicochemical analysis

In control and treated wines, immediately after plasma treatments, the concentrations of dissolved oxygen, free and total SO_2 and electrical conductivity were measured. The measurement of dissolved O_2 concentration was carried out using the oxygen measurement device (Nomasense O2 P6000, Nomacorc, Belgium), which is based on luminance principle. The device corrects the concentration of oxygen in terms of sugar and alcohol content and the temperature of the wine. Determination of dissolved oxygen was carried out immediately after treatment with plasma using an immersion probe with a detection limit of 15 μg/L of oxygen. The measurement of the free and total SO_2 concentration was performed on a SO_2 measurement device (LDS Sulfilyser, Laboratoires Dujardin-Salleron, Noizay, France) by titration with iodide/iodate solution whereby the iodine was reduced and SO_2 oxidized, with the potentiometric determination of the titration point via the LED indicator. Electrical conductivity was measured using a digital pH-meter HANNA edge (HANNA instruments, Croatia, Zagreb, Croatia). The measurement was performed by immersing the electrode for measuring electrical conductivity in the sample and after the stabilization the measured values were recorded.

Statistical analysis

Statistical analysis was carried out using analysis of variance (ANOVA) of Statistica V.10 software (Statsoft Inc., Tulsa, USA). Tukey's HSD test was used as comparison test when samples were significantly different after ANOVA ($p<0.05$).

RESULTS AND DISCUSSION

The results of the effects of various plasma treatments on the physicochemical parameters of wine are presented in Tables 1 (Graševina) and 2 (Cabernet Sauvignon). The results showed that physicochemical characteristics of wines were significantly influenced ($p \leq 0.05$) by the plasma treatment. As can be seen in Table 1, after different plasma treatments, the concentration of dissolved oxygen and total SO_2 values in white wine were reduced, while the concentration of free SO_2 was highly variable and independent of applied processing parameters. On the other hand, the value of electrical conductivity of the treated wine increased after the applied treatment compared to the untreated (control) wine. Regarding the SO_2 in wine, it is important to control its concentration after applied plasma treatments because SO_2 has antioxidant and antimicrobial effects on wine (Usseglio – Tomasset, 1992; Oliveira *et al*., 2011; Ugliano, 2013; Guerrero and Cantos – Villar, 2015). Except the SO_2, the concentration of dissolved oxygen also represents a crtical

parameter for the control of various processing treatments (Castellari *et al*., 2004). Among these, the reaction of SO_2 with oxygen is slow and has a crucial role in SO_2 antioxidant activity (Ugliano, 2013). It is known that the amount of SO_2 that binds with other substances in wine, or that remains free, depends on the wine temperature and pH. These observations can be related to changes in temperature of samples after the plasma exposure and the fact that the plasma is oxidative method (Vukušić, 2016). From the processing parameters, the treatment duration had a greater impact on physicochemical parameters of wines, but also the influence of plasma discharge frequency should not be neglected. These data show that the largest decrease in the examined parameters (dissolved oxygen and total SO_2 concentrations) occurred at treatment at 120 Hz for 10 minutes. Furthermore, the highest reduction of free SO_2 concentration was observed at treatment at 90 Hz for 10 minutes, while the highest concentration of free SO_2 was determined in the sample treated at a frequency of 60 Hz for 3 minutes. It has been demonstrated that by increasing the plasma frequency a large number of discharges occur leading to the generation of numerous radicals. In addition, by creating a strong photoionization effect the part of energy transfers to the surrounding medium and warms it (Vukušić, 2016). Regarding the electrical conductivity of the treated wine, the values increased along with increasing the treatment duration, but also by increasing the plasma discharge frequency. By increasing the electrical conductivity, the voltage required for the initiation of the discharge reduces (Zhu *et al*., 2009). Also, the electron density in the liquid increases. That is why the electricity discharge is larger, resulting in plasma higher density and temperature and more intense UV radiation (Locke *et al*., 2006). When the conductivity values are above 5 mS/cm plasma bullets are shorter than 1 mm and strong acoustic waves are created (Šunka, 2001). Although the physical processes are more intense, shorter bullets also means that the smaller volume of the liquid will be in contact with the plasma (Vukušić, 2016). Furthermore, the similar effect of plasma treatments was also observed in red wine (Table 2), where the highest reduction of dissolved oxygen and total SO_2 concentrations, but also the highest concentration of free SO_2 occurred in the sample treated at 120 Hz for 10 minutes. Moreover, the highest reduction of free SO_2 concentration was observed at treatment at 90 Hz for 10 minutes. The values of electrical conductivity determined in red wine also increased by increasing plasma processing parameters. Overall, the characteristics of the obtained wines showed that the applied plasma treatments resulted in wines with different physicochemical parameters compared to the untreated wines.

Table 1. Effects of plasma treatments on physicochemical parameters in white wine Graševina

Treatments	Dissolved oxygen (mg/L)	Free SO_2 (mg/L)	Total SO_2 (mg/L)	Conductivity (μS/cm)
Untreated (control)	3.03 ± 0.04^{f}	12.67 ± 0.58^{bc}	65.00 ± 0.00^{d}	1409.33 ± 6.03^{a}
60 Hz/3 min	2.80 ± 0.02^{e}	14.67 ± 0.58^{d}	65.00 ± 0.00^{d}	1528.00 ± 15.10^{b}
90 Hz/3 min	2.53 ± 0.09^{c}	12.67 ± 0.58^{d}	60.83 ± 1.44^{b}	1570.67 ± 3.06^{c}
120 Hz/3 min	2.17 ± 0.04^{c}	12.67 ± 0.58^{bc}	62.50 ± 0.00^{bcd}	1614.00 ± 3.61^{d}
60 Hz/5 min	2.96 ± 0.06^{f}	12.67 ± 0.58^{bc}	64.17 ± 1.44^{cd}	1576.33 ± 12.50^{c}

90 Hz/5 min	2.56±0.09^{d}	13.67±0.58^{cd}	60.00±0.00^{ab}	1556.33±13.32^{c}
120 Hz/5 min	2.14±0.03^{bc}	11.33±0.58^{ab}	60.83±1.44^{b}	1627.00±8.89^{d}
60 Hz/10 min	2.50±0.02^{d}	11.67±0.58^{ab}	61.67±1.44^{bc}	1583.00±7.94^{c}
90 Hz/10 min	2.01±0.02^{ab}	10.33±0.58^{a}	60.00±0.00^{ab}	1628.00±5.00^{d}
120 Hz/10 min	1.97±0.06^{a}	11.33±0.58^{ab}	57.50±0.00^{a}	1664.67±9.81^{e}

Data presented as average value of three analytical repetitions with stadard deviation. ANOVA to compare data; different letters indicate statistical differences between wines of all treatments at the same time (Tukey's test, $p < 0.05$).

Table 2. Effects of plasma treatments on physicochemical parameters in red wine Cabernet Sauvignon

Treatments	Dissolved oxygen (mg/L)	Free SO_2 (mg/L)	Total SO_2 (mg/L)	Conductivity (μS/cm)
Untreated (control)	1.64±0.01^{e}	16.67±0.58^{c}	40.00±0.00^{d}	1756.00±1.00^{a}
60 Hz/3 min	1.55±0.04^{d}	13.67±0.58^{ab}	39.67±0.58^{d}	1829.67±1.53^{de}
90 Hz/3 min	1.49±0.02^{cd}	14.67±0.58^{b}	42.00±0.87^{e}	1833.67±1.53^{e}
120 Hz/3 min	1.36±0.03^{ab}	13.67±0.58^{ab}	37.00±0.87^{c}	1820.33±1.53^{c}
60 Hz/5 min	1.40±0.02^{ab}	17.67±0.58^{cd}	30.33±0.58^{b}	1806.33±4.04^{b}
90 Hz/5 min	1.51±0.02^{d}	13.67±0.58^{ab}	30.00±0.00^{b}	1830.33±1.53^{de}
120 Hz/5 min	1.54±0.04^{d}	14.67±0.58^{b}	30.00±0.00^{b}	1811.33±3.21^{b}
60 Hz/10 min	1.33±0.03^{a}	18.33±0.58^{cd}	27.67±0.29^{a}	1823.00±3.61^{c}
90 Hz/10 min	1.41±0.02^{bc}	12.67±0.58^{a}	29.67±0.58^{b}	1830.33±2.52^{de}
120 Hz/10 min	1.33±0.03^{a}	18.67±0.58^{d}	27.00±0.87^{a}	1863.67±3.51^{f}

Data presented as average value of three analytical repetitions with stadard deviation. ANOVA to compare data; different letters indicate statistical differences between wines of all treatments at the same time (Tukey's test, $p < 0.05$).

CONCLUSION

In summary, this study showed that plasma treatments have influenced the physicochemical characteristics of wines. Compared to untreated wines, plasma treatments resulted in changes of all measured parameters, namely in reduction of dissolved oxygen and total SO_2. The concentration of free SO_2 was either decreased or increased, while electrical conductivity of wines increased after applied plasma treatments. Altogether, our results demonstrated the importance of determining the changes in wine physicochemical parameters after exposure to plasma treatments. Finally, these data are crucial precondition for possible application of plasma technology in wine industry. However, these parameters only provide overall quality of the wine. Future studies on the current topic are therefore required, particularly on sensory and chemical characteristics (phenolic and aroma compounds), in order to evaluate the plasma efficiency in winemaking.

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4. GENERAL DISCUSSION

At the beginning of this doctoral thesis, the following hypothesis was defined: the assumption that non-thermal techniques are potential methods for application in production of wine with reduced SO_2 concentration due to efficiency in inactivation of microorganisms, acceleration of oxidation-reduction reactions and preservation and improvement of product quality. Extensive research has been proven to test the initial hypothesis. The possibility of using high power ultrasound (HPU), high hydrostatic pressure (HHP) and high voltage electrical discharge plasma – cold plasma (CP) to reduce the use of SO_2 in the production of high quality red and white wines was investigated. In order to preserve the quality of the wine, the short-term impact of these techniques on the chemical and sensory characteristics of both wines were examined, and the optimal experimental parameters for each individual technique were defined. Then, wines treated under optimal conditions were stored in bottles and aged for 12 months to examine the long-term impact of these techniques on the overall wine quality. Additionaly, the synergistic effect of these techniques along with addition of antioxidants (SO_2 and glutathione) during 12 months of aging in bottles was investigated. Finally, the effectiveness of these three physical techniques in combination with antioxidants in the production of wine with a reduced concentration of SO_2 was determined.

4.1. Short-term impact of applied non-thermal techniques on the quality of red and white wines

The first phase of this study included investigation of the short-term effect of HPU, HHP and CP treatments on the stability of phenolic and aroma compounds and physicochemical, chromatic, and sensory characteristics of red and white wines, produced with reduced SO_2 concentration.

4.1.1. Influence of high power ultrasound on the quality characteristics of red and white wines

Investigation of the effect of high power ultrasound (HPU) on the quality of red and white wines involved the application of two different ultrasound generation systems: an ultrasonic bath and an ultrasonic probe. Experimental designs for both wines are shown in Tables 1 of PAPER 1 (Lukić et al., 2019a), PAPER 2 (Lukić et al., 2020a) and PAPER 3 (Lukić et al., 2019b).

Ultrasonic bath treatments were performed at different amplitudes (40, 60, 100%), frequencies (37 and 80 kHz), bath temperatures (20, 40 and 60°C) and treatment duration (20, 50, 65 and 90 min), while ultrasound probe treatments involved variations in probe diameter (12.7, 19.1, and 25.4 mm), amplitude (25, 50, 75, and 100%), and treatment duration (3, 6, and 9 min). The analysis of wine quality immediately after the applied treatments referred to total and individual phenolic compounds, aroma compounds, physicochemical, chromatic and sensory characteristics. The obtained results for phenolic, chromatic and aroma composition of red and white wines are shown in Supplementary Tables S1-S3 of PAPER 1 and in Supplementary Tables S3-S4 of PAPER 2. The results of the influence of HPU technique on the physicochemical characteristics (SO_2 and dissolved oxygen) of both wines are shown in Figure 1 of PAPER 3, while the results of sensory evaluation of HPU treated wines are shown in Figures S1-S6 of APPENDIX 1. Considering the large number of analyzed variables, statistical data processing was performed by analysis of variance (ANOVA), and the results (*F* values) are given in Tables 2 of PAPER 1 and PAPER 2. In this way, the influence of process parameters (independent variables) and their interactions on wine quality characteristics (dependent variables) were analyzed. The results showed that the significance of the applied process parameters is different depending on the observed dependent variable, but also on the type of treated wine.

4.1.1.1. Phenolic, chromatic and aroma composition

From the results given by PAPER 1 and PAPER 2, it can be observed that both ultrasonic bath and probe treatments influenced the chemical comoposition of red and white wines. Generally speaking, the mild ultrasound conditions showed a more favorable and lighter impact on the phenolic, chromatic and aroma composition of treated wines, while contrarily higher process conditions resulted in a decrease of analyzed variables.

Regarding ultrasonic bath treatments of red wine, lower concentrations of total phenolics, total and individual anthocyanins and total tannins were observed at higher conditions of frequency (80 kHz) and bath temperature (60°C) (PAPER 1 - Table S1). A clear influence of these HPU processing variables was confirmed by performed statictical analysis showed in Table 2 of PAPER 1. This is in correlation with other literature data which revealed the reduction of total phenolics in red wine with higher HPU process conditions (100 kHz, 300 W, 60°C, 100 min), emphasizing the greatest influence of frequency and treatment duration (Zhang et al., 2016). Briefly, the ultrasonic degradation of phenolic compounds is frequency-dependent, and their

stability is not compromised at lower frequencies (Ashokkumar et al. 2008). Similarly, higher bath temperature (60°C), amplitude (100%) and longer treatment duration (90 min) resulted in lower values of chromatic characteristics L*, a*, b* and C*, while the value of H* remained constant (PAPER 1 - Table S1). The calculated ΔE* values, in the range of 2 – 6 CIELab units, further confirmed the existence of the total color differences between the treated and control red wine samples (PAPER 1 - Table S3). When discussing the meaning of ΔE*, values around 3 are considered as visually acceptable level of color tolerance in red wines (Martinez et al., 2001). The color of red wine is primarily related to the presence of anthocyanins which are very sensitive, unstable and susceptible to degradation. As can be seen, the same HPU process parameters influenced both anthocyanins and chromatic characteristics, namely bath temperature, amplitude and frequency. Furthermore, the statistical analysis (PAPER 1 – Table 2) revealed that aroma of treated wine was mostly affected by applied bath temperature and ultrasound amplitude. From the results for aroma (PAPER 1 - Table S1), clear trends could not be fully observed (especially in the case of volatile fatty acids and terpenes), probably due to low ultrasound intensity that is not evenly distributed in ultrasound systems like ultrasonic bath. However, certain links were evident such as lower concentrations of total esters and total higher alcohols after applying higher bath temperatures (40-60°C) and treatment durations (65-90 min). A combination of cavitation and heating effect of ultrasound probably resulted in a more intense sonochemical effect on the stability of aroma compounds. More detailed information on ultrasound effects on red wine aroma are given in PAPER 1.

Regarding ultrasonic probe treatments, the smaller probe diameter (12.7 mm), higher amplitude (100%) and longer treatment duration (9 min) resulted in reduced concentration of analyzed phenolic compounds (total and individual) in treated red wine samples compared to control (PAPER 1 – Table S2). The significance of these processing variables was confirmed by analysis of variance (PAPER 1 – Table 2). Briefly, higher amplitudes can lead to higher ultrasound intensities, which can trigger the side effect of phenolic compounds degradation. However, there is no clear trend depending on the applied HPU conditions, which is most likely due to enhanced reactions of polymerization/depolymerization, copigmentation, isomerization and oxidation of phenolic compounds during wine exposure to HPU. Moreover, the obtained CIELab values for HPU treated samples differed from those of untreated wine, depending on the applied process conditions. For example, all HPU treatments with a 19.1 mm diameter probe resulted in the lowest values of chromatic characteristics (PAPER 1 - Table S2).

Also, from Table S3 of PAPER 1, it can be seen tha ΔE* values were in the range of 0.5 to 3 CIELab units, which is lower than in the case of ultrasonic bath experiment. Generally, the observed color changes in both cases are probably due to the already mentioned cavitation mechanism that triggers certain chemical reactions such as the isomerization of colored pigments (Alighourchi et al., 2013). From the results given by statistical analysis (PAPER 1 – Table 2), it can be concluded that the probe diameter was a key factor in affecting the aroma composition of red wine, more precisely total esters, higher alcohols, and terpenes. The obtained results showed that a smaller probe diameter (12.7 mm) together with higher amplitudes (75-100%) or longer exposure to ultrasound (6-9 min) resulted in lower concentrations of compounds responsible for wine aroma (PAPER 1 – Table S2). Briefly, the observed changes may be related to various HPU mechanisms, such as the thermal effect of cavitation bubble implosion and consequent free radical formation, mechanical effects of microcurrents, implosions and shock waves (Brnčić et al., 2010; Carbonell- Capella et al., 2017). More details on HPU effects on phenolic, chromatic and aroma composition of red wine can be found in PAPER 1.

PAPER 2 deals with the changes in chemical composition of white wine induced by HPU processing. Statistical results (Table 2) from this research revealed that the bath temperature, followed by ultrasound amplitude were crucial factors in affecting chemical composition of white wine in ultrasonic bath experiment. Namely, HPU treated samples showed lower concentrations of total phenolics and total flavan-3-ols, whereas the slight increase or decrease of total phenolic acids was observed compared to untreated wine (PAPER 2 – Table S3). As mentioned earlier, HPU effect can be manifested through various physical (cavitation, mechanical effects, micro-mechanical shocks) and chemical effects (creation of free radicals due to sonochemical reactions), which can act simultaneously or separately on components of treated medium (Marić et al., 2018). Detailed information on ultrasound effect on phenolic compounds in white wine is well explained in PAPER 2. Furthermore, only slight differences in CIELab values were observed between treated and untreated samples (PAPER 2 – Table S3). Additionaly, most of the ΔE* values ranged from 0.1 to 1.8, which is not noticeable to the human eye. Data on aroma composition (PAPER 2 – Table S3) showed that higher bath temperature (60°C) and treatment duration (90 min) resulted in a decrease of total esters and total higher alcohols in treated samples. Esters are particularly sensitive to temperature changes and their degradation is usually consequence of hydrolysis which is accelerated by a temperature increse (Scrimgeour et al., 2015). The mechanical effects of ultrasound have been

reported to have an influence on the degradation rate of higher alcohols (Zhang et al., 2020). However, under the same conditions, an increase in the concentration of total fatty acids was observed, while the results for total terpenes did not show a clear trend.

Experiment with ultrasonic probe showed that the probe diameter, followed by amplitude were the key factors affecting chemical composition of white wine (PAPER 2 – Table 2). In general, HPU treated samples were characterized by slightly lower concentrations of phenolics (with some deviations in the case of total phenolic acids) and aroma compounds compared to untreated wine. Compared to red wine, the unclear trend was here even more pronounced. Interestingly, a larger probe diameter (19.1 mm) in combination with higher ultrasound amplitudes (50–100%) resulted in a more favorable effect on the phenolic and aroma composition (PAPER 2 – Table S4). As noted earlier, the application of HPU to wine can accelerate a variety of chemical reactions involving various groups of phenolic and aroma compounds. Moreover, only slight changes were recorded in chromatic characteristics of HPU treated samples compared to control, which was confirmed by low values of ΔE* ranging from 0.2 to 3.8 (PAPER 2 – Table S4). The observed changes in wine chemical composition are probably related to the previously mentioned HPU mechanisms, which are well explained in PAPER 2. Taken together, these findings implicate that applied ultrasonic bath and probe treatments affected differently the phenolic and aroma composition of white wine, while there were no major changes in color.

4.1.1.2. Application of artificial neural networks for modeling and prediction of HPU wine processing

This section brings some important aspects in predicting wine quality and artificial neural networks (ANN), which are currently a subject undergoing intense study. Due to the complexity of ultrasonic wine processing and the preservation of wine quality, the selection of optimal process parameters is a very challenging task. Given the large number of input (independent) variables such as ultrasound frequency, amplitude, processing time, probe diameter, temperature, etc., the classical mathematical approach or empirical mathematical models show reduced ability to predict HPU treatment. Also, a lack of flexibility is a problem in optimization of parameters of the process itself. Therefore, ANN have proven to be the best alternative solution for modeling HPU process (Dahmoune et al., 2015; Roselló-Soto et al., 2015). Consequently, the aim was to ensure the required output quality of red and white wines (chemical composition) as a function of the above-mentioned input variables for both HPU systems used (ultrasonic bath and ultrasonic probe).

In the case of red wine, the experimental data in Tables S1 and S2 (PAPER 1) were used for the purpose of ANN modeling. In Table 3 of PAPER 1 are presented 5 neural networks and their properties for both HPU experiments. The networks with the best properties (marked "bold") were selected based on the coefficient of determination (R^2) and root mean square error (RMSE) for learning, testing and validation. The performance of selected ANN models is shown in Table 4 of PAPER 1. Also, for each tested parameter in terms of ANN prediction, the results for both HPU experiments are presented as correlations between experimental and model predicted data in Figures 1 and 2 (PAPER 1). Detailed information on ANN modeling and prediction of phenolic, chromatic and aroma composition of red wine for both HPU experiments is well explained in PAPER 1. Briefly, the ANN models created in both cases showed the best predictions for chromatic characteristics and partly for phenolic composition (especially total phenolics and total anthocyanins in ultrasonic probe experiment), while at the same time same developed models did not give satisfactory predictions for aroma composition of wine.

The ANN modeling approach was also used to describe and predict the phenolic and aroma composition of white wine after both HPU experiments. For this purpose, the experimental data in Tables S3 and S4 were used (PAPER 2). The results of chromatic characteristics were not included in the ANN modeling since HPU treatments did not have a significant effect on the color of white wine. For each HPU experiment, two ANN models were developed, one for predicting phenolic composition and one for predicting aroma composition. The properties of the proposed neural networks are shown in Table 3 of PAPER 2. The optimal ANNs for predicting the phenolic and aroma composition of white wine were marked "bold". Further, the performance of selected ANNs to predict phenolic and aroma composition are given in Table 4 (PAPER 2). Additionally, a comparison between observed and ANN predicted data for performed HPU experiments is given in Figures 1 and 2 (PAPER 2). Briefly, when discussing ANN predictions, the best fit between observed and model data were obtained for total flavan-3-ols, total esters and total higher alcohols, respectively. More details on ANN modeling and predicting white wine quality can be found in PAPER 2.

4.1.1.3. Physicochemical characteristics

It is well known that levels of SO_2 and dissolved oxygen are very important information for analyzing wines condition. Therefore, to determine the possibility of using HPU on wine, it was necessary to assess its impact on these characteristics as well. In Figure 1 of PAPER 3 are

presented the results of HPU effect on the concentration of SO_2 and dissolved oxygen in red and white wines. Briefly, both HPU experiments (ultrasonic bath and probe) had a minimal effect on the dissolved oxygen concentration and the concentration of total and free SO_2. Compared to untreated wine, the dissolved oxygen increased slightly in HPU treated samples, while no clear trend was seen in the results for SO_2. Generally, any operation involving contact with air significantly accelerates the dissolution of oxygen (Ribéreau-Gayon et al., 2000). So, the observed changes are probably due to the dissolution of a certain amount of oxygen in the wine during the oxygen measurement procedure itself. Furthermore, among the various HPU systems applied, slightly larger changes in the physicochemical characteristics of both wines were observed after ultrasonic bath treatments (PAPER 3 – Figure 1). The obtained results agree with the previous research of García et al. (2016) who also reported inconsistent data on the change in SO_2 concentration in wine after HPU treatment, highlighting the degassing effect of ultrasound as a cause of free SO_2 reduction.

4.1.1.4. Sensory characteristics

The results of the sensory analysis of HPU treated samples of red and white wine are shown in Figures S1-S6 (APPENDIX 1). Figures S1 and S2 show the influence of different ultrasound amplitudes and treatment durations at the probe diameter of 25.4 mm (graphs S1A and S2A), 19.1 mm (graphs S1B and S2B) and 12.7 mm (graphs S1C and S2C) for samples of red and white wine treated with an ultrasonic probe. The graphs presented in Figures S1 and S2 revealed that there were significant differences between HPU treatments, depending on the applied amplitude, treatment duration and probe diameter, and that each of these parameters significantly affected the change in sensory characteristics and overall sensory evaluation of wine. From the obtained results it can be concluded that HPU treatments with larger probe diameter (25.4 mm) had a more favorable and milder effect on the sensory quality of treated red and white wines (Figures S1A and S2A) compared to those samples treated with 19.1 and 12.7 mm probe diameter. It should be emphasized that the sensory quality of the wine varied depending on applied amplitude and treatment duration, regardless of the probe diameter. Samples were rated by a panel group using a hedonic scale with 9 possible responses (1-9). Namely, HPU treatments at the lowest amplitude (25%) and the shortest treatment duration (3 min) were marked as “like moderately” to “like very much”, except for the treatment of white wine with a probe diameter of 12.7 mm (Figure S2C) which was marked as “like slightly”. The lowest sensory quality scores had those samples treated at the highest amplitude (100%) and the longest treatment duration (9 min), which is especially evident in the case of white wine

where samples were marked as “dislike slightly”. Under the same HPU process conditions, higher sensory quality scores were assigned to red wine (Figure S1) compared to white wine (Figure S2).

In Figures S3 and S4 (APPENDIX 1) are presented red wine samples treated with ultrasonic bath at frequencies 37 and 80 kHz, where the effects of different bath temperatures and treatment durations at amplitudes of 40, 60 and 100% are shown respectively in graphs S3A and S4A, S3B and S4B, and S3C and S4C. Furthermore, Figures S5 and S6 show white wine samples treated with ultrasonic bath at frequencies 37 and 80 kHz, where the effects of different bath temperatures and treatment durations at amplitudes of 40, 60 and 100% are shown in graphs S5A and S6A, S5B and S6B, and S5C and S6C, respectively. These results indicate that there are significant differences between individual HPU treatments, depending on the applied frequency, amplitude, bath temperature and treatment duration. Namely, HPU treatments at 80 kHz had a more favorable and milder effect on the sensory quality of treated red (Figures S3 and S4) and white (Figures S5 and S6) wine compared to those samples treated at frequency 37 kHz. Moreover, it can be seen that the lowest sensory quality scores were assigned to those samples treated at the highest amplitude (100%) and temperature (60°C) and the longest treatment duration (90 min), which is especially evident in the case of red wine where the samples are marked as “dislike slightly” (Figures S3C and S4C). On the other hand, the best marks were assigned to samples of red (Figures S3A and S4A) and white (Figures S5A and S6A) wine treated at the lowest amplitude (40%) and temperature (20°C) and the shortest treatment duration (20 min), and they are marked as “like very much”. In addition, the results of sensory analysis (Figure S3-S6) clearly showed that HPU ultrasonic bath treatments had a lighter and less negative effect on the sensory characteristics of white wine compared to red wine.

4.1.2. Influence of high hydrostatic pressure on the quality characteristics of red and white wines

The study of the short-term influence of high hydrostatic pressure (HHP) on the quality of red and white wines included the application of different pressures (200, 400 and 600 MPa) and treatment durations (5, 15 and 25 min), and analysis of phenolic and chromatic composition (Lukić et al., 2020b (PAPER 5)), aroma composition (Tomašević et al., 2017 (PAPER 4)), physicochemical (Lukić et al., 2019b (PAPER 3)) and sensory (APPENDIX 2) characteristics of treated wines. Figure 1 of PAPER 5 gives a schematic representation of wine processing

with HHP. The diagram shows the actual values of pressure and temperature in the system at the given process parameters during HHP treatment.

4.1.2.1. Phenolic, chromatic and aroma composition

From the results in Tables 1 and 2 of PAPER 5, it can be seen that HHP treatments slightly influenced phenolic and chromatic composition of red and white wines. Briefly, a slight decrease of total (except total tannins) and individual phenolic compounds was determined in HHP treated red wine samples compared to control wine (PAPER 5 - Table 1). On the other hand, HHP treatments resulted in a slight increase of chromatic characteristics of red wine. The downward trend was also found for total and individual (except some phenolic acids) phenolic compounds in HHP treated white wine samples compared to untreated wine (PAPER 5 – Table 2). Also, negligible changes in the chromatic characteristics were observed for white wine after HHP treatments, which was also confirmed by calculated ΔE* values. Generally, the main variations in the examined parameters were related to applied pressure. The observed changes were most pronounced at higher pressure levels and longer treatment durations, especially at 600 MPa/25 min. This is in correlation with other literature data which emphasized the importance of both processing parameters, where the pressure also had a greater impact on the wine quality (Tao et al., 2013). More details on these results can be found in PAPER 5.

Detailed information on the influence of HHP on aroma composition of red and white wines are well explained in PAPER 4. Briefly, the applied conditions of HHP treatment resulted in slight changes in the aroma composition of white and red wine, which can be seen from Tables 1 and 2 of PAPER 4. These changes are primarily related to a slight decrease in the concentration of esters, followed by volatile fatty acids and terpenes, and an increase in the concentration of higher alcohols in treated samples. However, in most of HHP treatments performed, the observed differences were not significant. Despite the similarities in trends, after HHP processing there were more pronounced changes in the aroma composition of red wine. Furthermore, principal component analysis (PCA) projections (PAPER 4 - Figures 1 and 2) were done using aroma composition attributes to examine possible grouping of samples with respect to different applied HHP conditions. In both cases, as demonstrated for phenolic compounds, higher pressures (400 – 600 MPa) and longer treatment durations (15 – 25 min) resulted in a greater loss of most analyzed aroma compounds (except higher alcohols), which was confirmed by positioning of these samples far away from untreated (control) one.

4.1.2.2. Physicochemical characteristics

The results of HHP effect on the concentration of SO_2 and dissolved oxygen in red and white wines are shown in Table 2 of PAPER 3. It can be seen that there was no statistically significant difference in the concentration of total and free SO_2 between untreated and HHP treated wines, while slight changes were observed in the dissolved oxygen concentration. The observed trends are the same in the case of red and white wines. The results obtained are consistent with previous studies that have shown that HHP had no effect on the concentration of SO_2 in wine (Tabilo-Munizaga et al., 2014; Santos et al., 2016). Furthermore, the dissolved oxygen concentration was slightly increased in both wines after HHP treatment, but there was no clear trend among the applied treatments. Since very little is known about the effect of HHP on oxygen concentration in wine, it is assumed that this effect is a combination of HHP treatment and high oxygen permeability of polyethylene bottles used as packaging during HHP processing (Dombre et al., 2015; Santos et al., 2019).

4.1.2.3. Sensory characteristics

The results of sensory analysis of HHP treated samples of red and white wine are shown in Figure S8 (APPENDIX 2). It was shown that the application of HHP treatment at selected process parameters (pressure and treatment duration) did not have a negative impact on the sensory characteristics of both, red and white wines, namely the color, odor and taste. As in the case of HPU effect, a hedonic scale with 9 possible responses was used. Most HHP treated samples of red and white wine were marked as "like very much" and "like extremely", confirming that HHP did not affect these sensory characteristics in the short-term. The highest scores on the hedonic scale were given to samples treated at a pressure of 200 MPa for 5 min, while the lowest scores were given to the samples treated at processing conditions of 600 MPa for 25 min.

4.1.3. Influence of cold plasma on the quality characteristics of red and white wines

Investigation of the short-term effect of cold plasma (CP) on the quality of red and white wines included the application of different frequencies (60, 90 and 120 Hz) and treatment durations (3, 5 and 10 min) at positive polarity with argon gas injection, and analysis of phenolic and chromatic composition (Lukić et al., 2019c (PAPER 6)), physicochemical characteristics (Lukić et al., 2017 (PAPER 7)), aroma composition and sensory characteristics (APPENDIX 3) of treated wines. A schematic representation of the experimental setup of CP processing of wines is given in Figure 1 of PAPER 6.

4.1.3.1. Phenolic and chromatic composition

In PAPER 6 is explained how plasma treatments have influenced the phenolic and chromatic composition of red and white wines. Respectively, CP treatments resulted in slight changes of chromatic composition and in reduction of phenolic compounds in both, red and white wines, including total phenolics, total anthocyanins, total tannins and certain individual anthocyanins, while the concentration of most analyzed phenolic acids and flavan-3-ols in white wine slightly increased (PAPER 6 – Tables 2–5). The highest process conditions (120 Hz/10 min) resulted evidently in the biggest changes. Moreover, statistical analysis (MANOVA) revealed a significant influence of applied process parameters, especially treatment duration on wine chemical composition, which was additionally confirmed by performed PCA (PAPER 6 - Figure 2). The observed changes can be associated with different plasma mechanisms such as generation of free radicals, development of shock waves, UV light, cavitation and electric fields, which are already well explained in PAPER 6.

4.1.3.2. Aroma composition

Since the results for the influence of CP treatments on aroma composition of red and white wines have not been published and discussed, more emphasis was placed on them. The results are presented in Tables S2 and S3 (APPENDIX 3), according to which aroma of analyzed red and white wine is characterized by several chemical groups: esters, higher alcohols, volatile fatty acids, terpenes and aldehydes. The obtained data were analyzed with statistical software (Statistica, Vers. 10.0, StatSoft Inc., USA). The multivariate analysis of variance (MANOVA) was used to explore the effect of each factor separately and their interaction. The analysis of variance revealed that treatment duration and frequency of CP treatment, as well as their interaction, had significant effect on changes in wine aroma ($p < 0.05$). Considering each process parametar (Tables S2-S3), it can be observed that treatment duration represents factor which highly affected aroma compounds of both, red and white wines, since the lowest concentrations were found in samples treated by the longest treatment duration (10 min). When observing the influence of frequency, it can be concluded that wines treated at higher frequency (120 Hz) had lower concentrations of all analyzed aroma compounds. At the same time, the synergistic effect of both treatment duration and frequency of CP treatment on *i*-butyl acetate ($p = 0.017$), *i*-amyl alcohol ($p = 0.000$) and benzaldehyde ($p = 0.000$) in red wine was significant, but for other aroma compounds that was not a case. Regarding the aroma composition of white wine, mentioned interaction of both process parameters (MANOVA analysis) had significant effect on ethyl hexanoate ($p = 0.020$), 2-phenylethyl acetate

(p = 0.029), *i*-amyl alcohol (p = 0.000), 1-hexanol (p = 0.002), hexanoic acid (p = 0.001), decanoic acid (p = 0.001) and linalool (p = 0.045), while did not affect other aroma compounds. There are no available studies regarding changes in wine aroma after CP treatments and in general, studies about changes in wine composition after applying plasma technology are rare. The observed reduction of aroma compounds could be due to electrohydraulic cavitation induced by plasma discharge that promotes the formation of hydroxyl radicals, which leads to chemical degradation (Joshi et al., 1995; Hoffmann et al., 1996). During the plasma discharge in liquid media, several mechanisms can act simultaneously: thermal effects of implosion of electrohydraulic cavitation bubbles, the mechanical stresses produced by shock waves and the generation of free radicals (Vukušić, 2016). Furthermore, a rise in temperature after the plasma exposure can be also one of the reasons for lower concentrations of aroma compounds. Namely, aroma is an important aspect of quality in wines and it is influenced by many factors. Volatile esters are a major constituent, contributing fruity and floral aromas to wine and they are more sensitive to temperature effects than other volatile components (Scrimgeour et al., 2015). According to Grymonpré et al. (2004), the presence of oxygen in the area above the surface of treated fluid, despite the spraying of argon during the gas phase discharge plasma and the treatment in a hybrid gas-liquid discharge reactor, causes the formation of ozone and its dissolution in a liquid that additionally increases the concentration of active species. Ozone in reaction with aroma compounds originates the formation of hydroxylated compounds and quinones, which are generated by firing a benzene ring (Langlais et al., 1991). In the present literature, only few results of plasma effect on food volatile composition could be observed. For example, Ma and Lan (2015) found that CP treatment has less effect on aroma composition of tomato juice compared to heat processes, with an increase in the contents of *trans*-2-hexenal and n-hexanal. Furthermore, Amini et al. (2017) reported changes in volatile oils of saffron and crocin esters after cold plasma treatment, namely a decrease in saffranal and crocin esters and increase in isophorone and 4-ketoisophorone. Vukušić (2016) investigated the application of high voltage electrical discharge plasma on fruit juices (apple juice and Marasca sour cherry nectar) and its influence on the physicochemical and sensory characteristics. This author found that the aroma profile of fruit juices was less affected by cold plasma technique compared to heat processing, emphasizing that the main changes in aroma composition were observed after the longer plasma exposure (9 min) and higher frequencies (90 and 120 Hz) as well as at higher temperature (50°C).

To compare analyzed wine samples according to aroma compounds, PCA was also conducted. The projection of analyzed aroma variables and the distribution of control and CP treated red wine samples in the two-dimensional coordinate system defined by first two variables explaining 89.84% of the total variance is shown in Figure S9A (APPENDIX 3). First variable (PC1) was strongly negatively correlated with the content of majority of the analyzed aroma compounds: ethyl acetate (-0.97), ethyl butyrate (-0.98), ethyl hexanoate (-0.98), ethyl octanoate (-0.98), ethyl decanoate (-0.90), diethyl succinate (-0.94), *i*-amyl acetate (-0.99), *i*-amyl alcohol (-0.96), phenylethyl alcohol (-0.99), 1-hexanol (-0.97), hexanoic acid (-0.99), octanoic acid (-0.99), decanoic acid (-0.98), linalool (-0.99), benzaldehyde (-0.97) and highly negatively correlated with *i*-butyl acetate (-0.73). Second variable (PC2) was strongly positively correlated with 2-phenylethyl acetate (0.90). Furthermore, control sample and CP treated samples at 60, 90 and 120 Hz for 3 min were placed on the left side of PC1 and displaced from all other treated wines due to higher concentrations of aroma compounds, which correlate negatively with PC1. The CP samples treated for 5 and 10 min were placed in first and fourth quadrant since they are characterized by lower concentrations of aforementioned aroma compounds. The projection of white wine samples, as well as analyzed aroma variables in the two-dimensional coordinate system defined by first two variables, explaining 96.1%, is shown in Figure S9B (APPENDIX 3). First variable, that explains 94.08% of the total variance (PC1), showed strong negative correlation with the content of all analyzed aroma compounds: ethyl acetate (-0.99), ethyl butyrate (-0.97), ethyl hexanoate (-0.99), ethyl octanoate (-0.97), ethyl decanoate (-0.99), diethyl succinate (-0.97), *i*-butyl acetate (-0.96), *i*-amyl acetate (-0.97), hexyl acetate (-0.91), 2-phenylethyl acetate (-0.97), *i*-amyl alcohol (-0.96), phenylethyl alcohol (-0.98), 1-hexanol (-0.98), *cis*-3-hexenol (-0.99), hexanoic acid (-0.96), octanoic acid (-0.97), decanoic acid (-0.99), linalool (-0.91) and benzaldehyde (-0.99). Moreover, the distribution of wine samples in the coordinate system indicates clear separation of CP treated samples, primarily, according to the length of treatment. In accordance with mentioned, samples treated at 60, 90 and 120 Hz during 5 and 10 min were positioned on the right side of PC1 and characterized by less significant content of all analyzed aroma compounds.

4.1.3.3. Physicochemical characteristics

In PAPER 7 are presented results of the effect of CP treatments on the physicochemical characteristics of red and white wines. From Tables 1 and 2 it can be clearly seen that CP treatments influenced the physicochemical characteristics of treated wines. Moreover, the treatment duration had a greater impact on analyzed characteristics, but also the influence of frequency should not be excluded. Namely, the concentration of dissolved oxygen and total SO_2 were reduced, while the concentration of free SO_2 was highly variable and independent of applied processing parameters. The largest decrease in the examined parameters, dissolved oxygen and total SO_2, occurred in the samples treated at 120 Hz for 10 min, while the lowest concentration of free SO_2 was observed after applying conditions of 90 Hz/10 min. These observations can be related to the fact that plasma is oxidative method and that by increasing the frequency many plasma discharges occur leading to the production of free radicals. On the other hand, the value of electrical conductivity of the treated wines increased after applied treatments compared to the untreated (control) wine. Recently, the incresement in wine conductivity was also observed by Sainz-García et al. (2019) after batch atmospheric pressure cold plasma treatments of 60-90 W for 3-5 min. In summary, the applied CP treatments resulted in wines with different physicochemical characteristics compared to untreated wines.

4.1.3.4. Sensory characteristics

The influence of cold plasma on wine sensory quality with the results represented as the average scores of the panelists are showed in Figure S10 (APPENDIX 3), for red and white wines, respectively. The sensory characteristics (color, odor and taste) of wines were analyzed by nine-point hedonic scale method. The results indicated the modification of sensory characteristics of red and white wines after CP treatments, particularly in terms of odor and taste. Generally, both wines showed similar trends in liking rating after exposure to plasma treatments. Among the treated wine samples, the samples treated during the shortest time were evaluated with higher scores (6 = "like slightly") compared to those treated for the longest time (scores in the range from 2 = "dislike very much" to 4 = "dislike slightly"). Especially, the plasma treatment during 10 min resulted with changes in odor and taste hard to explain, but panelists described it as „unpleasant" and „foreign-metal". It can be observed that treatment duration is crucial factor in affecting the sensory attributes of wines, which agrees with previously described results for aroma compounds, as well as the chromatic characteristics and phenolic composition. Among the red and white wines, the white wine had slightly lower ratings compared to the red wine treated at the same CP processing conditions. Generally, the

degradation rate of red wine aroma is slower compared to that of white wine due to higher concentration of phenolic compounds, which have the antioxidant properties. According to Fuhrman et al. (2001) limited antioxidant character of white wines makes them more susceptible to oxidation in contrast to red wines, which was probably the reason why cold plasma affected more the white wine sensory attributes than those of red wine. Moreover, it seems that the changes in aroma and phenolic composition, influenced by CP treatments, significantly modify the sensory quality of wines. However, the relationship between chemical composition and sensory attributes is not always easy to evaluate, due to complexity of wine chemical composition and its numerous interacting components (Forde et al., 2011).

4.2. Long-term impact of applied non-thermal techniques and antioxidants on the quality of red and white wines

The second phase of the study relies on the results obtained in the first part of this thesis. The most favorable operating conditions, which ensured the optimal wine quality, were selected to perform small scale experiments. This section covers the impact of high power ultrasound (HPU), high hydrostatic pressure (HHP) and cold plasma (CP) treatments along with different concentrations of antioxidants (SO_2 and glutathione) on the chemical and sensory characteristics of red and white wines during 12 months of bottle aging. The following Figures 5 and 6 illustrate wine variations and process conditions of HPU, HHP and CP techniques used for investigation of their long-term impact. The differences in experimental wines reffer to the concentration of antioxidants used (Table 2).

Table 2. The wine variations used in this part of study.

		Free SO_2 (mg/L)	
Variations	Description	Red wine	White wine
Control	Untreated wine with standard SO_2	25	45
↓SO_2	Wine with low SO_2	10	25
↓SO_2+GSH	Wine with low SO_2 and 20 mg/L of glutathione	10	25
↔SO_2	Wine with standard SO_2	25	45

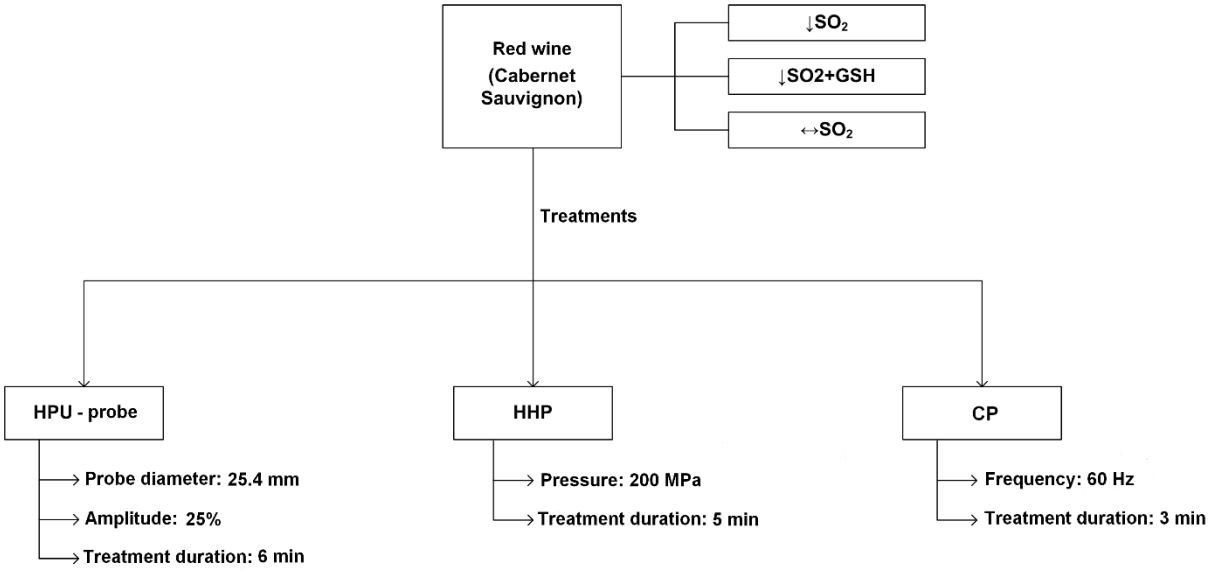

Figure 5. Red wine variations and selected process conditions of HPU, HHP and CP techniques.

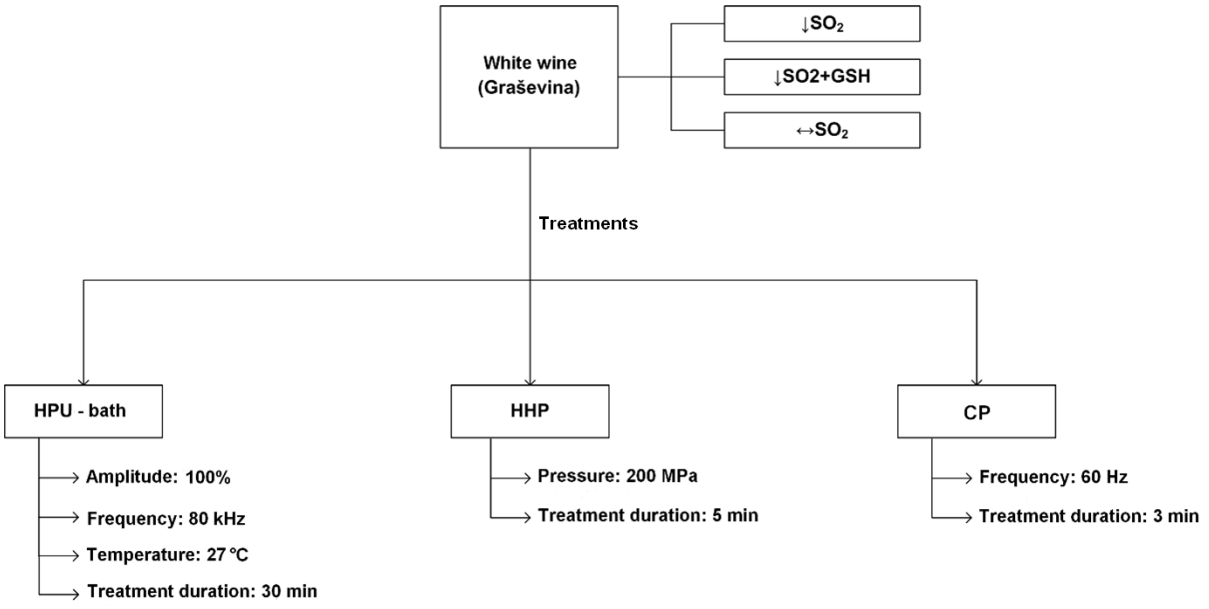

Figure 6. White wine variations and selected process conditions of HPU, HHP and CP techniques.

4.2.1. Impact of high power ultrasound and antioxidants addition (SO_2 and glutathione) on the quality of red and white wines during 12 months of aging

4.2.1.1. Phenolic, chromatic and aroma composition

In PAPER 1 (Lukić et al., 2019a), the effect of HPU treatment in combination with antioxidants (SO_2 and glutathione) on the phenolic, chromatic and aroma composition of red wine during 6 months of bottle aging is presented (Table 5). A remaining unpublished data for 12 months of aging are shown in Table S1 (APPENDIX 1). Briefly, these results demonstrated that both HPU and antioxidants treatments slightly affected chemical composition of red wine during 12 months of aging. Namely, most of phenolic and aroma compounds in analyzed wine samples decreased during observed period of aging, while chromatic characteristics increased. Respectively, these changes were evidently enhanced after applying HPU to wine (PAPER 1 - Table 5). Namely, HPU treated samples were characterized by lower concentrations of total phenolics, total and individual anthocyanins, and total flavan-3-ols compared to untreated wine. Furthermore, the addition of higher concentration of antioxidants (SO_2 and GSH) slowed the reduction in concentrations of aforementioned compounds. Similar effect occurred with lightness (L*) and total fatty acids. However, there was no statistically significant difference found among treated samples in the rest of chromatic characteristics, total tannins, total esters, total higher alcohols and total terpenes after 6 months of aging. Detail information on storage stability and changes in the chemical composition of red wine processed by HPU is well explained in PAPER 1. Additionaly, data from Table S1 (APPENDIX 1) showed that a decrease in concentration of phenolic and aroma compounds continued after 12 months of aging independently of the treatment applied, while no greater change was found in chromatic composition. Moreover, in HPU treated samples were observed lower concentrations of most phenolic (except total tannins) and aroma compounds compared to untreated wine, which suggests that HPU slightly accelerated certain chemical changes during aging. Also, significant differences can be observed in lightness (L*), redness/greenness (a*) and yellowness/blueness (b*) among the different treatments of the wine samples. The observed changes were particularly pronounced in wine with low SO_2 concentration, while aging with higher concentration of SO_2 and GSH allowed to produce wines with significantly higher concentration of analyzed compounds as well as chromatic characteristics (APPENDIX 1 – Table S1). In summary, this research highlighted that HPU treatment is able to assist and accelerate the aging process of wine.

Data on the impact of HPU combined with antioxidants (SO_2 and GSH) treatments on the chemical composition of white wine during 12 months of aging can be found in Table 5 of PAPER 2 (Lukić et al., 2020a). In this study, the observations on the phenolic, chromatic and aroma composition of white wine were conducted even over a longer period of aging in the bottles, namely 18 months. Briefly, a constant decrease of total phenolics and total flavan-3-ols was detected throughout the all period of aging independently of the treatment applied, while an increase was observed in the concentration of total phenolic acids. The observed changes in the phenolic composition were accompanied with the decrease in chromatic characteristics L* and a*, whereas b* and C* slightly increased. Similarly, the loss of total esters (except ethyl acetate and diethyl succinate) and total terpenes was determined in all presented wines throughout monitored period, while the concentration of total fatty acids and total higher alcohols slightly increased. When discussing the influence of HPU treatment, sonicated wines compared to control wine were slightly lower in the concentration of both total and individual phenolic and aroma compounds along aging period, while there was no significant impact observed on the chromatic composition. These significantly lower concentrations of analyzed compounds were clearly evident in wine with low SO_2 concentration. Analysis also showed no significant difference in the concentration of phenolic and aroma compounds between sonicated wine samples with higher SO_2 and GSH levels (PAPER 2 – Table 5). Detailed information on the influence of HPU and antioxidants on the quality characteristics of white wine during aging are provided in PAPER 2. Finally, from these results can be concluded that combination of HPU and low SO_2 and GSH treatment could be a good alternative to decrease SO_2 addition in wine production.

4.2.1.2. Physicochemical characteristics

Generally, from the results in Figure 3 of PAPER 3 (Lukić et al., 2019b) it can be observed a decreasing trend in the concentration of dissolved oxygen, free and total SO_2 in all presented wines during 12 months of aging, independently of treatments applied. Furthermore, data for ultrasonic probe treatment of red wine (PAPER 3 – Figure 3a-c) showed that HPU treated sample with standard SO_2 concentration had slightly higher reduction in dissolved oxygen and free SO_2 compared to untreated wine. On the other hand, sonicated samples with low SO_2 concentration were characterized by slightly lower reduction in aforementioned parameters after 12 months of aging. These observations are substantiated by calculated percent reduction between initial (0 months) and final (12 months) values of analyzed physicochemical characteristics for each wine, respectively (data provided in the Results and discussion section

of PAPER 3). Similar trends can be observed in the case of ultrasonic bath treatment of white wine (PAPER 3 – Figure 3d-f). Compared to untreated wine, HPU treated sample with standard SO_2 concentration showed slightly higher reduction in free and total SO_2, while slightly lower reduction in dissolved oxygen and total SO_2 was observed in those samples with low SO_2 concentration after 12 months of aging. The observed results might be explained by the degassing effect as well as free radicals produced by ultrasound, which is well explained in PAPER 3. When discussing the effect of antioxidants used in these experiments, higher concentration of SO_2 proved to be more protective than glutathione as these samples showed the lowest concentration of dissolved oxygen. This confirms that the consumption speed of the oxygen in wine is dependent on the concentration of reducing agents, primarily SO_2. More details on this can be found in PAPER 3.

4.2.1.3. Sensory characteristics

The influence of combined HPU and antioxidants (SO_2 and GSH) treatments on the sensory characteristics of red and white wines during 12 months of aging are shown in Figure S7 (APPENDIX 1). The preformed sensory evaluation of wine samples demonstrated that at the beginning of storage there were no great differences in presented red and white wines as all these samples were evaluated with the highest scores (9 = "like extremely" and 8 = "like very much"). After 3 and 6 months of aging the numbers assigned to wine samples were slightly lower (in the range from 7 = "like moderately" to 8 = "like very much"). When sensory analysis was conducted after 12 months, the assigned average scores for presented wines were the lowest throughout whole observed period. Particularly, this was evident in white wine samples with low SO_2 concentration as they were rated with 6 = "like slightly" (Figure S7B). In fact, it is clearly seen that higher concentration of SO_2 resulted in higher scores in both red and white wine samples, which indicates its better protective effect on sensory attributes (color, odor and taste) during aging compared to glutathione. Moreover, it seems that combined HPU and antioxidants treatments influenced the sensory characteristics of white wine slightly more than those of red wine, since these samples showed slightly lower hedonic ratings. The main reason is probably that white wines contain lower concentrations of phenolic compounds and thus they are more susceptible to oxidation compared to red wines. Also, in view of the fact that ultrasound effects arise from the cavitation phenomenon, during which highly reactive species are formed, organic and inorganic compounds in the solution undergo oxidation or reduction processes depending on their reactivity (Babu et al., 2016).

4.2.2. Impact of high hydrostatic pressure and antioxidants addition (SO_2 and glutathione) on the quality of red and white wines during 12 months of aging

4.2.2.1. Phenolic, chromatic and aroma composition

Detailed information on the effect of combined HHP and antioxidants (SO_2 and glutathione) treatments on the phenolic, chromatic and aroma composition of red and white wines during 12 months of bottle aging is well explained in PAPER 5 (Lukić et al., 2020b). The results for red wine are presented in Figures 2 and 3, while those of white wine in Figures 4 and 5. Briefly, the general observation to emerge from given data was that HHP treatment affected most of phenolic compounds (except total tannins) of red wine after 12 months of aging. Contrary, no great HHP impact was found in red wine chromatic and aroma composition after observed period, except in parameters L*, a* and total fatty acids (PAPER 5 – Figures 2-3). As regards white wine, HHP treatment showed an effect on aroma composition after 12 months of aging, whereas most of phenolic compounds (except total phenolics) and chromatic characteristics (except L*) were not influenced (PAPER 5 – Figures 4-5). These observations can be explained by the fact that HHP processing possesses the effect of changing the equilibrium of chemical reactions and consequently accelerating the aging process of wine. Namely, Le Chatelier's principle describes how an increase in pressure favors those reactions, which tend to reduce the volume (Martinez-Monteagudo and Saldana, 2014). Regarding the effect of antioxidants used, the results demonstrated that higher concentration of SO_2 and glutathione resulted in higher concentration of phenolic and aroma compounds in both HHP treated red and white wine samples, respectively. Taken together, these findings suggested that HHP treatment in combination with glutathione and lower concentration of SO_2 can potentially complement the multiple action of SO_2 and in this way preserve wine from deterioration.

4.2.2.2. Physicochemical characteristics

The results of the effect of combined HHP and antioxidants (SO_2 and GSH) treatments on the physicochemical characteristics of red and white wines during 12 months of aging are presented (PAPER 3 - Figure 2) and in detail discussed in PAPER 3 (Lukić et al., 2019b). The given results referred to the concentration of dissolved oxygen, total and free SO_2 in control (untreated) and treated red (Figure 2a-c) and white (Figure 2d-f) wine samples determined after 0, 3, 6 and 12 months of aging in the bottles. Briefly, from obtained results, it can be seen the decrease of dissolved oxygen and SO_2 concentrations in all presented wine samples,

independently of treatments applied. A significant drop in analyzed physicochemical characteristics was mostly evident immediately after bottling and in the first 3 months of aging, while after 6 and 12 months the reduction rate was slowed down. When comparing red and white wines, it seems that these changes occurred slightly faster in red wine. Namely, it has been determined that the oxygen absorption capacity of a wine is in positive correlation with its total phenolic content (Karbowiak et al., 2009). Regarding the effect of HHP, slightly higher decrease of total and free SO_2 was found in HHP treated red and white wines with standard concentration of SO_2 compared to untreated (control) wines. Moreover, the results revealed the effect of used antioxidants, primarily higher concentration of SO_2 led to lower concentration of dissolved oxygen in wine samples. This also highlighted the greater protective effect of sulfur dioxiode addition in wine compared to glutathione. Already well-known protection mechanisms of SO_2 against oxidation are responsible for such effect in wine.

4.2.2.3. Sensory characteristics

Generally, from the results of sensory analysis presented in Table 3 of PAPER 5 (Lukić et al., 2020b), it can be observed that HHP treatment along with antioxidants addition (SO_2 and GSH) did not have a negative effect on the sensory characteristics (color, odor and taste) of red and white wines during 12 months of aging in the bottles. Namely, all presented red and white wine samples were rated with 7 = "like moderately" and 6 = "like slightly" after 12 months of aging. Among HHP treated samples, the lowest average scores were assigned to those red and white wines with low SO_2 concentration during observed aging period. Again, as already demonstrated for other quality characteristics, higher concentration of antioxidants (SO_2 and GSH) showed better protective effect on wine sensory attributes. Moreover, slightly lower ratings in sensory characteristics were determined for white wine in regard to red wine. A more detailed discussion is provided in the Results and Discussion section of PAPER 5.

4.2.3. Impact of cold plasma and antioxidants addition (SO_2 and glutathione) on the quality of red and white wines during 12 months of aging

The results of the long-term effect of combined cold plasma and antioxidants (SO_2 and GSH) treatments on the chemical and sensory characteristics of red and white wines are provided in APPENDIX 3 as supplementary data. The obtained results of chemical composition were analyzed with statistical software (Statistica, Vers. 10.0, StatSoft Inc., USA). One-way

ANOVA and post-hoc Tukey's HSD test were used to evaluate the significant differences in aged wines.

4.2.3.1. Phenolic, chromatic and aroma composition

In supplementary Tables S4 and S5 of APPENDIX 3 are shown the results of phenolic, chromatic and aroma composition of red and white wines treated by CP and antioxidants (SO_2 and GSH) during 12 months of aging. Regarding red wine, it can be seen a decreasing trend in the concentration of total phenolics, total anthocyanins, total free anthocyanins and total flavan-3-ols during observed period, independently from treatments applied (Table S4). After 6 and 12 months of aging, significant differences ($p < 0.05$) among the different treatments were observed indicating that CP treatment affected both total and individual phenolic compounds, except total flavan-3-ols. Particularly, after 12 months of storage CP treated samples showed significantly lower concentration of phenolic compounds compared to untreated (control) wine (Table S4). Additionally, the significant differences in the phenolic composition of CP treated samples were found depending on the concentration of antioxidants used. Namely, wine with low SO_2 concentration had the lowest concentration of analyzed phenolic compounds. Moreover, the addition of GSH resulted in slightly higher concentration of phenolics (except total tannins) during observed period compared to wine with low SO_2 concentration aged without GSH. However, these differences were diminished in the case of total flavan-3-ols after 6 and 12 months of aging (Table S4). Regarding white wine, the decrease of total phenolics and total flavan-3-ols was observed over time, whereas the concentration of total phenolic acids increased (Table S5). Along storage period, CP treated samples showed lower concentration of both total and individual phenolic compounds compared to control wine. This was particularly evident in the case of total phenolics and total flavan-3-ols after 12 months of aging, while there was no significant difference in the concentration of total phenolic acids (Table S5). The relation of plasma effect and observed changes in phenolic composition of both red and white wines is well explained in PAPER 6 (Lukić et al., 2019c). Briefly, the observed changes are probably the result of the action of CP technique, such as the formation of hydrogen peroxide, hydroxyl and oxygen radicals (chemical effect), the creation of shock waves and UV radiation (physical effect), and electrical effect (Vukušić et al., 2016; Mandal et al., 2018). Also, after 6 months of aging, the statistical analysis showed a significant difference ($p < 0.05$) among applied antioxidants (SO_2 and GSH) treatments of white wine in both total and individual phenolics (Table S5). After 12 months, these differences were

observed in total phenolics, while in the case of total flavan-3-ols they only referred to different concentration of SO_2 in treated wines. In addition, the lowest concentrations of both total and individual phenolic compounds were found in CP treated wine with low SO_2 concentration. However, there was no great difference in total phenolic acids and total flavan-3-ols among treated samples aged with GSH and those with only low SO_2 concentration.

When discussing the chromatic composition of presented red wine samples during bottle aging (Table S4), the results showed a slight increase in the chromatic characteristics in the first 6 months followed by a slight decrease after 12 months of aging. Furthermore, it can be observed that there was no significant difference ($p < 0.05$) in the chromatic characteristics between untreated (control) and CP treated standard SO_2 wines. This indicates that CP treatment did not affect the color of red wine. Regarding the antioxidants effect, significant differences were found in analyzed color parameters between CP treated red wine samples during observed period. Primarily, higher concentration of SO_2 resulted in higher values of all chromatic characteristics, whereas the effect of GSH was most pronounced in the case of parameters b* and C* after 12 months of aging. These samples had the value of the total color difference (ΔE*) between 3 and 6 CIELab units after 12 months, while wine with low SO_2 concentration showed significantly higher ΔE* value around 12 CIELab units. From the results given for the white wine (Table S5), it can be seen that lightness (L*) slightly decreased during 12 months of aging, while the rest of the chromatic characteristics, a*, b* and C*, slightly increased in the presented wine samples. With respect to the effect of CP treatment, there was no significant influence of this technique on the color of white wine after 12 months. As regards the impact of SO_2 and GSH treatments, there was no significant difference ($p < 0.05$) among the different treatments of wine samples, except in parameter L*, which was especially evident in wine sample with low SO_2 concentration after 6 and 12 months of aging. Namely, higher concentration of SO_2 and GSH resulted in higher values of lightness. These treatments resulted in ΔE* values between 1 and 2 CIELab units, while for the combined CP and low SO_2 treatment the value was 3.5 after observed period.

In relation to aroma composition of red wine (Table S4), the results showed an increase in the concentration of total esters and total higher alcohols, while the concentration of total fatty acids and total terpenes decreased in all wine samples during 12 months of aging. Although esters are known to decrease during aging, the observed opposite trend is due to increase in the content of ethyl acetate and diethyl succinate, whereas other individual aroma compounds included in sum of total esters in fact decreased. Moreover, slightly lower concentration of

almost all analyzed groups of aroma compounds (except total higher alcohols) was found in CP treated samples in comparison with control (untreated) wine during observed period. As discussed in the previous section (4.1.3. Influence of cold plasma on the quality characteristics of red and white wines), the CP effect on wine aroma can be attributed to electrohydraulic cavitation phenomenon, shock waves and the formation of free radicals. Briefly, these mechanisms can act simultaneously and result in loss of aroma compounds. In the first 3 months, the significant differences were mainly observed in the case of total higher alcohols, while after 6 months these differences were determined in total esters, total fatty acids and total terpenes, respectively. However, after 12 months of aging, there was no significant difference in the concentration of total esters compared to control, indicating that CP treatment did not affect this group of aroma compounds (Table S4). Further, regarding the effect of antioxidants (SO_2 and GSH), the lowest concentration of total esters, total fatty acids and total terpenes was found in wine sample with low SO_2 concentration. Although some differences existed in aroma composition of treated samples in the first 6 months of aging, after 12 months there was no great distinction in the concentration of total fatty acids and total terpenes among standard SO_2 and low SO_2 and GSH samples (Table S4). Regarding the changes in aroma composition of white wine (Table S5), it can be observed that the concentration of total esters, total fatty acids and total terpenes decreased in all wine samples during 12 months of aging, while at the same time the concentration of total higher alcohols increased. Furthermore, CP treated samples showed slightly lower concentrations of almost all aroma compounds (except total higher alcohols) compared to untreated wine during observed period. Immediately after CP treatment, there were significant differences in all analyzed groups of aroma compounds, while after 3 months these changes continued only in the case of total higher alcohols and total fatty acids (Table S5). Additionally, after 6 and 12 months the effect of CP treatment was significant in the case of total esters, total higher alcohols and total terpenes, while there was no great difference in the concentration of total fatty acids compared to control sample (Table S5). During aging period, the clear difference can be seen among the different treatments of wine samples that is greatly conditioned by addition of antioxidants (SO_2 and GSH). Namely, the highest concentration of analyzed aroma compounds (except total higher alcohols) can be found in a sample with standard SO_2 concentration, following by a sample with low SO_2 aged with GSH and lastly in a sample with only low SO_2 (Table S5). These changes were particularly noticeable after 6 and 12 months of aging. Generally, it can be said that higher concentration of antioxidants (SO_2 and GSH) showed more protective effect on wine aroma.

4.2.3.2. Physicochemical characteristics

The results of the long-term impact of the combined CP and antioxidants (SO_2 and GSH) treatments on the physicochemical characteristics of red and white wines during 12 months of aging are presented in supplementary Figure S11 of APPENDIX 3. The presented graphs referred to the concentration of dissolved oxygen, total and free SO_2 in red (Figure S11A-C) and white (Figure S11D-F) wine determined after 0, 3, 6 and 12 months of aging in the bottles. As expected, it can be seen a decreasing trend in the concentration of all measured physicochemical parameters in both red and white wines during observed period of storage, independently of treatments applied. Especially, it was evident a rapid decrease of dissolved oxygen in the first 3 months of aging in all treatments performed, and the values were quite similiar or slightly higher than of those observed in untreated (control) wine. Prior to bottling, the average concentrations of dissolved oxygen in wines were around 1.2-2.1 mg/L, while at the end of storage the concentrations amounted around 0.4 mg/L (Figure S11C and F). Since an invasive method for oxygen measurement was used, ie a dipping probe with the sensor was immersed in the bottle, it is likely that a certain amount of oxygen was dissolved in the wine during this procedure. Namely, when oxygen comes in the contact with wine, it dissolves very quickly and reacts with phenolic compounds to form highly reactive chemical forms such as quinones, free radicals and hydrogen peroxide which further stimulate oxidation reactions (Waterhouse and Laurie, 2006; Dimkou et al., 2013). As a result, numerous modifications occur in the wine, such as a reduction in the concentrations of dissolved oxygen and SO_2. When discussing the effect of CP treatment on both wines, no great difference among untreated and CP treated samples with standard SO_2 concentration was observed for all analyzed parameters, indicating that CP did not affect the SO_2 and dissolved oxygen concentrations during observed period of storage (Figure S11). When comparing all variations of wine samples regardless of CP treatment, the addition of antioxidants (SO_2 and GSH) did not influence in major extent the oxygen and SO_2 consumption rate in both red and white wines, since all these samples showed similar concentrations of dissolved oxygen after 12 months of aging. However, based on determined values for free SO_2 in presented wine samples, we can conclude that better protected wines were those aged with a higher concentration of SO_2 (Figure S11).

4.2.3.3. Sensory characteristics

The results of the sensory analysis are presented in supplementary Figure S12 of APPENDIX 3. On given graphs are presented the scores assigned to red (Figure S12A) and white (Figure S12B) wines treated by combined CP and antioxidants (SO_2 and GSH) treatments and aged for 12 months. According to the nine-point hedonic scale method, in the first 3 months, both red and white wines were evaluated with the highest scores (in the range from 6= "like slightly" to 8 = "like very much"), while after 6 and 12 months the ratings were much lower (in the range from 5="neither like nor dislike" to 7="like moderately"). Regarding the antioxidants effect, the best protection was achieved with higher SO_2 concentration, since these samples were marked with the highest scores (Figure S12). Additionally, the samples with lower SO_2 concentration as well as with GSH were described as "oxidized" and "reduced" wines. Moreover, slightly lower hedonic ratings were assigned to white wine samples over observed period of storage, suggesting that applied treatments influenced slightly more the sensory characteristics of white wine compared to those of red wine. Regarding the application of CP in food processing, the non-thermal nature of this technique has been shown to protect the nutritional and sensory characteristics of fruit juices and help to extend their shelf life (Pankaj and Keener, 2018). On the other hand, the influence of CP on the sensory characteristics of wine has not been investigated so far. The research of Križanović et al. (2018) showed that after the treatment of red wine with cold plasma, larger changes occur compared to the treatments with high hydrostatic pressure and ultrasound. First of all, same authors pointed out that there is a negative effect of CP on the sensory characteristics of wine. It follows that, as in the case of any other technology, it is necessary to pay attention to the selection of appropriate process parameters since inadequate treatments can lead to negative sensory changes and impairment of the quality of treated wine.

5. CONCLUSIONS

Based on the investigation conducted in this study, the following conclusions were drawn.

- All three applied non-thermal techniques (HPU, HHP and CP) influenced the chemical composition of both red and white wines.
- Higher operating conditions of applied HPU, HHP and CP treatments showed less favorable effect on the phenolic and aroma composition as well as sensory characteristics of treated red and white wines.
- Regarding the short-term effect of HPU, treatments with ultrasonic bath showed that especially higher frequency and bath temperature resulted in lower values of phenolic compounds and chromatic characteristics in HPU treated red wine, while no clear trend was established in the case of aroma composition. Particularly, higher bath temperature affected more the aroma composition of white wine, especially total esters and total higher alcohols, and to a lesser degree phenolic composition.
- In HPU treatments with ultrasonic probe, a smaller probe diameter along with higher amplitude/longer treatment duration resulted in lower concentrations of phenolic and aroma compounds in both red and white wines. However, no great changes were observed regarding chromatic and physicochemical characteristics. Also, a lighter and less negative effect of this technique was determined on the sensory characteristics of white wine compared to red wine.
- Artificial neural networks (ANNs) proved to be a good approach for modeling and prediction of HPU processing of wine, where best ANN predictions were achieved for chromatic characteristics of red wine and for total flavan-3-ols, total esters and total higher alcohols of white wine.
- Regarding the short-term effect of HHP, only slight changes occurred in the phenolic, chromatic and aroma composition of both red and white wines. These changes were more pronounced at higher pressures and longer treatment durations, where pressure had a greater impact on analyzed parameters.
- Moreover, HHP did not affect physicochemical characteristics of treated wines, primarily the concentration of SO_2. Also, HHP treatments did not have any negative effect on the sensory characteristics of red and white wines.
- As for the short-term effect of CP, the changes manifested as reduction in most of phenolic and aroma compounds, as well as in the concentrations of oxygen and SO_2. Additionally,

there was no significant impact on the chromatic characteristics of both red and white wines. The exception was a slight increase of phenolic acids and flavan-3-ols in CP treated white wine, which indicated that this technique might be used in improving the oxidative stability of wine and consequently the wine quality during aging.

- However, CP negatively modified the sensory characteristics of both red and white wines, especially in terms of odor and taste, immediately after treatments. All these changes were more pronounced at higher frequencies and longer treatment durations, where duration contributed to a larger part of total variation in analyzed parameters.
- Independently of all treatments applied (non-thermal techniques and antioxidants), a general trend for analyzed quality characteristics of red and white wines during 12 months of aging can be observed. There was a decreasing trend in the concentration of phenolic and aroma compounds (with some exceptions) of both red and white wines with time. Regarding chromatic characteristics, their increase was observed in red wine during aging, whereas white wine showed the opposite trend, primarily in the case of lightness.
- The long-term effect of HPU, HHP and CP treatments manifested primarily in a slight reduction of phenolic and aroma compounds in treated red and white wines, while no significant effect was observed in chromatic composition.
- SO_2 and oxygen concentrations did not differ drastically in HPU, HHP and CP treated red and white wines compared to those in untreated samples during aging.
- Conducted sensory evaluation demonstrated that HPU, HHP and CP along with antioxidants (SO_2 and GSH) affected more the sensory characteristics of white wine compared to red wine. But, in general, these techniques did not have a detrimental effect on the sensory characteristics of both red and white wines during 12 months of aging.
- Regarding antioxidants, higher concentration of SO_2 and GSH delayed the loss of phenolic and aroma compounds in HPU, HHP and CP treated red and white wines during aging. In particular, HPU and HHP treated wines with standard SO_2 and low SO_2 and GSH concentrations showed similar chemical composition, indicating that these techniques in combination with GSH and lower concentration of SO_2 might potentially preserve wine.
- Finally, although these techniques do not possess antioxidant properties, they could be combined with antioxidants treatments and thus help to reduce the need for SO_2 in wine production. In the future, to verify the effectiveness of this approach, it is important to carry out large-scale experiments.

- From analysis of all three non-thermal techniques on lab scale level, it can be concluded that HPU is the most affordable, cheapest and applicable technique with good impact on wine quality for achieving aforementioned. The biggest disadvantage of HHP is its high cost, whereas CP technique shown to be negative for the sensory characteristics of wine.
- Additionally, the managing of undesirable microorganisms to avoid wine spoilage needs to be fundamental for preserving wine quality. Hence, the important aspect is also the antimicrobial effect of this physical treatments on wine. Further investigations should include all these aspects to accomplish adequate overall quality of wine.

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7. AUTOBIOGRAPHY

Katarina Lukić, mag. ing., was born in Zagreb, 26th September 1992, where she finished elementary and general grammar school. She graduated at the Faculty of Food Technology and Biotechnology, University of Zagreb, in 2016, and in the same year she started to work as an assistant on the project of Croatian Science Foundation entitled "New enological tools for the reduction of sulfur dioxide and production of high-quality wine". As a part of her assistant position, she enrolled Postgraduate University Doctoral Study at the Faculty of Food Technology and Biotechnology, University of Zagreb. Currently, she is working as a research assistant in the research and higher education in the Laboratory for Technology and Analysis of Wine at the Department of Food Engineering of the same Faculty. Her scientific research is focused on application of new non-thermal techniques such as physical and chemical methods on wine as an alternative for conventional methods in terms of improvement overall wine quality and achieving satisfying microbiological stability of wine. Her working skills are primarily related to spectrophotometric methods for determining phenolic compounds and wine color, as well as work on sophisticated devices (HPLC and GC) for determining phenolic and aroma composition and sensory analysis of wine. She presented the results of her research related to the application of non-thermal techniques as an alternative to sulfur dioxide in wine production by attending numerous international conferences. To date, her research work has resulted in the publication of eight papers in highly indexed journals, six papers indexed in secondary publications and one paper in conference Proceeding. She is the winner of the annual award of the Biotechnical Foundation for the achieved results in the field of biotechnology with potential application in the economy in the academic year 2020/2021, and in 2018/2019 she was awarded with the support of the same foundation of the Faculty of Food Technology and Biotechnology, University of Zagreb.

8. SENSORY AND ANALYTICAL DATA SUPPLEMENT

Appendix 1

The sensory and analytical results of the influence of high power ultrasound on wine quality

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Figure S1-S7

Table S1

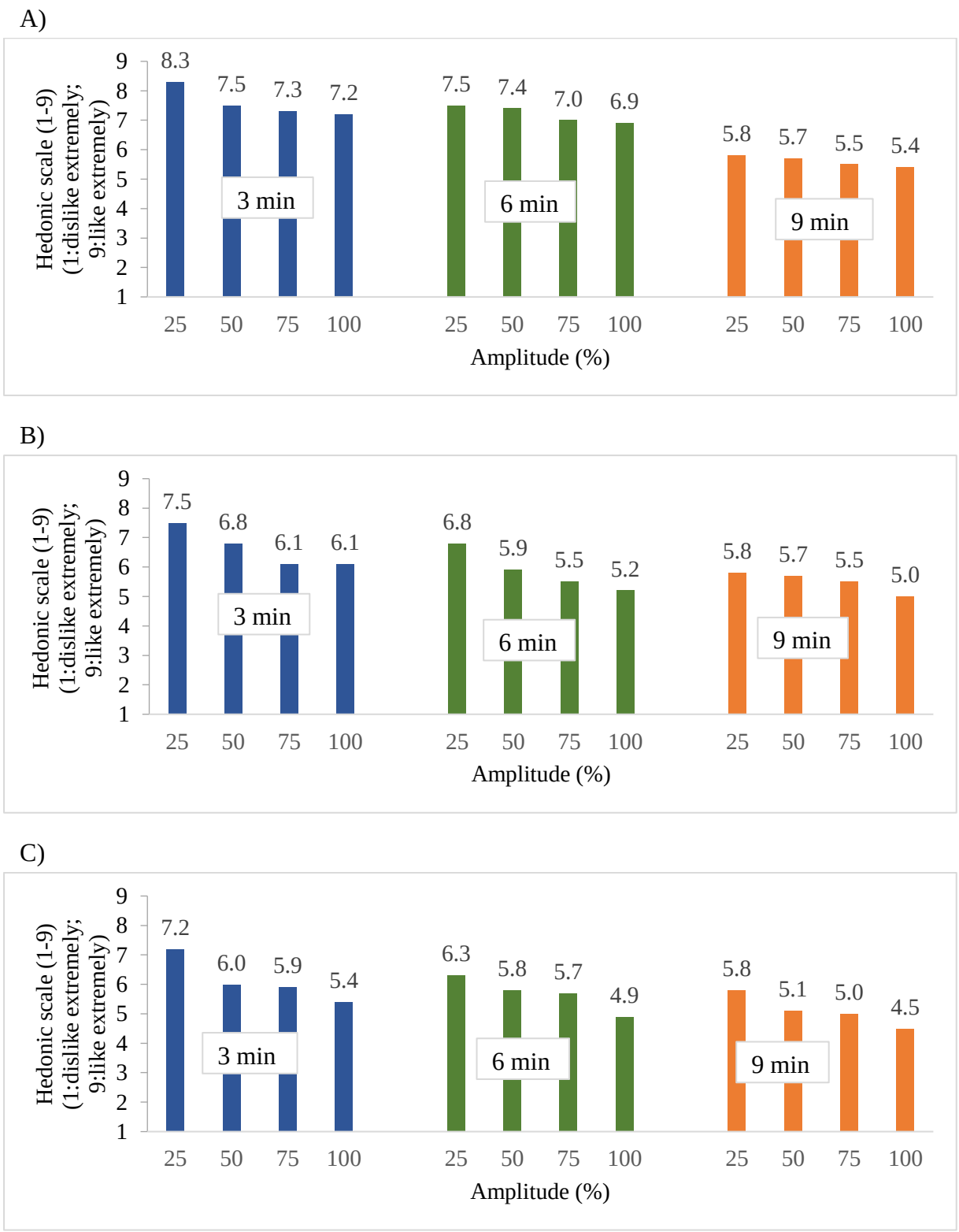


Figure S1. The influence of different high power ultrasound (ultrasonic probe) process parameters on the sensory characteristics of red wine; (A) probe diameter 25.4 mm; (B) probe diameter 19.1 mm; (C) probe diameter 12.7 mm

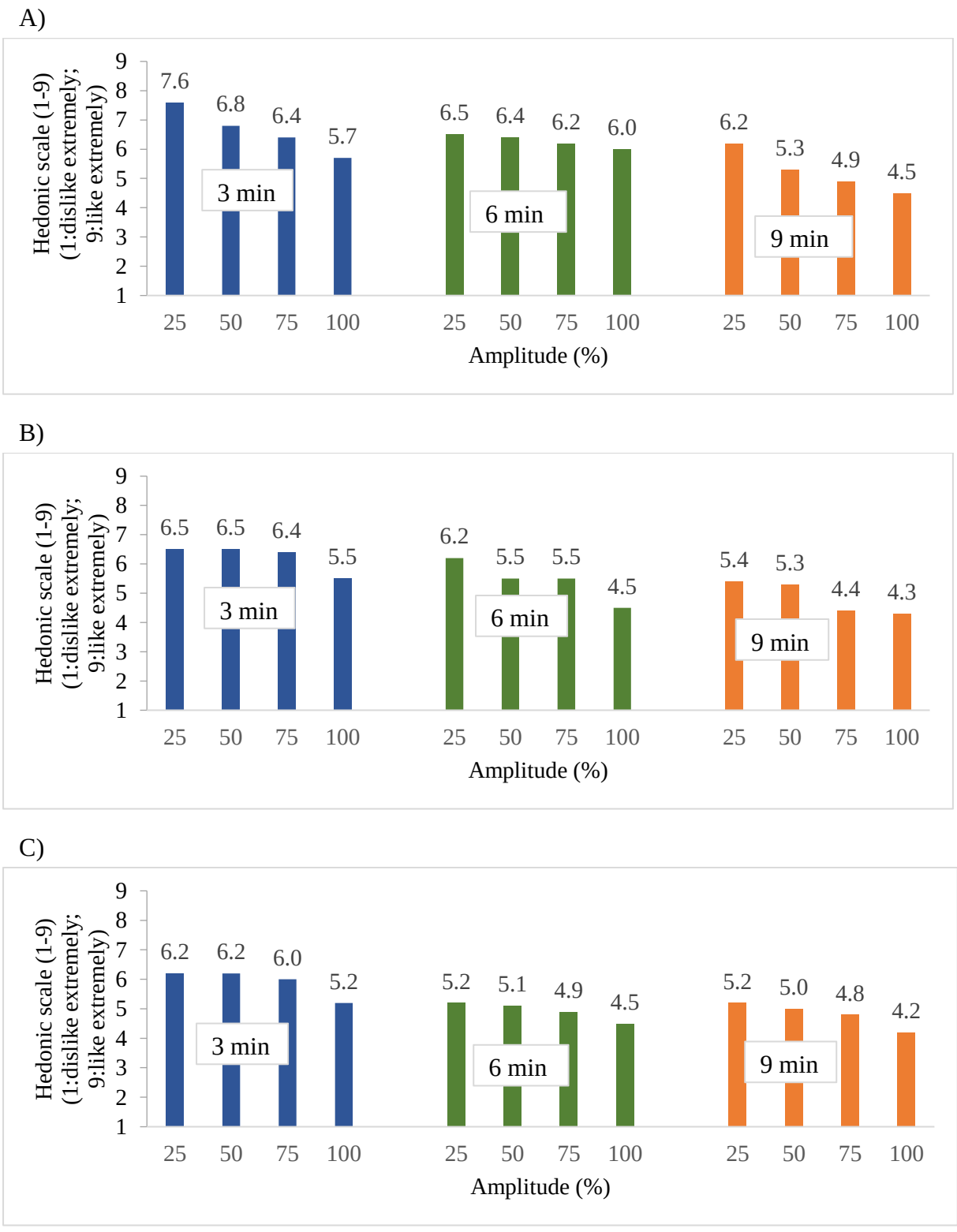


Figure S2. The influence of different high power ultrasound (ultrasonic probe) process parameters on the sensory characteristics of white wine; (A) probe diameter 25.4 mm; (B) probe diameter 19.1 mm; (C) probe diameter 12.7 mm

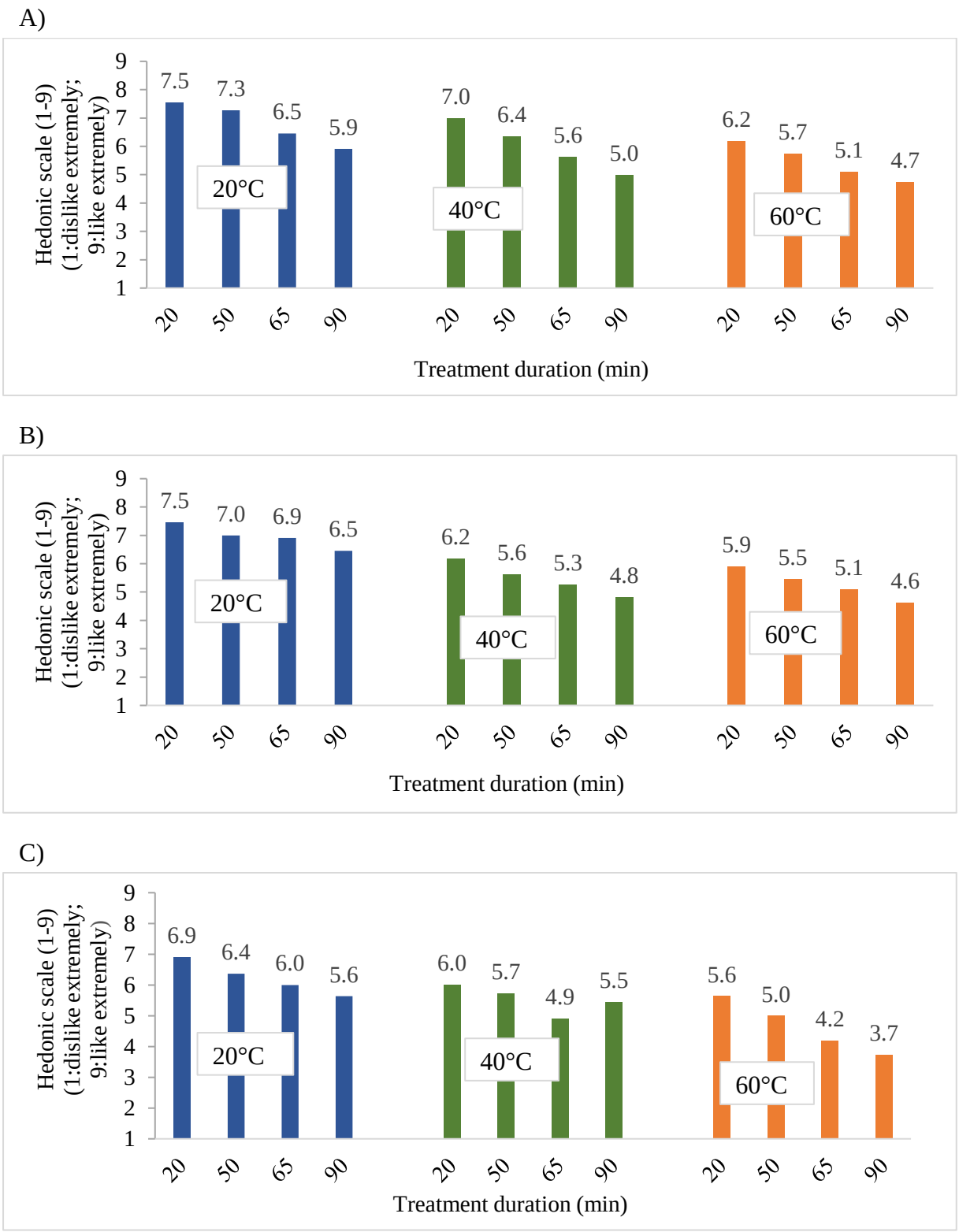


Figure S3. The influence of different high power ultrasound (ultrasonic bath) process parameters on the sensory characteristics of red wine at frequency of 37 kHz; (A) amplitude 40%; (B) amplitude 60%; (C) amplitude 100%

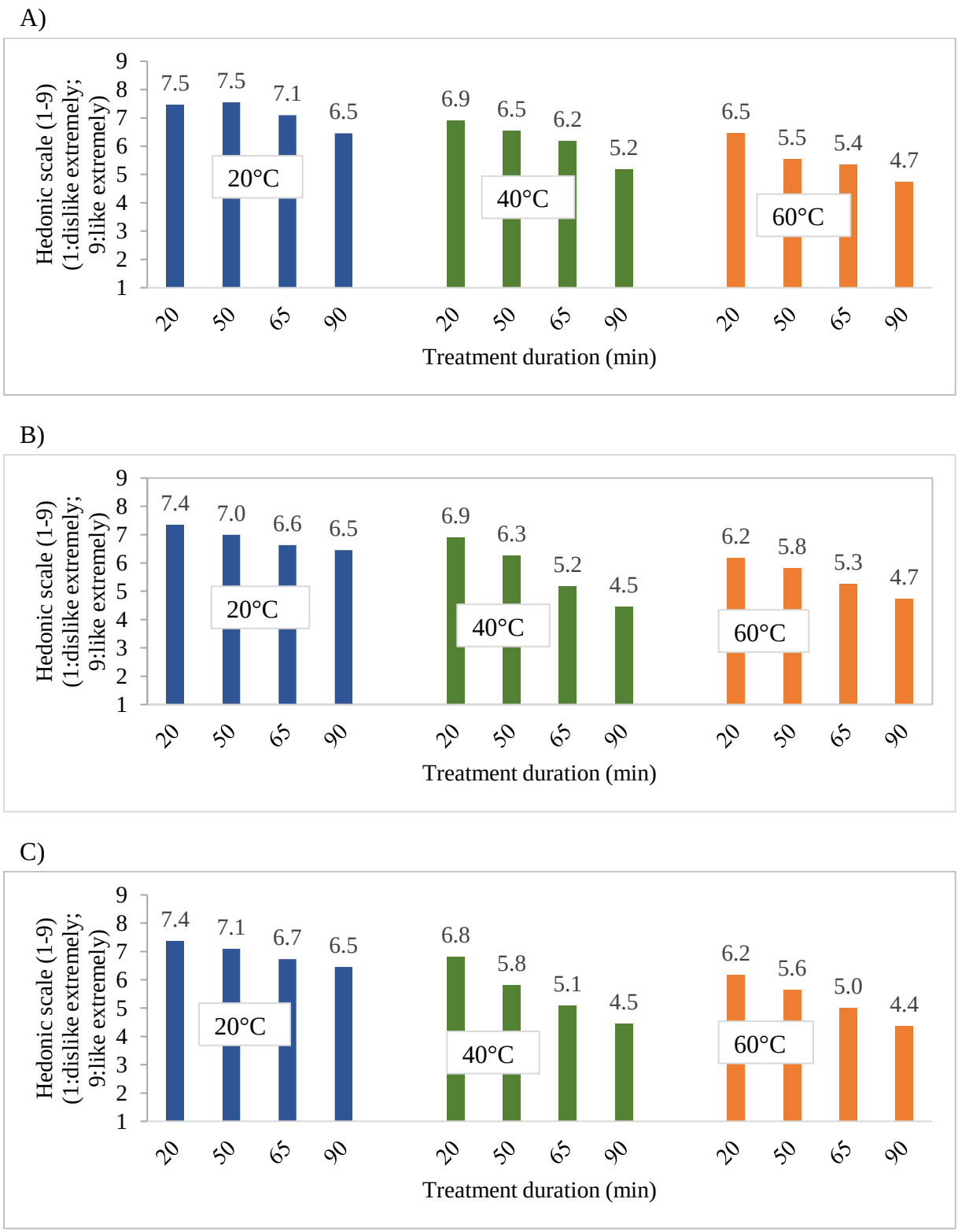


Figure S4. The influence of different high power ultrasound (ultrasonic bath) process parameters on the sensory characteristics of red wine at frequency of 80 kHz; (A) amplitude 40%; (B) amplitude 60%; (C) amplitude 100%

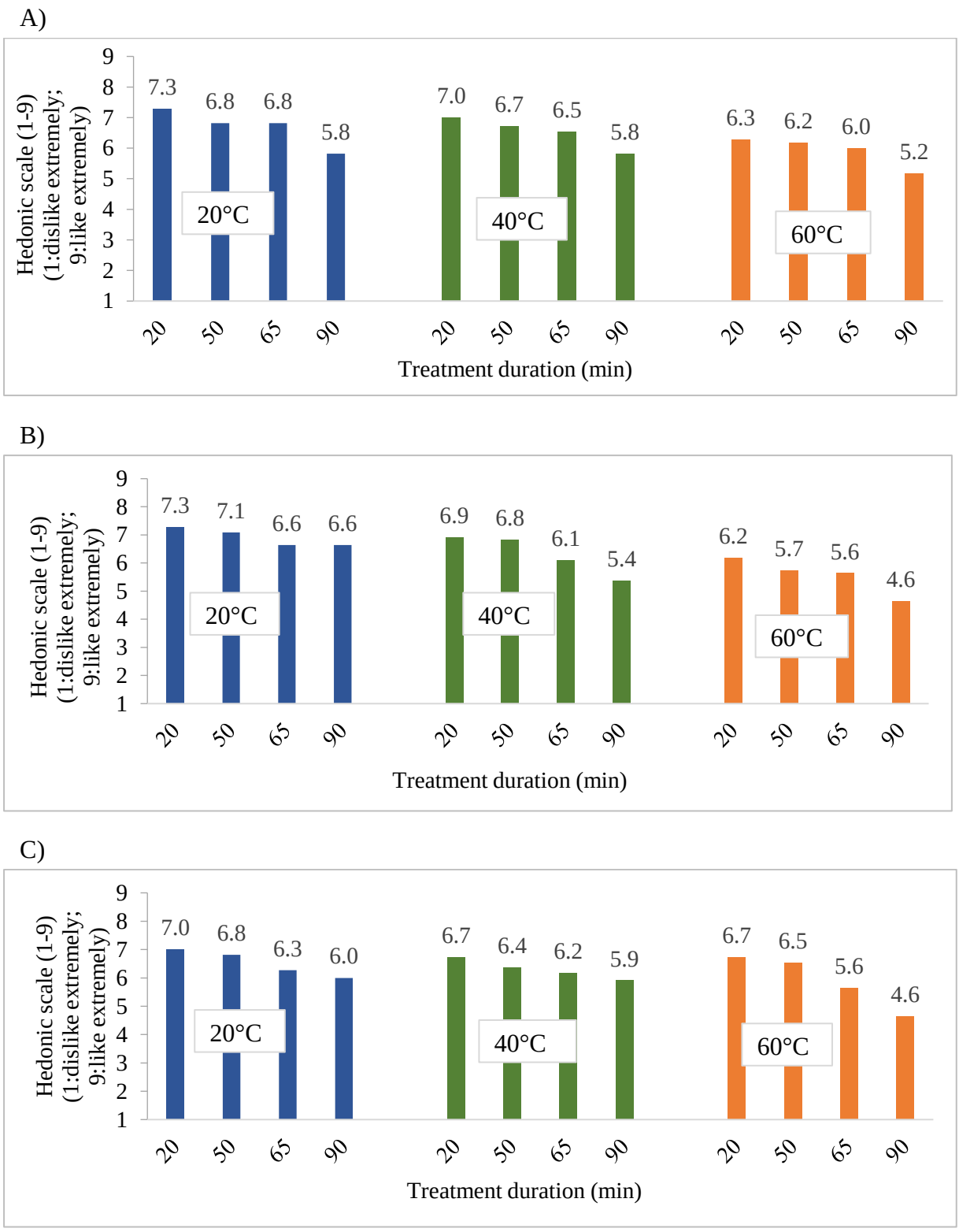


Figure S5. The influence of different high power ultrasound (ultrasonic bath) process parameters on the sensory characteristics of white wine at frequency of 37 kHz; (A) amplitude 40%; (B) amplitude 60%; (C) amplitude 100%

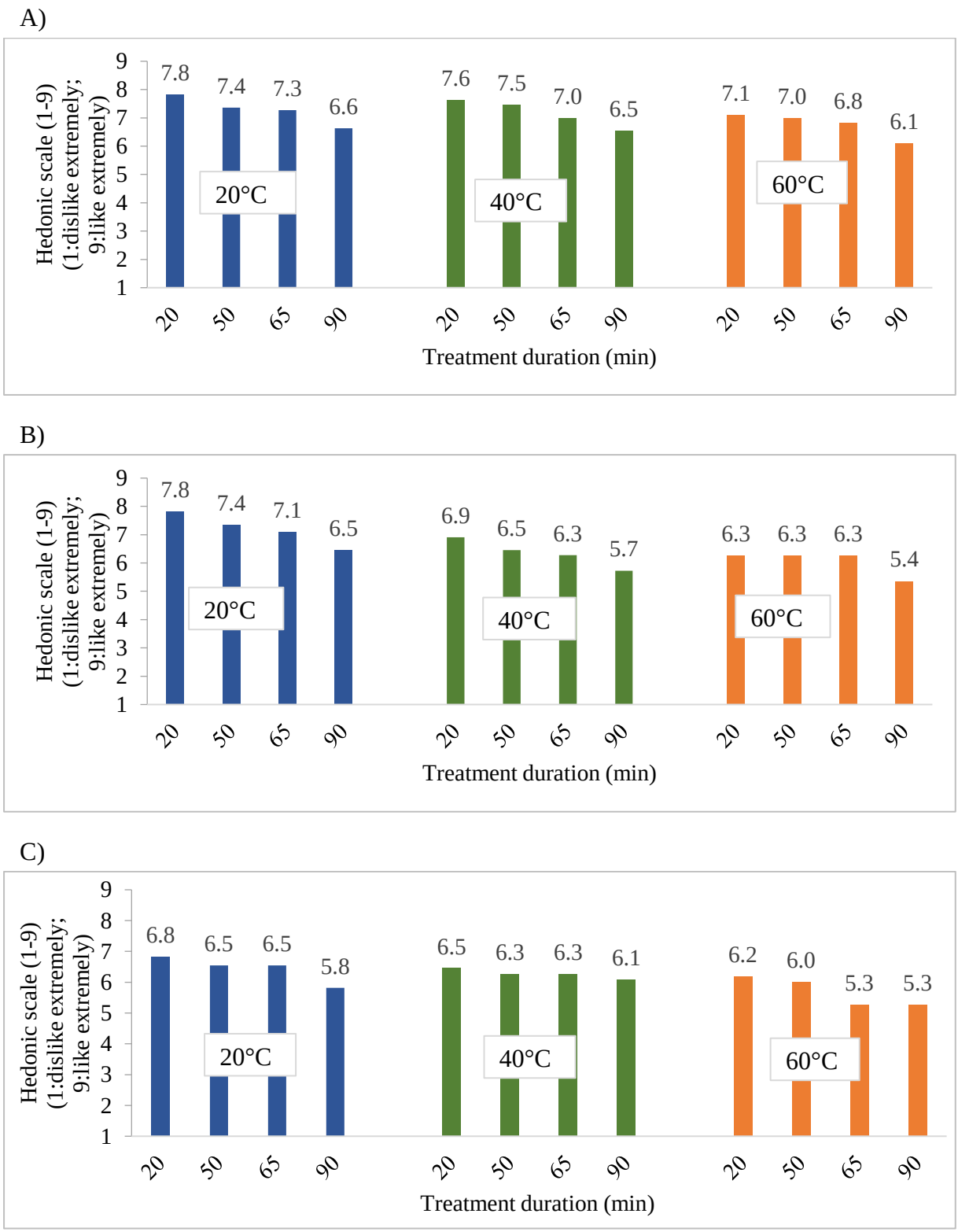
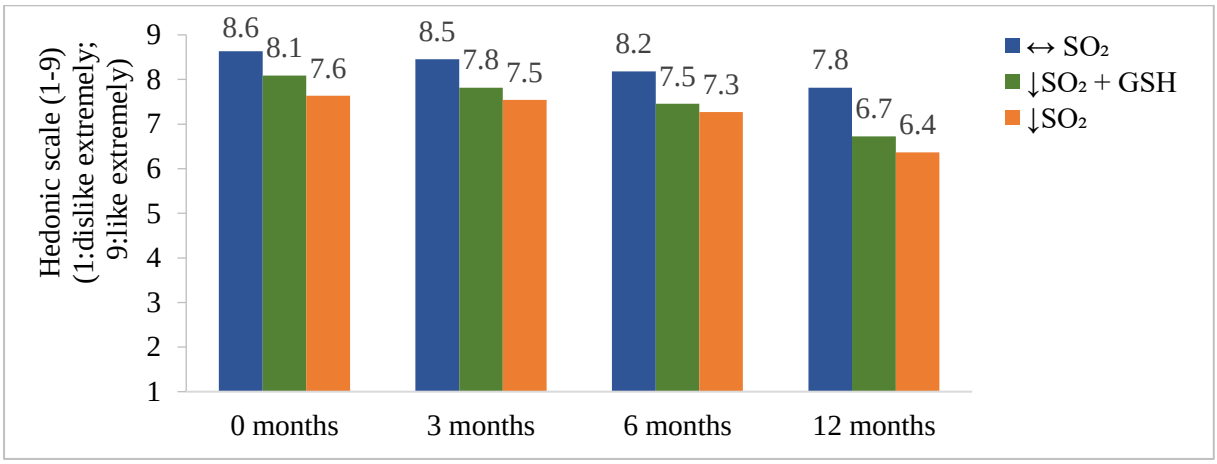


Figure S6. The influence of different high power ultrasound (ultrasonic bath) process parameters on the sensory characteristics of white wine at frequency of 80 kHz; (A) amplitude 40%; (B) amplitude 60%; (C) amplitude 100%

A)



B)

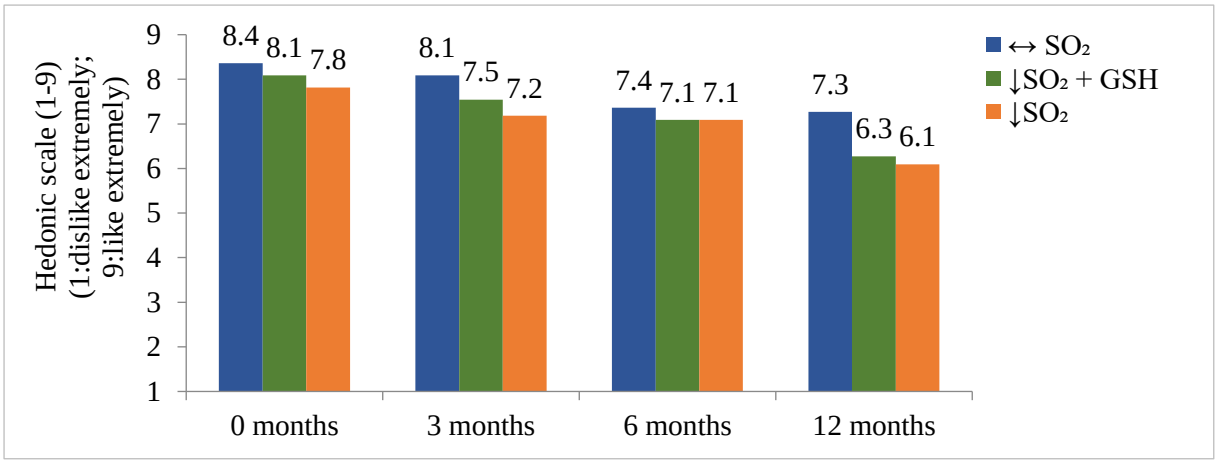


Figure S7. The effect of high power ultrasound and antioxidants addition (SO_2 and GSH) on the sensory characteristics of red (A-ultrasonic probe) and white (B-ultrasonic bath) wine during 12 months of bottle aging

Table S1. The effect of high power ultrasound and antioxidants addition (SO_2 and GSH) on the phenolic, chromatic and aroma composition of red wine Cabernet Sauvignon after 12 months of bottle aging

Analyzed characteristics	**Wine sample**			
	Control	Standard SO_2	↓SO_2 + GSH	↓SO_2
Total phenolics (mg/L)	2610.63 ± 15.03^{a}	2559.79 ± 16.20^{a}	2340.63 ± 13.88^{b}	2223.75 ± 4.12^{c}
Total anthocyanins (mg/L)	228.35 ± 2.28^{a}	216.27 ± 3.35^{b}	148.07 ± 0.40^{c}	118.21 ± 1.48^{d}
Total tannins (g/L)	2.75 ± 0.01^{a}	2.72 ± 0.01^{a}	2.69 ± 0.00^{ab}	2.64 ± 0.03^{b}
Total free anthocyanins (mg/L)	102.54 ± 2.03^{a}	74.41 ± 0.04^{b}	57.53 ± 0.37^{c}	55.51 ± 0.04^{c}
Total flavan-3-ols (mg/L)	333.75 ± 0.72^{a}	273.92 ± 2.60^{b}	253.96 ± 0.19^{c}	233.43 ± 0.07^{d}
L^{*}	26.88 ± 0.02^{a}	25.56 ± 0.13^{b}	23.81 ± 0.07^{c}	20.96 ± 0.04^{d}
a^{*}	54.13 ± 0.03^{a}	54.68 ± 0.16^{b}	53.59 ± 0.10^{c}	50.96 ± 0.07^{d}
b^{*}	40.74 ± 0.11^{a}	41.03 ± 0.18^{b}	39.02 ± 0.12^{c}	34.51 ± 0.15^{d}
C^{*}	67.75 ± 0.09^{a}	68.36 ± 0.23^{a}	66.29 ± 0.15^{b}	61.55 ± 0.14^{c}
H^{*}	0.65 ± 0.00^{a}	0.64 ± 0.00^{a}	0.63 ± 0.00^{b}	0.60 ± 0.00^{c}
ΔE*	-	1.48 ± 0.03^{c}	3.55 ± 0.10^{b}	9.16 ± 0.08^{a}
Total esters (mg/L)	25.99 ± 0.11^{a}	24.13 ± 0.74^{b}	21.40 ± 0.01^{c}	14.61 ± 0.05^{d}
Total higher alcohols (mg/L)	107.73 ± 0.66^{a}	104.02 ± 0.17^{b}	100.54 ± 0.61^{c}	96.76 ± 0.14^{d}
Total fatty acids (mg/L)	1.63 ± 0.05^{a}	1.38 ± 0.10^{b}	1.10 ± 0.03^{c}	0.87 ± 0.04^{c}
Total terpenes (μg/L)	8.81 ± 0.35^{a}	7.86 ± 0.63^{ab}	6.23 ± 0.10^{bc}	5.45 ± 0.42^{c}

Results are expressed as mean value ± standard deviation (N=6). ANOVA to compare results; different letters indicate a statistical difference between analyzed wine samples at the same time of analysis (Tukey's HSD test, $p < 0.05$).

Appendix 2

The sensory results of the influence of high hydrostatic pressure on wine quality

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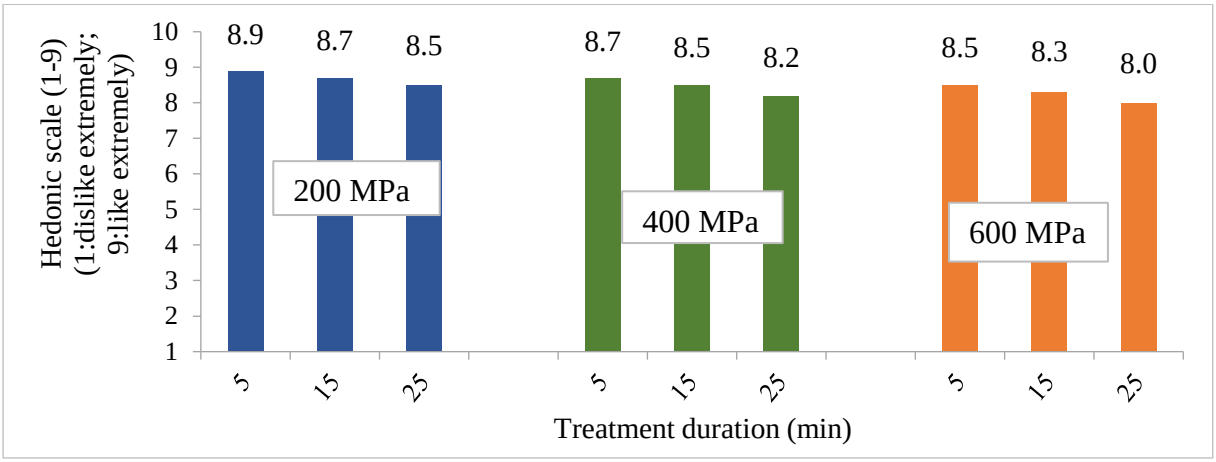
Figure S8

A)



B)

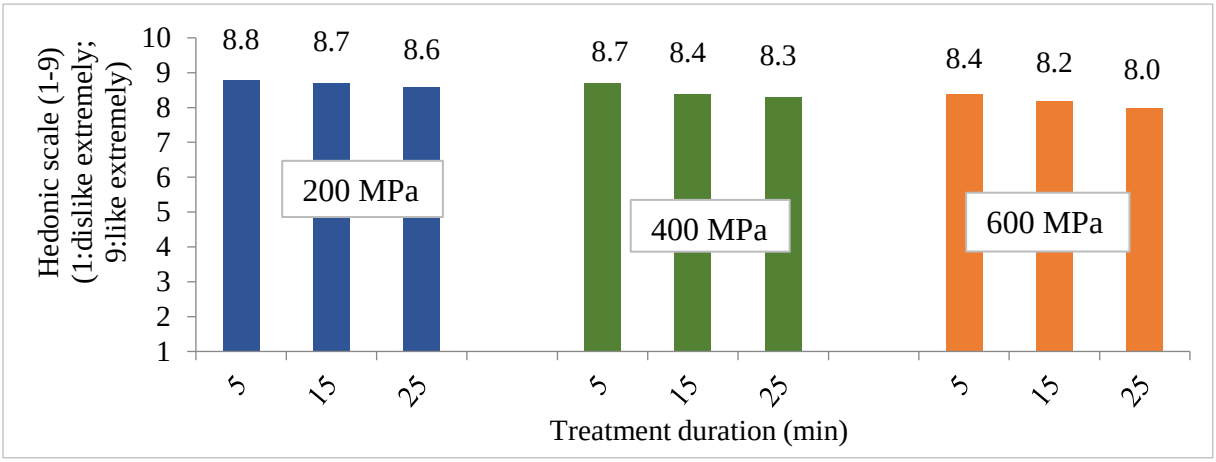


Figure S8. The influence of high hydrostatic pressure process parameters on the sensory characteristics of red (A) and white (B) wines

Appendix 3

The sensory and analytical results of the influence of cold plasma on wine quality

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Table S2-S5

Figure S9-S12

Table S2. The influence of cold plasma process parameters on aroma composition of red wine Cabernet Sauvignon

Source of variation		Treatment duration (min)			Frequency (Hz)		
	Control	3 min/60,90,120 Hz	5 min/60,90,120 Hz	10 min/60,90,120 Hz	60 Hz/3,5,10 min	90 Hz/3,5,10 min	120 Hz/3,5,10 min
Esters (mg/L)							
ethyl acetate	$31.44 \pm 1.81^{d,C}$	27.47 ± 1.64^{c}	23.76 ± 2.24^{b}	16.48 ± 2.92^{a}	24.34 ± 4.62^{B}	22.98 ± 4.86^{B}	20.39 ± 5.71^{A}
ethyl butyrate	$0.38 \pm 0.04^{d,C}$	0.31 ± 0.05^{c}	0.22 ± 0.02^{b}	0.16 ± 0.02^{a}	0.26 ± 0.08^{B}	0.22 ± 0.08^{A}	0.21 ± 0.06^{A}
ethyl hexanoate	$0.55 \pm 0.08^{d,C}$	0.42 ± 0.04^{c}	0.32 ± 0.03^{b}	0.27 ± 0.03^{a}	0.38 ± 0.08^{B}	0.33 ± 0.07^{A}	0.31 ± 0.07^{A}
ethyl octanoate	$0.39 \pm 0.06^{d,C}$	0.28 ± 0.04^{c}	0.21 ± 0.02^{b}	0.16 ± 0.02^{a}	0.24 ± 0.06^{B}	0.21 ± 0.06^{B}	0.19 ± 0.04^{A}
ethyl decanoate	$0.13 \pm 0.06^{b,A}$	0.11 ± 0.02^{b}	0.09 ± 0.01^{b}	0.07 ± 0.03^{a}	0.09 ± 0.03^{A}	0.09 ± 0.03^{A}	0.08 ± 0.03^{A}
diethyl succinate	$0.61 \pm 0.09^{c,B}$	0.57 ± 0.06^{c}	0.47 ± 0.03^{b}	0.38 ± 0.05^{a}	0.50 ± 0.10^{A}	0.47 ± 0.08^{A}	0.45 ± 0.10^{A}
i-butyl acetate	$0.07 \pm 0.00^{c,C}$	0.07 ± 0.00^{b}	0.06 ± 0.01^{a}	0.06 ± 0.01^{a}	0.06 ± 0.00^{B}	0.06 ± 0.00^{B}	0.06 ± 0.01^{A}
i-amyl acetate	$0.78 \pm 0.10^{d,C}$	0.64 ± 0.03^{c}	0.51 ± 0.07^{b}	0.36 ± 0.04^{a}	0.54 ± 0.13^{B}	0.51 ± 0.12^{AB}	0.45 ± 0.13^{A}
hexyl acetate	$0.00 \pm 0.01^{a,A}$	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}
2-phenylethyl acetate	$0.05 \pm 0.01^{a,A}$	0.04 ± 0.01^{a}	0.05 ± 0.01^{a}	0.04 ± 0.00^{a}	0.04 ± 0.01^{A}	0.04 ± 0.01^{A}	0.05 ± 0.01^{A}
Alcohols (mg/L)							
i-amyl alcohol	$259.11 \pm 8.30^{d,D}$	216.69 ± 15.06^{c}	196.90 ± 4.83^{b}	182.01 ± 6.09^{a}	207.99 ± 22.26^{C}	197.05 ± 12.34^{B}	190.55 ± 12.07^{A}
phenylethyl alcohol	$51.85 \pm 1.04^{d,C}$	43.54 ± 2.83^{c}	34.64 ± 3.12^{b}	25.69 ± 2.53^{a}	37.78 ± 7.95^{B}	33.65 ± 7.40^{A}	32.44 ± 8.23^{A}
1-hexanol	$1.76 \pm 0.08^{d,C}$	1.49 ± 0.10^{c}	1.34 ± 0.04^{b}	1.25 ± 0.02^{a}	1.41 ± 0.15^{B}	1.36 ± 0.11^{AB}	1.31 ± 0.08^{A}
cis-3-hexenol	nd	nd	nd	nd	nd	nd	nd
Acids (mg/L)							
hexanoic acid	$1.87 \pm 0.13^{d,C}$	1.54 ± 0.12^{c}	1.34 ± 0.08^{b}	1.01 ± 0.15^{a}	1.41 ± 0.22^{B}	1.29 ± 0.22^{A}	1.19 ± 0.28^{A}
octanoic acid	$3.37 \pm 0.22^{d,C}$	2.63 ± 0.21^{c}	2.26 ± 0.14^{b}	1.69 ± 0.17^{a}	2.35 ± 0.44^{B}	2.18 ± 0.45^{AB}	2.06 ± 0.39^{A}
decanoic acid	$0.37 \pm 0.03^{d,C}$	0.31 ± 0.02^{c}	0.26 ± 0.02^{b}	0.18 ± 0.03^{a}	0.27 ± 0.05^{B}	0.25 ± 0.05^{B}	0.23 ± 0.07^{A}
Terpenes (µg/L)							
linalool	$6.81 \pm 0.79^{d,C}$	5.90 ± 0.20^{c}	5.25 ± 0.12^{b}	4.66 ± 0.31^{a}	5.45 ± 0.49^{B}	5.32 ± 0.50^{AB}	5.05 ± 0.65^{A}
Aldehydes (µg/L)							
benzaldehyde	$1467.83 \pm 6.15^{d,D}$	1352.29 ± 104.38^{c}	1065.00 ± 39.32^{b}	818.64 ± 116.44^{a}	1172.57 ± 206.34^{C}	1075.69 ± 280.32^{B}	987.67 ± 214.71^{A}

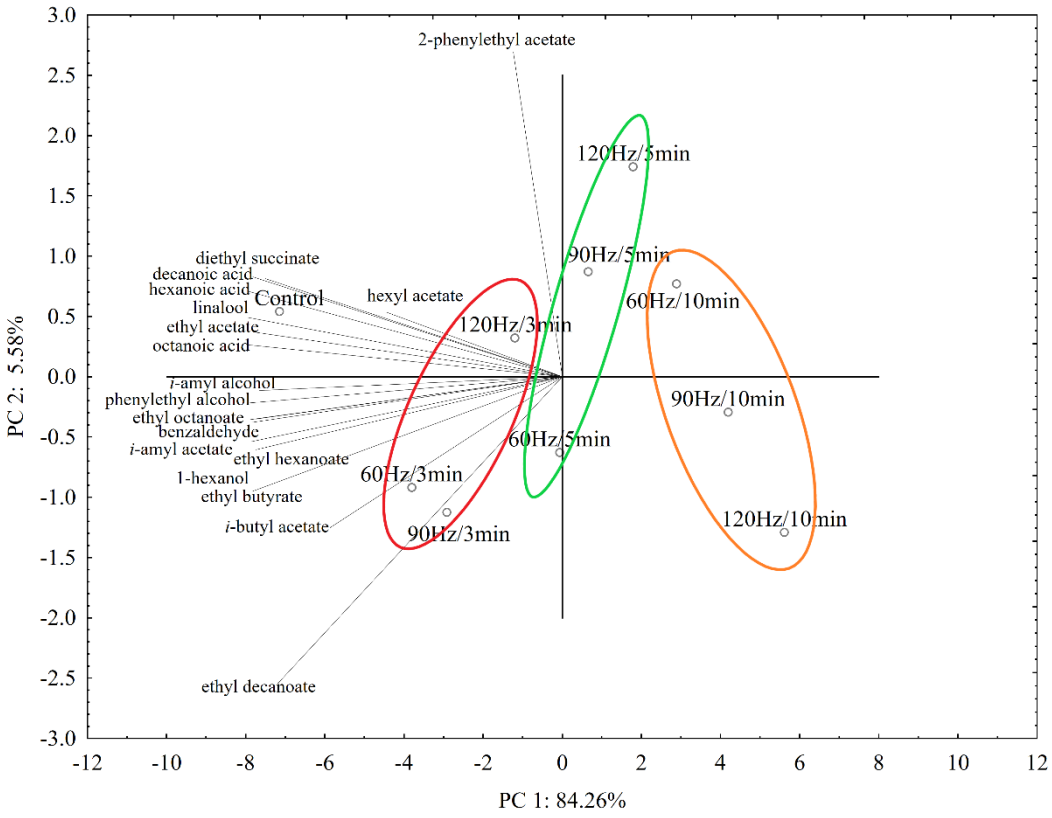
Data presented as average value of three analytical repetitions with standard deviation. MANOVA to compare data; different superscript letters indicate statistical differences between wines of all treatments (Tukey's test, $p < 0.05$): the lowercase letters refer to the effect of applied treatment duration, while the uppercase letters refer to the effect of applied frequency. nd – not detected.

Table S3. The influence of cold plasma process parameters on aroma composition of white wine Graševina

Source of variation		Treatment duration (min)			Frequency (Hz)		
	Control	3 min/60,90,120 Hz	5 min/60,90,120 Hz	10 min/60,90,120 Hz	60 Hz/3,5,10 min	90 Hz/3,5,10 min	120 Hz/3,5,10 min
Esters (mg/L)							
ethyl acetate	$29.80 \pm 3.20^{d,C}$	23.51 ± 1.91^{c}	17.14 ± 2.03^{b}	12.66 ± 1.70^{a}	19.78 ± 4.86^{B}	17.48 ± 4.68^{A}	16.05 ± 4.90^{A}
ethyl butyrate	$1.27 \pm 0.20^{d,C}$	1.00 ± 0.06^{c}	0.86 ± 0.08^{b}	0.61 ± 0.04^{a}	0.87 ± 0.18^{B}	0.84 ± 0.18^{AB}	0.76 ± 0.16^{A}
ethyl hexanoate	$1.54 \pm 0.07^{d,C}$	1.43 ± 0.07^{c}	1.30 ± 0.04^{b}	1.22 ± 0.06^{a}	1.36 ± 0.11^{B}	1.32 ± 0.09^{B}	1.27 ± 0.09^{A}
ethyl octanoate	$2.06 \pm 0.20^{d,C}$	1.69 ± 0.10^{c}	1.38 ± 0.11^{b}	1.21 ± 0.19^{a}	1.50 ± 0.21^{B}	1.47 ± 0.20^{B}	1.30 ± 0.30^{A}
ethyl decanoate	$1.42 \pm 0.07^{d,C}$	1.26 ± 0.08^{c}	1.10 ± 0.04^{b}	0.95 ± 0.10^{a}	1.16 ± 0.14^{B}	1.12 ± 0.14^{B}	1.03 ± 0.15^{A}
diethyl succinate	$0.18 \pm 0.01^{d,C}$	0.16 ± 0.02^{c}	0.14 ± 0.01^{b}	0.11 ± 0.01^{a}	0.15 ± 0.03^{B}	0.14 ± 0.03^{B}	0.13 ± 0.02^{A}
i-butyl acetate	$0.12 \pm 0.00^{d,C}$	0.11 ± 0.01^{c}	0.09 ± 0.01^{b}	0.06 ± 0.01^{a}	0.09 ± 0.02^{C}	0.09 ± 0.02^{B}	0.08 ± 0.02^{A}
i-amyl acetate	$2.16 \pm 0.27^{c,B}$	1.83 ± 0.08^{b}	1.70 ± 0.05^{b}	1.54 ± 0.07^{a}	1.74 ± 0.15^{A}	1.70 ± 0.12^{A}	1.64 ± 0.14^{A}
hexyl acetate	$0.29 \pm 0.01^{c,B}$	0.27 ± 0.02^{bc}	0.25 ± 0.02^{ab}	0.23 ± 0.02^{a}	0.26 ± 0.02^{A}	0.24 ± 0.02^{A}	0.25 ± 0.02^{A}
2-phenylethyl acetate	$0.22 \pm 0.01^{d,C}$	0.19 ± 0.02^{c}	0.15 ± 0.01^{b}	0.14 ± 0.01^{a}	0.17 ± 0.03^{B}	0.16 ± 0.03^{AB}	0.15 ± 0.02^{A}
Alcohols (mg/L)							
i-amyl alcohol	$65.15 \pm 0.60^{d,D}$	57.78 ± 8.98^{c}	41.65 ± 1.83^{b}	36.48 ± 2.61^{a}	49.14 ± 11.44^{C}	46.58 ± 12.69^{B}	40.18 ± 5.39^{A}
phenylethyl alcohol	$7.69 \pm 0.53^{d,D}$	6.31 ± 0.62^{c}	5.00 ± 0.42^{b}	3.33 ± 0.57^{a}	5.36 ± 1.42^{C}	4.90 ± 1.14^{B}	4.37 ± 1.43^{A}
1-hexanol	$1.76 \pm 0.10^{d,C}$	1.57 ± 0.14^{c}	1.34 ± 0.04^{b}	1.22 ± 0.03^{a}	1.43 ± 0.20^{B}	1.40 ± 0.19^{B}	1.31 ± 0.10^{A}
cis-3-hexenol	$0.13 \pm 0.01^{d,C}$	0.11 ± 0.01^{c}	0.10 ± 0.01^{b}	0.08 ± 0.01^{a}	0.10 ± 0.02^{B}	0.10 ± 0.01^{B}	0.09 ± 0.02^{A}
Acids (mg/L)							
hexanoic acid	$8.09 \pm 0.08^{d,C}$	7.16 ± 0.48^{c}	6.18 ± 0.42^{b}	4.98 ± 0.60^{a}	6.37 ± 1.04^{B}	6.05 ± 0.83^{AB}	5.90 ± 1.25^{A}
octanoic acid	$20.32 \pm 0.64^{d,C}$	18.64 ± 1.29^{c}	16.59 ± 0.99^{b}	14.19 ± 1.58^{a}	17.63 ± 1.95^{B}	16.63 ± 2.05^{B}	15.16 ± 2.20^{A}
decanoic acid	$4.87 \pm 0.08^{d,D}$	4.30 ± 0.41^{c}	3.60 ± 0.21^{b}	2.94 ± 0.29^{a}	3.87 ± 0.72^{C}	3.62 ± 0.63^{B}	3.36 ± 0.54^{A}
Terpenes (µg/L)							
linalool	$7.10 \pm 0.79^{c,C}$	5.63 ± 0.75^{b}	4.78 ± 0.35^{a}	4.61 ± 0.25^{a}	5.23 ± 0.86^{B}	5.12 ± 0.54^{AB}	4.67 ± 0.43^{A}
Aldehydes (µg/L)							
benzaldehyde	$153.84 \pm 22.44^{d,C}$	108.24 ± 16.62^{c}	74.52 ± 7.58^{b}	54.33 ± 8.31^{a}	88.75 ± 27.86^{B}	79.14 ± 24.50^{AB}	69.20 ± 21.91^{A}

Data presented as average value of three analytical repetitions with standard deviation. MANOVA to compare data; different superscript letters indicate statistical differences between wines of all treatments (Tukey's test, $p < 0.05$): the lowercase letters refer to the effect of applied treatment duration, while the uppercase letters refer to the effect of applied frequency.

A)



B)

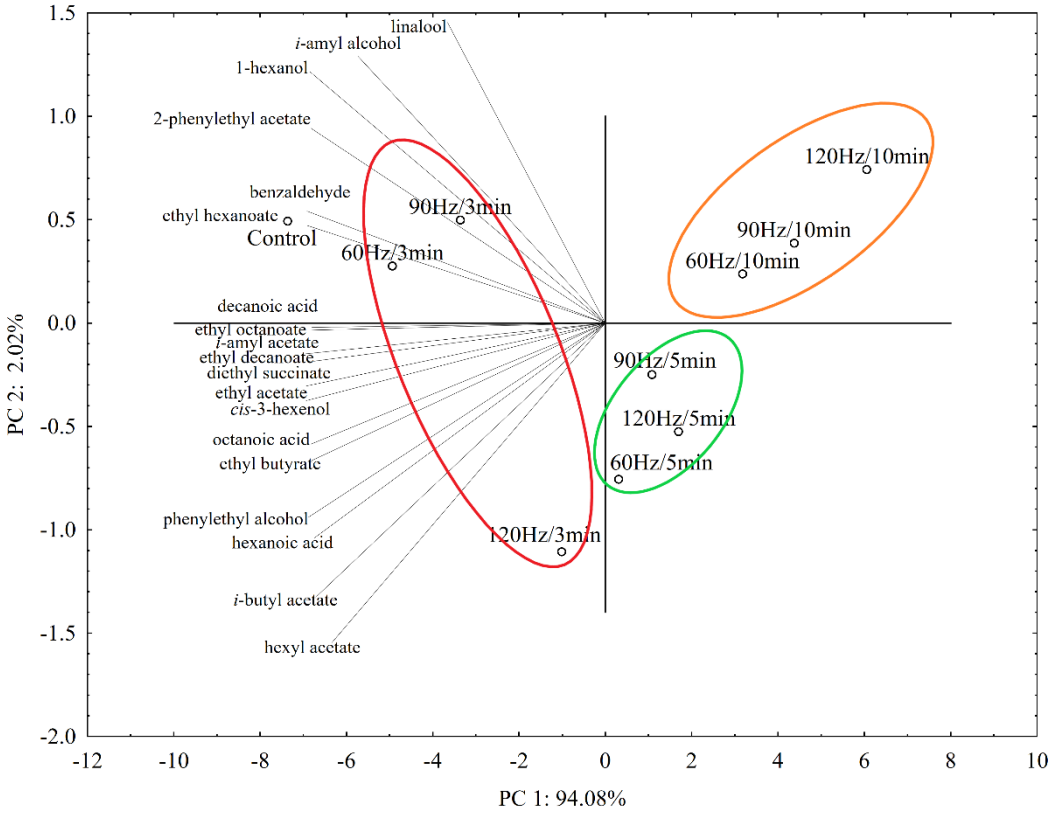
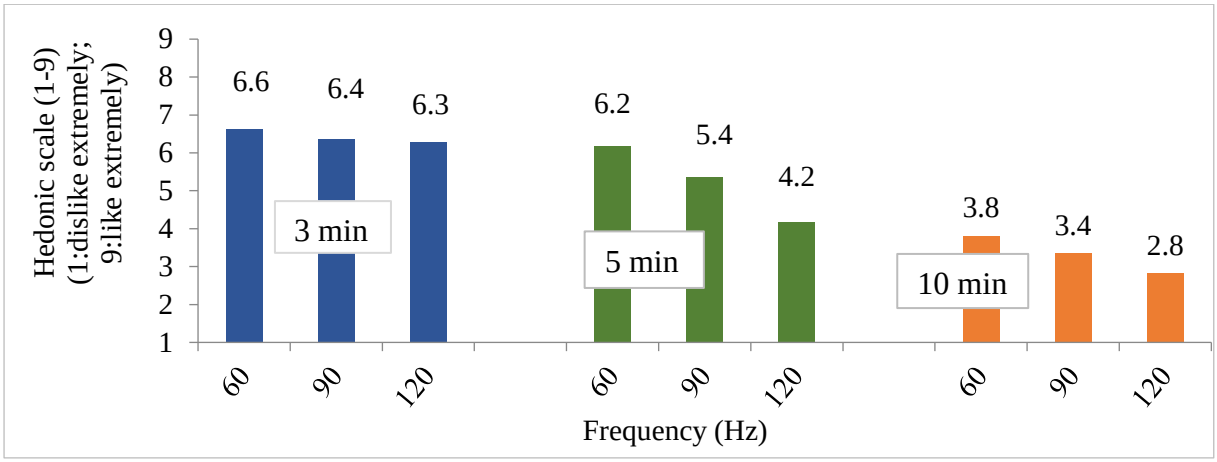


Figure S9. Distribution of the wine samples in the two-dimensional coordinate system defined by first two principal components (PC 1 and PC 2) according to applied cold plasma treatments and aroma composition. (A) red wine; (B) white wine

A)



B)

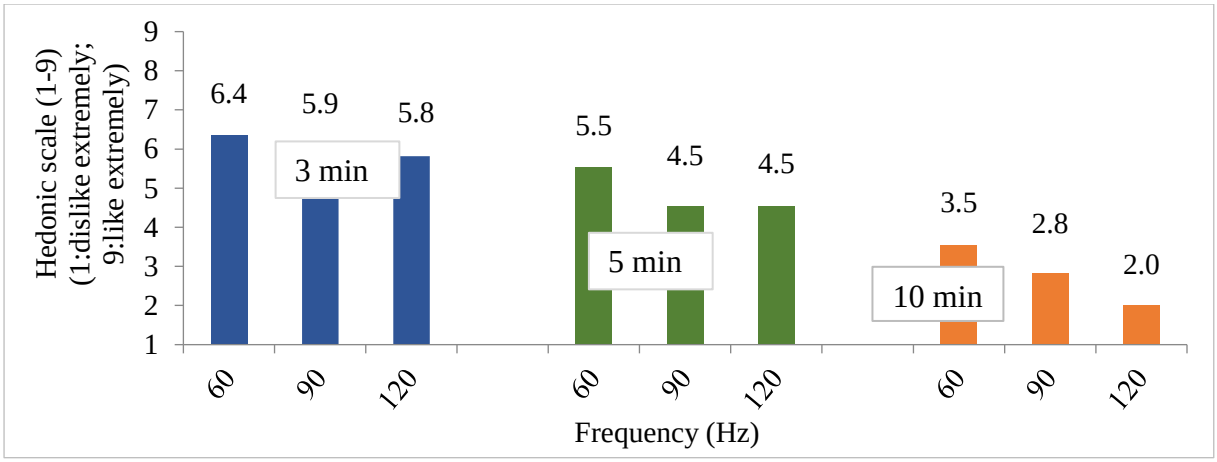


Figure S10. The influence of cold plasma process parameters on the sensory characteristics of red (A) and white (B) wines

Table S4. The effect of cold plasma and antioxidants addition (SO_2 and GSH) on the phenolic, chromatic and aroma composition of red wine Cabernet Sauvignon during 12 months of bottle aging

Time (months)		**Wine sample**			
		Control	↔SO_2	↓SO_2+GSH	↓SO_2
Total phenolics (mg/L)	0	2435.45 ± 2.57^{a}	2257.27 ± 1.29^{b}	2157.27 ± 2.57^{c}	2137.27 ± 2.57^{d}
	3	2337.92 ± 2.95^{a}	2191.25 ± 2.95^{b}	2105.83 ± 1.18^{c}	2097.92 ± 1.77^{c}
	6	2207.50 ± 1.18^{a}	2087.08 ± 0.59^{b}	2014.58 ± 1.77^{c}	1996.25 ± 0.59^{d}
	12	2137.92 ± 10.02^{a}	2042.50 ± 3.54^{b}	1985.00 ± 5.89^{b}	1570.42 ± 43.02^{c}
Total anthocyanins (mg/L)	0	366.71 ± 0.49^{a}	364.57 ± 3.53^{a}	325.33 ± 0.87^{b}	300.17 ± 1.42^{c}
	3	330.23 ± 3.71^{a}	326.51 ± 2.78^{a}	301.26 ± 0.12^{b}	279.39 ± 0.87^{c}
	6	313.86 ± 1.61^{a}	288.75 ± 2.60^{b}	270.33 ± 0.19^{c}	260.09 ± 4.39^{c}
	12	287.22 ± 0.93^{a}	272.34 ± 1.30^{b}	235.68 ± 0.31^{c}	210.44 ± 2.60^{d}
Total tannins (g/L)	0	2.94 ± 0.00^{a}	2.81 ± 0.01^{b}	2.72 ± 0.01^{c}	2.71 ± 0.02^{c}
	3	2.86 ± 0.03^{a}	2.80 ± 0.01^{a}	2.61 ± 0.02^{b}	2.61 ± 0.06^{b}
	6	2.81 ± 0.01^{a}	2.74 ± 0.04^{a}	2.59 ± 0.03^{b}	2.58 ± 0.01^{b}
	12	2.26 ± 0.01^{a}	2.06 ± 0.03^{b}	2.05 ± 0.02^{b}	1.98 ± 0.05^{b}
Total free anthocyanins (mg/L)	0	187.60 ± 1.22^{a}	162.87 ± 0.40^{b}	134.11 ± 0.55^{c}	125.32 ± 0.93^{d}
	3	142.58 ± 1.88^{a}	140.38 ± 1.69^{a}	108.08 ± 0.24^{b}	94.80 ± 0.57^{c}
	6	141.24 ± 1.01^{a}	131.12 ± 2.88^{b}	100.24 ± 0.55^{c}	86.64 ± 0.52^{d}
	12	119.44 ± 1.91^{a}	107.98 ± 0.08^{b}	73.28 ± 1.19^{c}	63.82 ± 0.34^{d}
Total flavan-3-ols (mg/L)	0	301.93 ± 4.64^{a}	294.92 ± 2.21^{a}	287.13 ± 7.37^{ab}	269.15 ± 0.24^{b}
	3	296.33 ± 1.86^{a}	286.67 ± 0.87^{b}	272.19 ± 0.63^{c}	266.51 ± 1.26^{d}
	6	288.60 ± 2.99^{a}	282.22 ± 2.64^{a}	261.97 ± 1.09^{b}	258.43 ± 1.99^{b}
	12	282.05 ±1.36^{a}	281.45 ± 0.67^{a}	254.38 ± 0.55^{b}	252.98 ± 3.43^{b}
L*	0	19.94 ± 0.22^{a}	18.53 ± 0.44^{a}	16.08 ± 0.50^{b}	15.79 ± 0.32^{b}
	3	20.55 ± 0.37^{b}	21.56 ± 0.26^{a}	16.45 ± 0.01^{c}	16.24 ± 0.03^{c}
	6	22.25 ± 0.10^{a}	21.70 ± 0.40^{a}	18.20 ± 0.04^{b}	18.15 ± 0.10^{b}
	12	18.94 ± 1.28^{a}	18.04 ± 0.58^{a}	15.94 ± 0.96^{ab}	12.89 ± 0.10^{b}
a*	0	49.41 ± 0.34^{a}	49.07 ± 0.76^{a}	45.92 ± 0.86^{b}	45.51 ± 0.48^{b}
	3	50.82 ± 0.53^{b}	52.90 ± 0.37^{a}	47.55 ± 0.03^{c}	47.26 ± 0.04^{c}
	6	52.74 ± 0.12^{a}	52.81 ± 0.32^{a}	49.62 ± 0.07^{b}	49.50 ± 0.15^{b}
	12	48.52 ± 0.35^{a}	46.22 ± 2.66^{ab}	45.19 ± 0.60^{ab}	41.53 ± 0.29^{b}
b*	0	30.68 ± 0.37^{a}	29.89 ± 0.70^{a}	26.17 ± 0.91^{b}	25.82 ± 0.51^{b}
	3	32.32 ± 0.57^{b}	34.28 ± 0.40^{a}	27.32 ± 0.03^{c}	27.03 ± 0.05^{c}
	6	32.69 ± 0.06^{a}	32.80 ± 0.18^{a}	30.35 ± 0.10^{b}	30.23 ± 0.14^{b}
	12	29.35 ± 0.49^{a}	27.98 ± 1.39^{ab}	25.82 ± 0.85^{b}	21.36 ± 0.20^{c}
C*	0	58.16 ± 0.48^{a}	57.46 ± 1.01^{a}	52.82 ± 1.20^{b}	52.32 ± 0.67^{b}
	3	60.23 ± 0.76^{b}	63.04 ± 0.53^{a}	54.84 ± 0.04^{c}	54.45 ± 0.06^{c}
	6	62.05 ± 0.13^{a}	62.17 ± 0.36^{a}	58.17 ± 0.11^{b}	58.02 ± 0.22^{b}
	12	57.50 ± 0.57^{a}	56.23 ± 0.09^{a}	52.05 ± 0.94^{b}	46.70 ± 0.34^{c}
H*	0	0.56 ± 0.00^{a}	0.55 ± 0.00^{a}	0.52 ± 0.01^{b}	0.52 ± 0.00^{b}
	3	0.57 ± 0.00^{b}	0.58 ± 0.00^{a}	0.52 ± 0.00^{c}	0.52 ± 0.00^{c}
	6	0.56 ± 0.00^{a}	0.56 ± 0.00^{a}	0.55 ± 0.00^{b}	0.55 ± 0.00^{b}
	12	0.52 ± 0.04^{a}	0.54 ± 0.00^{a}	0.52 ± 0.01^{a}	0.48 ± 0.00^{a}
ΔE*	0	-	1.82 ± 0.11^{b}	6.89 ± 1.31^{a}	7.49 ± 0.76^{a}
	3	-	3.03 ± 0.60^{b}	7.25 ± 0.05^{a}	7.69 ± 0.07^{a}
	6	-	0.65 ± 0.27^{b}	5.62 ± 0.11^{a}	5.77 ±0.21^{a}
	12	-	3.42 ± 1.39^{b}	5.71 ± 1.38^{b}	12.22 ± 0.34^{a}

Table S4. *(continued)*

Time (months)		Wine sample			
		Control	↔SO_2	↓SO_2+GSH	↓SO_2
Total esters (mg/L)	0	23.23 ± 0.11^{a}	22.02 ± 0.24^{ab}	20.71 ± 0.84^{bc}	19.44 ± 0.66^{c}
	3	27.64 ± 0.32^{a}	27.04 ± 0.20^{ab}	26.61 ± 0.05^{b}	22.21 ± 0.33^{c}
	6	33.72 ± 0.73^{a}	31.47 ± 0.55^{b}	28.30 ± 0.20^{c}	27.38 ± 0.44^{c}
	12	44.49 ± 0.45^{a}	43.89 ± 0.05^{a}	34.83 ± 1.17^{b}	28.54 ± 0.05^{c}
Total higher alcohols (mg/L)	0	175.86 ± 0.59^{d}	180.45 ± 0.31^{c}	189.50 ± 0.73^{b}	192.28 ± 0.91^{a}
	3	194.33 ± 0.15^{d}	197.72 ± 0.63^{c}	202.25 ± 0.37^{b}	215.35 ± 0.01^{a}
	6	200.96 ± 0.13^{b}	204.69 ± 0.88^{b}	206.28 ± 1.79^{ab}	211.95 ± 2.06^{a}
	12	228.76 ± 0.16^{b}	236.69 ± 1.84^{ab}	243.89 ± 4.70^{a}	243.28 ± 1.86^{a}
Total fatty acids (mg/L)	0	5.41 ± 0.34^{a}	4.95 ± 0.14^{ab}	4.14 ± 0.36^{b}	4.03 ± 0.14^{b}
	3	5.38 ± 0.13^{a}	4.87 ± 0.17^{ab}	3.93 ± 0.16^{bc}	3.17 ± 0.58^{c}
	6	3.97 ± 0.06^{a}	3.37 ± 0.02^{b}	3.04 ± 0.03^{c}	2.84 ± 0.01^{d}
	12	2.62 ±0.22^{a}	2.22 ± 0.08^{ab}	2.03 ± 0.18^{ab}	1.82 ± 0.21^{b}
Total terpenes (μg/L)	0	19.76 ± 0.06^{a}	18.06 ± 0.77^{ab}	17.23 ± 0.63^{b}	16.20 ± 0.16^{b}
	3	16.34 ± 0.45^{a}	15.20 ± 0.36^{ab}	14.86 ± 0.37^{b}	14.38 ± 0.14^{b}
	6	16.32 ± 0.51^{a}	14.90 ± 0.13^{b}	14.39 ± 0.42^{b}	12.03 ± 0.08^{c}
	12	15.79 ± 0.11^{a}	14.47 ± 0.35^{b}	13.86 ± 0.04^{b}	11.64 ± 0.05^{c}

Results are expressed as mean value ± standard deviation (N=6). ANOVA to compare results; different letters indicate a statistical difference between analyzed wine samples at the same time of analysis (Tukey's HSD test, $p < 0.05$).

Table S5. The effect of cold plasma and antioxidants addition (SO_2 and GSH) on the phenolic, chromatic and aroma composition of white wine Graševina during 12 months of bottle aging

Time (months)		**Wine sample**			
		Control	↔SO_2	↓SO_2+GSH	↓SO_2
Total phenolics (mg/L)	0	226.91 ± 0.39^{a}	217.95 ± 1.35^{b}	211.59 ± 0.32^{c}	209.45 ± 1.80^{c}
	3	220.33 ± 0.35^{a}	211.75 ± 0.12^{b}	201.38 ± 0.41^{c}	198.58 ± 0.12^{d}
	6	213.38 ± 0.06^{a}	205.75 ± 1.77^{b}	198.50 ± 0.59^{c}	176.96 ± 0.18^{d}
	12	212.21 ± 0.88^{a}	197.38 ± 0.88^{b}	191.63 ± 0.53^{c}	174.63 ± 0.29^{d}
Total phenolic acids (mg/L)	0	45.57 ± 0.10^{a}	45.19 ± 0.03^{b}	45.11 ± 0.06^{b}	44.34 ± 0.03^{c}
	3	46.67 ± 0.20^{a}	45.66 ± 0.02^{b}	45.19 ± 0.09^{c}	45.01 ± 0.01^{c}
	6	49.09 ± 0.01^{a}	48.47 ± 0.55^{ab}	47.78 ± 0.02^{bc}	46.62 ± 0.22^{c}
	12	62.24 ± 0.10^{a}	62.09 ± 0.16^{a}	61.36 ± 0.49^{ab}	60.63 ± 0.46^{b}
Total flavan-3-ols (mg/L)	0	23.69 ± 0.21^{a}	21.84 ± 0.20^{b}	21.12 ± 0.05^{c}	20.77 ± 0.08^{c}
	3	22.80 ± 0.24^{a}	21.71 ± 0.02^{b}	21.03 ± 0.05^{c}	20.60 ± 0.10^{c}
	6	22.65 ± 0.03^{a}	21.50 ± 0.12^{b}	20.80 ± 0.05^{c}	19.22 ± 0.05^{d}
	12	22.46 ± 0.30^{a}	20.41 ± 0.50^{b}	17.62 ± 0.36^{c}	16.54 ± 0.30^{c}
L*	0	99.72 ± 0.02^{a}	99.45 ± 0.16^{ab}	99.46 ± 0.04^{ab}	99.19 ± 0.00^{b}
	3	99.66 ± 0.20^{a}	99.49 ± 0.12^{ab}	98.91 ± 0.01^{bc}	98.66 ± 0.23^{c}
	6	98.46 ± 0.19^{a}	97.45 ± 0.19^{b}	96.34 ± 0.07^{c}	94.39 ± 0.41^{d}
	12	97.50 ± 0.00^{a}	96.21 ± 1.15^{a}	96.89 ± 0.49^{ab}	94.08 ± 0.47^{b}
a*	0	-0.72 ± 0.15^{a}	-0.92 ± 0.02^{a}	-0.90 ± 0.04^{a}	-0.91 ± 0.01^{a}
	3	-0.93 ± 0.06^{a}	-0.85 ± 0.07^{a}	-0.85 ± 0.01^{a}	-0.85 ± 0.07^{a}
	6	-0.79 ± 0.05^{a}	-0.80 ± 0.08^{a}	-0.70 ± 0.08^{a}	-0.74 ± 0.05^{a}
	12	-0.65 ± 0.00^{a}	-0.89 ± 0.15^{a}	-0.96 ± 0.15^{a}	-1.12 ± 0.38^{a}
b*	0	4.46 ± 0.25^{b}	5.14 ± 0.02^{a}	4.96 ± 0.09^{ab}	5.13 ± 0.12^{a}
	3	4.77 ± 0.13^{b}	5.90 ± 0.28^{a}	5.88 ± 0.16^{a}	5.38 ± 0.18^{ab}
	6	4.65 ± 0.23^{a}	4.74 ± 0.35^{a}	5.18 ± 0.86^{a}	5.08 ± 0.24^{a}
	12	6.31 ± 0.00^{a}	6.55 ± 1.30^{a}	7.17 ± 0.13^{a}	6.37 ± 0.43^{a}
C*	0	4.52 ±0.28^{b}	5.22 ± 0.02^{a}	5.04 ± 0.10^{ab}	5.21 ± 0.12^{a}
	3	4.86 ± 0.12^{b}	5.96 ± 0.27^{a}	5.94 ± 0.16^{a}	5.45 ± 0.17^{ab}
	6	4.72 ± 0.24^{a}	4.81 ± 0.36^{a}	5.23 ± 0.84^{a}	5.13 ± 0.23^{a}
	12	6.35 ± 0.00^{a}	6.62 ± 1.26^{a}	7.23 ± 0.10^{a}	6.47 ± 0.49^{a}
H*	0	-1.41 ± 0.02^{a}	-1.39 ± 0.00^{a}	-1.39 ± 0.00^{a}	-1.40 ± 0.00^{a}
	3	-1.40 ± 0.00^{a}	-1.43 ± 0.02^{a}	-1.43 ± 0.00^{a}	-1.41 ± 0.02^{a}
	6	-1.39 ± 0.00^{a}	-1.40 ± 0.01^{a}	-1.43 ± 0.04^{a}	-1.43 ± 0.02^{a}
	12	-1.47 ± 0.00^{a}	-1.43 ± 0.05^{a}	-1.44 ± 0.02^{a}	-1.40 ± 0.05^{a}
ΔE*	0	-	0.76 ± 0.07^{a}	0.59 ± 0.11^{a}	0.88 ± 0.10^{a}
	3	-	1.14 ± 0.30^{a}	1.34 ± 0.14^{a}	1.17 ± 0.29^{a}
	6	-	1.04 ± 0.21^{b}	2.26 ± 0.27^{b}	4.10 ± 0.43^{a}
	12	-	1.65 ± 1.06^{a}	1.14 ± 0.32^{a}	3.49 ± 0.41^{a}

Table S5. *(continued)*

Time (months)		Wine sample			
		Control	↔SO_2	↓SO_2+GSH	↓SO_2
Total esters (mg/L)	0	38.04 ± 0.50^{a}	36.63 ± 0.20^{b}	34.90 ± 0.10^{c}	30.28 ± 0.23^{d}
	3	36.94 ± 0.43^{a}	35.90 ± 0.33^{a}	33.59 ± 1.71^{a}	25.73 ± 2.20^{b}
	6	35.97 ± 0.11^{a}	35.24 ± 0.10^{b}	32.31 ± 0.01^{c}	23.36 ± 0.01^{d}
	12	28.81 ± 0.14^{a}	27.59 ± 0.29^{ab}	26.37 ± 0.81^{b}	23.08 ± 0.11^{c}
Total higher alcohols (mg/L)	0	79.95 ± 1.00^{b}	81.10 ± 0.10^{ab}	81.27 ± 0.02^{ab}	82.14 ± 0.10^{a}
	3	81.30 ± 0.23^{c}	83.58 ± 0.44^{b}	84.57 ± 0.13^{ab}	85.21 ± 0.12^{a}
	6	82.13 ± 0.16^{d}	85.24 ± 0.86^{c}	87.22 ± 0.05^{b}	90.33 ± 0.03^{a}
	12	92.17 ± 0.05^{d}	99.10 ± 0.05^{c}	101.00 ± 0.06^{b}	101.83 ± 0.01^{a}
Total fatty acids (mg/L)	0	22.14 ± 0.14^{a}	20.73 ± 0.18^{b}	19.33 ± 0.04^{c}	17.69 ± 0.09^{d}
	3	20.15 ± 0.05^{a}	19.08 ± 0.04^{b}	18.28 ± 0.11^{c}	16.66 ± 0.15^{d}
	6	18.66 ± 0.45^{a}	17.43 ± 0.12^{ab}	16.44 ± 0.40^{bc}	15.80 ± 0.24^{c}
	12	14.58 ± 0.13^{a}	13.68 ± 0.21^{a}	12.26 ± 0.26^{b}	10.76 ± 0.30^{c}
Total terpenes (μg/L)	0	14.15 ± 0.16^{a}	12.60 ± 0.10^{b}	11.83 ± 0.08^{c}	11.57 ± 0.01^{c}
	3	12.04 ± 0.05^{a}	11.71 ± 0.21^{a}	11.39 ± 0.23^{a}	10.41 ± 0.08^{b}
	6	11.58 ± 0.10^{a}	10.88 ± 0.04^{b}	9.68 ± 0.02^{c}	9.48 ± 0.04^{c}
	12	11.22 ± 0.04^{a}	10.13 ± 0.06^{b}	9.09 ± 0.01^{c}	8.21 ± 0.02^{d}

Results are expressed as mean value ± standard deviation (N=6). ANOVA to compare results; different letters indicate a statistical difference between analyzed wine samples at the same time of analysis (Tukey's HSD test, $p < 0.05$).

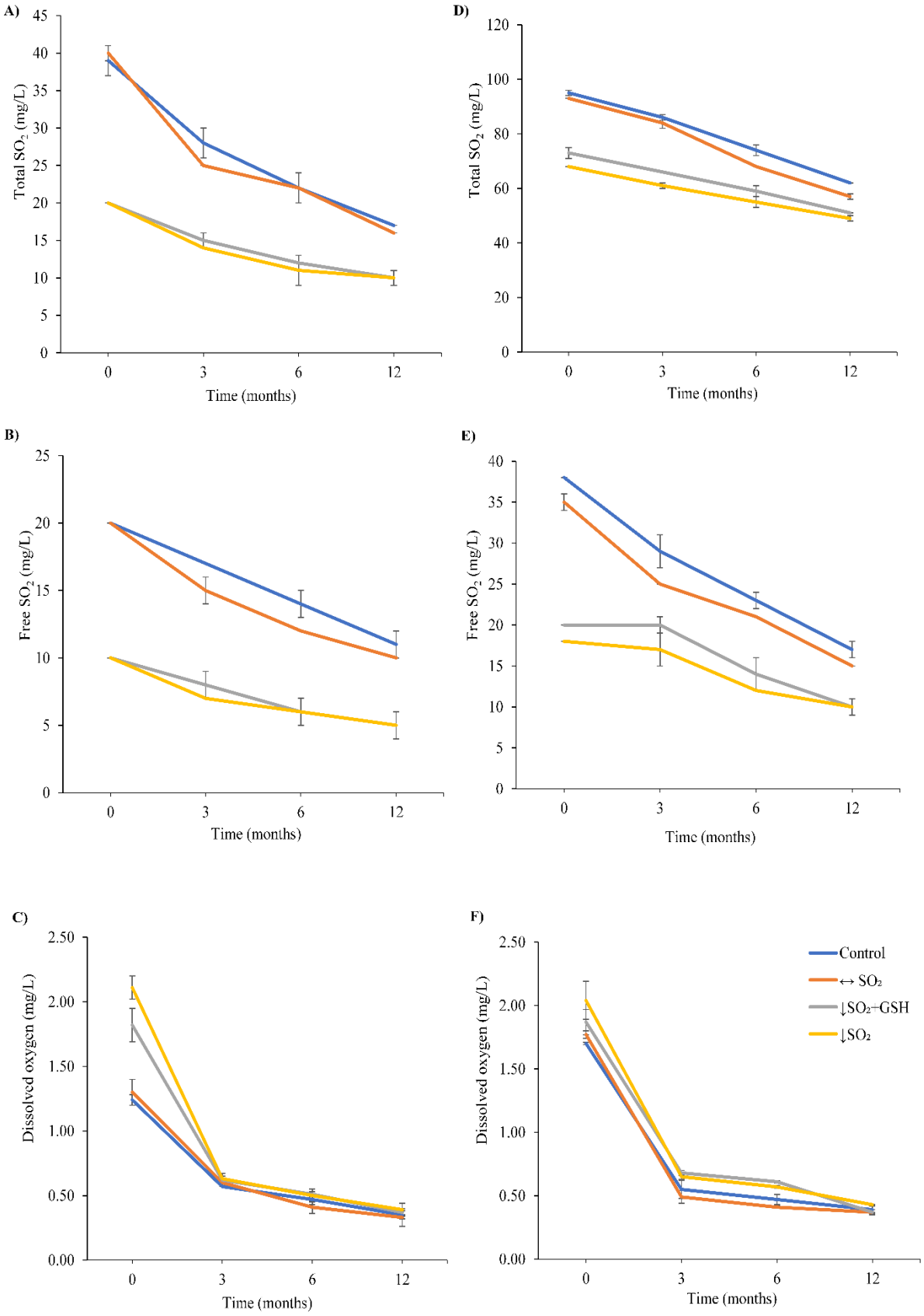
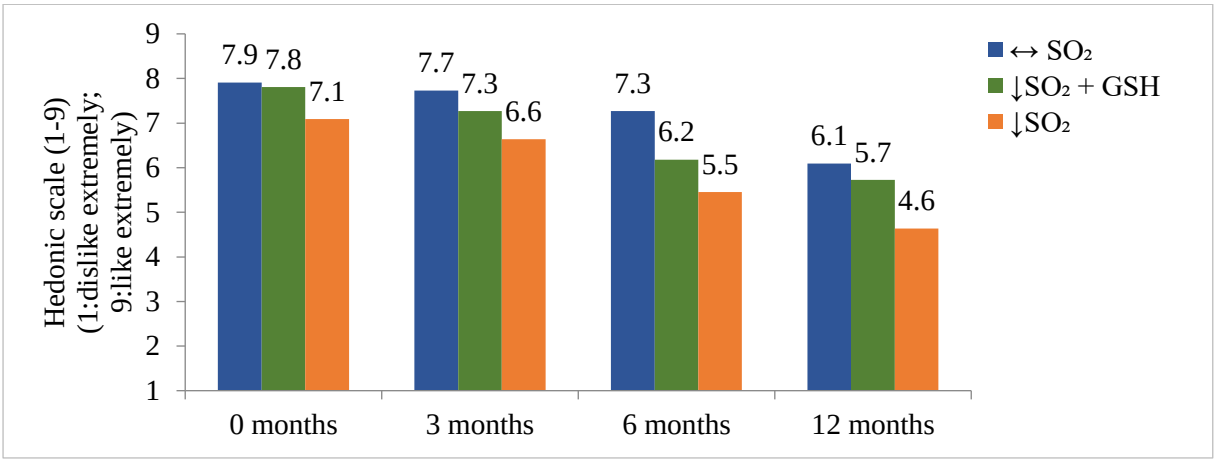


Figure S11. The effect of cold plasma and antioxidants addition (SO_2 and GSH) on the concentration of dissolved oxygen, total and free SO_2 in red and white wines during 12 months of aging. Red wine samples (A-C). White wine samples (D-F).

A)



B)

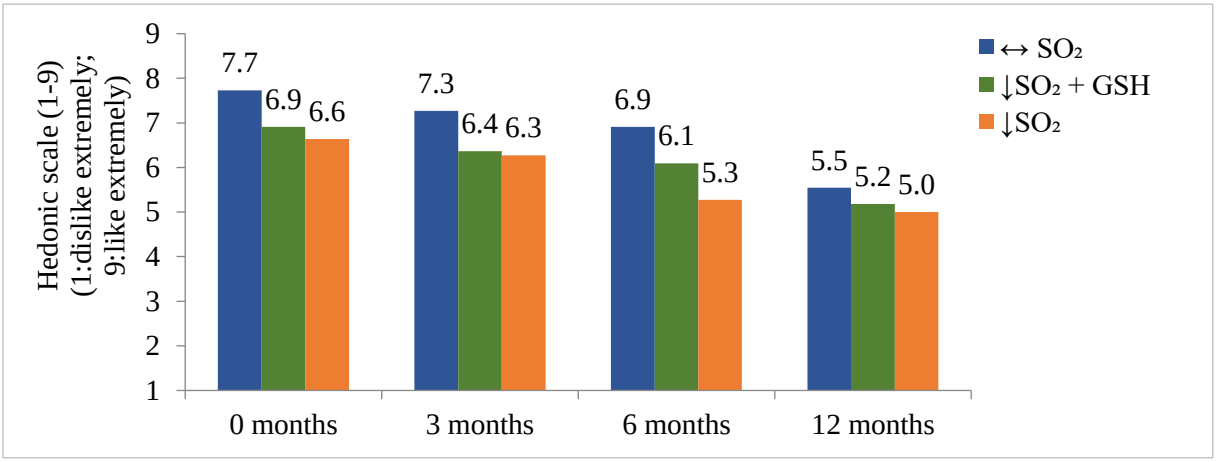


Figure S12. The effect of cold plasma and antioxidants addition (SO_2 and GSH) on the sensory characteristics of red (A) and white (B) wine during 12 months of bottle aging