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Review

Non-Thermal Technology Approaches to Improve Extraction, Fermentation, Microbial Stability, and Aging in the Winemaking Process

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Abstract: Research into non-thermal wine processing technologies is constantly evolving due to the increasing trend towards high-quality, minimally processed, and preservative-free wines. Technologies such as high-power ultrasound, high hydrostatic pressure, pulsed electric field, ultra-high pressure homogenization, and, more recently, cold plasma are some of the best examples currently being explored. This article provides an overview of the latest scientific research into these five non-thermal technologies and their current status in relation to winemaking. By exploring the potential applications of these technologies, it becomes possible to enhance extraction, shorten maceration time, inactivate microorganisms and oxidative enzymes, reduce the addition of chemical additives, accelerate aging, improve wine quality, and much more. However, further experiments are necessary to better comprehend the short- and long-term impacts on the overall quality of the wines produced, especially in terms of sensory characteristics and consumer acceptance. Optimizing processing conditions and scaling up are also of paramount importance to ensure better performance of these technologies at the various stages of winemaking. Additionally, a combined technologies approach has been highlighted as one of the future directions to overcome the limitations of processing with a single technology.

Keywords: non-thermal technologies; winemaking; ultrasound; high pressure; pulsed electric field; cold plasma; phenolics; aroma; color; quality

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1. Introduction

Wine is one of the most widely consumed alcoholic beverages. According to the World Viticulture Report [1], in 2023, the world wine production amounted to 247 million hectoliters, which indicates the importance of the wine market on the global scale. The winemaking process can be defined as a series of operations ranging from grape juice extraction to alcoholic fermentation, aging, clarification, and packaging [2]. Although winemaking is strongly linked to tradition, these various stages can be significantly improved through the application of innovation, which can result in high-quality wines with a longer shelf life. The consumer demand for such wines, and the openness of the wine industry to innovative technologies and approaches, has resulted in remarkable scientific coverage and developments over the last 20 years. In this context, non-thermal technologies have been recognized by the scientific community as a useful tool for the wine sector [3]. The ability to increase the extraction of bioactive compounds, enhance the color of wine, shorten maceration time, accelerate maturation and bottle aging, reduce spoilage microorganisms and oxidative enzymes, and maintain or even improve wine quality and sensory characteristics are key parameters allowed by these technologies [3–5]. In addition, lower treatment temperatures and shorter processing times make these technologies suitable for a heat-sensitive medium such as wine. Several recent publications summarize the main features of those most successful, like high power ultrasound [6–9], high hydrostatic pressure [10], pulsed electric field [9,11,12], ultra-high pressure homogenization [13–15], and, most recently, cold plasma [16]. The application of some of these non-thermal technologies for wine processing, preservation, and quality enhancement has been recently reviewed by Kumar et al. [3].

Among the aforementioned technologies, high-power ultrasound (HPU) has proven to be a very efficient for the extraction of grape compounds [17–23], the production of low-sulfite wines [24,25], the stabilization of wine proteins [26], and the preservation/improvement of wine quality characteristics [27–34]. High hydrostatic pressure (HHP) is another promising technology that can be used to alter wine composition [35], accelerate the aging process [36,37], reduce wild microorganisms [38], and thus minimize SO_2 addition during winemaking [39–41]. There is, at present, an increasing research interest in pulsed electric field (PEF) for several applications on grape, must, and wine, such as the control of the microbial population [42–50], the reduction in maceration time [51–53], the extraction enhancement of bioactive compounds [54–59], and assistance in the aging and enrichment of wines with aroma compounds from oak chips [60]. Furthermore, ultra-high-pressure homogenization (UHPH) was highlighted in view of grape must processing, inactivating microorganisms and oxidative enzymes [61–63], improving colloidal stability [64], and additionally allowing for the easier implementation of new fermentation biotechnologies [65]. Although cold plasma (CP) is the newest non-thermal technology presented in winemaking, its research is becoming very important. A strong benefit of using CP technology was demonstrated through the preservation or even improvement of wine quality and oxidative stability [66–68], the reduction in natural contaminants of wine-like biogenic amines [69], and in chemical additives such as SO_2 [70]. In addition to the direct application to must or wine, CP also showed effectiveness in the microbial sanitization of wine barrels [71].

2. Methodology and Analysis

Despite recent advances, an up-to-date overview of these non-thermal technologies with particular reference to their application in the winemaking process is lacking. Therefore, the present review aims to provide important information on five selected non-thermal technologies, namely HPU, HHP, PEF, UHPH, and CP, which will certainly be useful for all those involved in the wine sector. The applications, benefits, and challenges of selected technologies are listed in Table 1. To compose this review article, only scientific papers published between 2019 and 2024 (including those available at the time of writing this review in 2024) were retrieved from trusted and reputable online databases such as Google Scholar, Science Direct, Web of Science, and Scopus, using keywords such as non-thermal technologies, high-power ultrasound, high hydrostatic pressure, pulsed electric field, ultra-high pressure homogenization, cold plasma, winemaking, microbial safety, sulfur dioxide, and quality characteristics. Some other useful references have also been added to supplement this review. In total, a comprehensive and detailed analysis of the scientific repository resulted in 85 papers, including 55 research articles, 20 reviews, and 10 book chapters. Figure 1 shows the number of published articles per year and in relation to each technology about the analyzed topic during the last five years. As can be seen, 2020 and 2021 were the years with the highest number of published papers, while cumulatively the most studied technologies were pulsed electric field and high-power ultrasound, followed by high hydrostatic pressure. In addition, the strategy employed in composing this review article is presented in Figure 2. The next section provides some valuable insights into the latest applications of the aforementioned non-thermal technologies (HPU, HHP, PEF, UHPH, and CP) at different stages of winemaking and the changes in certain wine quality characteristics, particularly in phenolics, color, and aroma, based on the collected reports.

Table 1. Applications, benefits, and challenges of selected non-thermal technologies in winemaking.

Non-Thermal Technologies	Applications	Benefits	Challenges	References
High-power ultrasound (HPU)	• Extraction • Fermentation • Microbial stabilization • Aging	• Accelerated mass transfer • Shorter aging time • Accelerated fermentation metabolism • Reduction of SO_2 • Improved quality characteristics • Extended shelf life	• Uneven distribution • Selectivity • Equipment size/scalability • Risk of excessive cavitation • Cost-effectiveness	[7]
High hydrostatic pressure (HHP)	• Inactivation of microorganisms • Aging	• Reduction in microorganisms • Reduction of SO_2 • Accelerated aging • Improved sensory characteristics of red wine • Improved shelf life	• Undesirable sensory changes in white wine • Consumer acceptance • Equipment/operational costs	[10]
Pulsed electric field (PEF)	• Extraction • Malolactic fermentation • Inactivation of microorganinsm • Aging	• Increased phenolic content and anthocyanin extraction • Reduction in maceration time and temperature • Accelerated release of mannoproteins • Accelerated aging • Reduction of SO_2	• Variability in treatment efficacy • Selectivity • Limited scalability • Equipment complexity/cost	[11]
Ultra-high pressure homogenization (UHPH)	• Inactivation of microorganisms • Inactivation of oxidative enzymes • Extraction	• Reduction in microorganisms • Reduction of SO_2 • Improved extraction • Increased antioxidant activity • Preservation of sensory and nutritional quality	• Equipment cost/complexity • Limited scalability	[14]
Cold plasma (CP)	• Microbial inactivation	• Reduction in microorganisms • Reduction of SO_2 • Extended shelf life • Small impact on final quality	• Potential for sensory alterations • Consumer acceptance • Equipment complexity/cost • Process integration	[16,70]

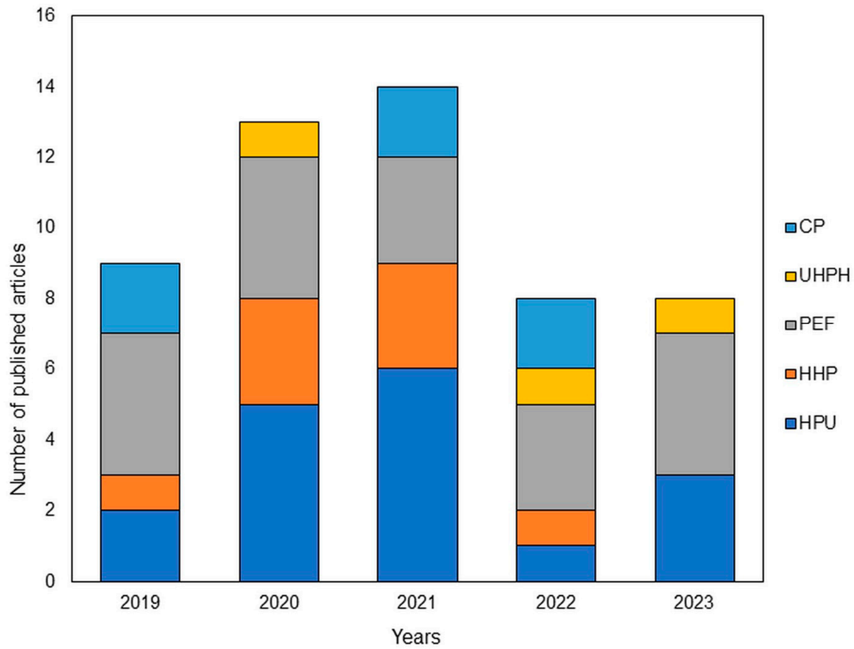

Figure 1. Number of published articles about non-thermal technologies applications in winemaking. CP: cold plasma; UHPH: Ultra-high pressure homogenization; PEF: Pulsed electric field; HHP: High hydrostatic pressure; HPU: High-power ultrasound.

Figure 2. Strategy employed in composing present review article.

3. Non-Thermal Technologies in Winemaking Process

3.1. High-Power Ultrasound (HPU) Effects on Varietal Aroma/Phenol Extraction and Wine Quality

High-power ultrasound technology with intensities above 1 W/cm^2 and frequencies of 20–100 kHz refers to the use of ultrasonic waves at power levels that are able to cause significant changes in various foods or beverages to which they are applied [72]. Most commonly, HPU is applied through liquid medium, and for this purpose there is commercially available equipment in the form of ultrasonic baths where probes are mounted in housing below the device (indirect system) and directly in liquid-immersed ultrasonic probes (direct system) (Figure 3) [73]. The acoustic cavitation is the main mechanism responsible for sonochemical effects when applied to a liquid (Figure 4). It is characterized by formation, growth, and violent bubble disruption [74]. As the consequence of the bubble disruption, i.e., collapse extreme conditions are generated (temperature and pressure), resulting in the production of free radicals, shock waves, and shear forces [75]. Both bath and probe systems can effectively trigger this phenomenon, which can lead to cell rupture and the release of bioactive compounds from the plant tissue [76]. However, the nature of the sample determines whether the ultrasonic bath will be used (gentle treatment) or directly immersed probe system (more aggressive application). In winemaking, HPU can be applied at different stages from extraction, fermentation, and microbial stabilization to aging, with very promising results [7,8]. Table 2 summarizes recent applications and effects of HPU technology in this field.

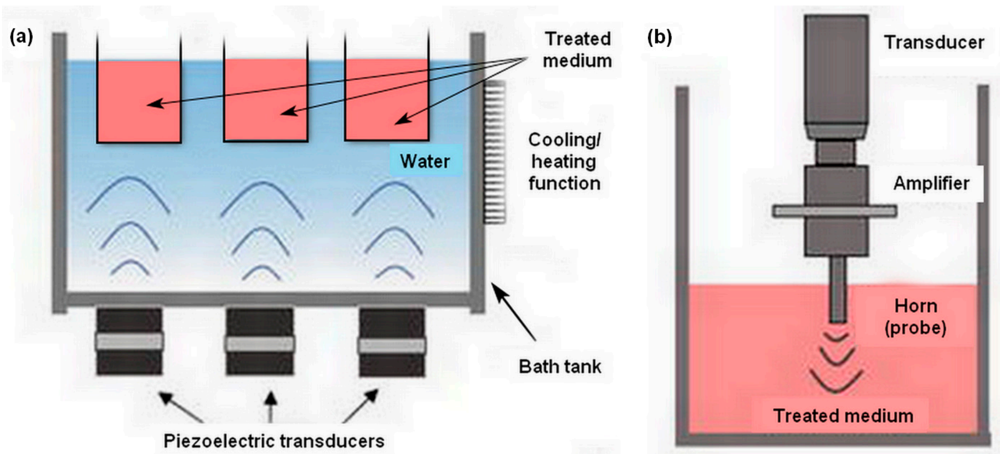

Figure 3. High-power ultrasound bath (**a**) and probe (**b**) systems. © 2020 Leire Astráin-Redín, Salomé Ciudad-Hidalgo, Javier Raso, Santiago Condón, Guillermo Cebrián, and Ignacio Álvarez. Adapted from Application of High-Power Ultrasound in the Food Industry; originally published under CC BY 3.0 license. Available from: 10.5772/intechopen.90444 https://www.intechopen.com/chapters/70675 (accessed on 14 March 2024) [73].

Recently, Lukić et al. [24] explored the effect of a HPU bath and probe on the quality composition of a young Cabernet Sauvignon red wine. Both HPU methods influenced phenolic, chromatic, and aroma composition of wine, whereby milder processing conditions resulted in a more beneficial effect. They found that ultrasound bath frequency had the greatest effect on red wine composition, followed by temperature and amplitude, while in HPU probe treatment, these were the following parameters: probe diameter, treatment time, and amplitude. In view of this, the authors pointed out that it is important to establish the most suitable HPU conditions to prevent excessive oxidation and reduction in phenolic and aroma compounds. In a same study, authors also explored the combined effect of HPU and antioxidants addition (SO_2 and glutathione) on wine composition during 6 months of aging, and found that HPU-treated wines showed lower concentration of phenolic compounds, but in combination with higher antioxidants addition this effect was delayed. Contrary, HPU did not induce major changes in large part of chromatic and aroma composition.

The same experimental methodology was applied on a young Graševina white wine in another study by Lukić et al. [25]. The both applied HPU systems influenced wine chemical composition, without notable changes in color. Namely, higher bath temperatures resulted in a reduction in aroma compounds, while larger probe diameter along with higher amplitude had less effect on phenolic and aroma composition. HPU effects were also determined after 18 months of aging, with greater changes in HPU-treated wines with low antioxidant content. In global, both studies showed that HPU can be considered as an efficient and reliable technology for production of low-sulfite wines. Furthermore, HPU (150 W, 30 °C, 30 min) was reported to be very efficient in reduction in higher alcohols in red wine, thus improving wine sensory characteristics [30]. The same authors also showed that this decrease was influenced by tartaric acid, ethanol concentration, mannitols, iron, and copper ions in the wine, which may be related to ultrasound-formed free radicals and their further reactions. Another study by Zhang et al. [34] also showed a reducing effect of HPU (59 kHz, 200 W, 30 °C, 30 min) on higher alcohols, as well as on phenolic compounds and color parameters, indicating the importance of selecting suitable ultrasound equipment (bath/probe) and conditions (frequency, power, time, temperature) during the processing of wine.

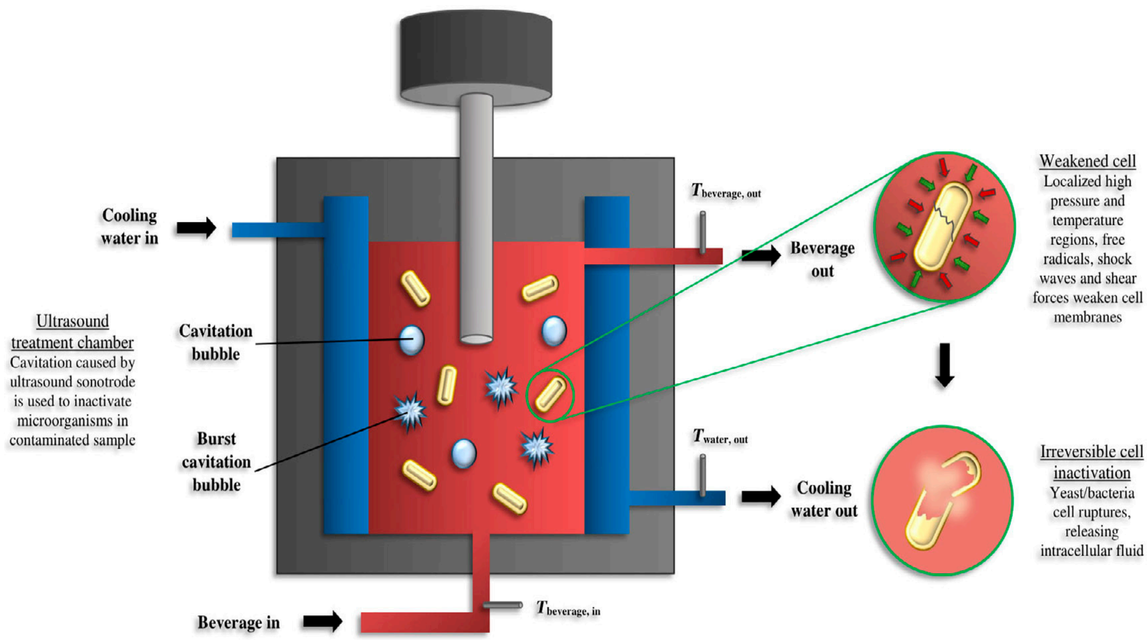

Figure 4. High-power ultrasound (HPU) mechanism of microbial inactivation in wine. T: temperature. This Figure was published in Preservatives and preservation approaches in beverages, Vol 15, Sanelle van Wyk and Filipa V.M. Silva, Nonthermal preservation of wine, 203–235, Copyright Elsevier (2019) [75].

Celotti et al. [27] studied the effect of HPU at various amplitudes (41 and 81%) and treatment times (1, 3, and 5 min) on the chemical composition of two young red wines and found no detrimental effect of HPU on anthocyanins and phenolic compounds, as well as chromatic characteristics with an increase in ultrasound parameters. However, higher amplitude (81%) induced greater reduction in tannins (15 and 40%) after wine storage (15 and 30 days). Recently, the effect of ultrasound amplitudes (30–90%) and treatment times (5–10 min) was also explored on the stability of proteins in two different Italian white wines [26]. The results demonstrated increased protein stability with an increase in ultrasound amplitude and treatment time, where HPU at 90% amplitude and 10 min achieved full stabilization effect on wines. Aside from that, HPU induced some positive changes in the conformational structure of wine proteins. Natolino and Celotti [28] used HPU at different amplitudes (30–90%) and treatment times (2, 6, and 10 min) to treat a young

Cabernet Sauvignon red wine. The results demonstrated no degradative effect of increased HPU processing conditions on wine phenolic profile. However, HPU led to an increment in the polymerization of tannins and a reduction in the astringency index and particle size. Similarly, Ahmad et al. [31] employed low-frequency ultrasound (24 kHz, 22 mm probe, 400 W, 100%, 3 min) to improve the taste quality of red wine, thus reducing tannins and astringency and increasing polyphenols and anthocyanins. Apart from improving wine organoleptic properties, HPU showed to be effective in increasing particle size distribution, viscosity coefficient, yield stress, and mass transfer in red wine [33]. On the other hand, Van Wyk et al. [29] applied HPU (24 kHz, 14 mm diameter probe, 125 µm amplitude, 0.73 mL/s flow rate) to five different table wines and revealed that antioxidant activity and total phenols of most wines decreased after HPU processing, while color density slightly increased. However, HPU did not influence the quality characteristics of wine during 2 months of storage.

In a study from Pérez-Porras et al. [20], a pilot-scale HPU system at two different frequencies (20 and 28 kHz) was applied during skin maceration (48 and 72 h) of Monastrell red grapes. Firstly, authors reported on morphological changes in grape cells induced by HPU processing. Secondly, HPU-treated grapes at 20 kHz showed a lower fermentation degree during the first period of fermentation, which was attributed to the HPU effect on the microbial population. After 72 h of maceration, the HPU-treated samples at 28 kHz presented the highest total phenol index, total anthocyanin content, and color intensity. However, at the end of fermentation and bottling, a 7-day macerated control sample showed the highest values of the abovementioned parameters, along with the content of polymeric anthocyanins and methyl cellulose precipitable tannins. In addition, all HPU-treated samples showed a higher extraction of total tannins in all three stages of wine production (after maceration, complete fermentation, and at bottling). No remarkable changes were observed in volatile acidity, tartaric, and malic acids. Finally, the higher alcohol and tannin content was observed in wines obtained from grapes treated by 20 kHz HPU, whereas higher anthocyanin content was achieved at 28 kHz. The same winery scale HPU system was also used to process Monastrell red grapes, favoring the extraction of grape phenolic compounds and allowing for the production of low-alcohol wines [19]. Moreover, HPU processing of red grapes at the same frequencies has been reported to increase free varietal aroma compounds (C6 alcohols, terpenes, and norisoprenoids) in musts and wines [22].

More recently, Labrador Fernández et al. [17] conducted a study on the effect of HPU on the color and aroma of rosé wines using a semi-industrial-scale system at 30 kHz, and found higher values of color intensity, total polyphenols, and anthocyanins in wines obtained from HPU-treated crushed grapes. The extraction of varietal aroma compounds in HPU musts was also enhanced, while finished HPU wines showed increased content of some norisoprenoids and terpenes. From a sensory aspect, HPU resulted in wines with more intense fruity-floral notes and aftertaste characteristics. Similarly, increased content of phenols and free thiols was observed after processing of Sauvignon Blanc white grape must with a HPU probe at 20 kHz and 153 µm amplitude [23]. In addition, Maier et al. [18] used a HPU probe device (20 kHz, 50–90%, 3–5 min) to treat crushed red Merlot grapes before maceration, and also observed increased the extraction rate of phenolic compounds and antioxidant activity.

Xie et al. [21] explored the effect of combined HPU and low temperature pretreatment on the quality parameters of Merlot dry red wine. In this study, low temperature, ultrasonic bath treatment (120, 240, and 400 W), and their combinations were applied to must before fermentation. The results demonstrated that HPU combined with low temperature pretreatment had a positive effect on the quality composition of produced wine, leading to an increase in the content of anthocyanins and phenolic acids, which additionally affected chromatic composition and improved antioxidant capacity. Furthermore, HPU of 240 W combined with low temperature pretreatment decreased unpleasant odors and enhanced fruity and floral aromas as well as the aftertaste of the wine. Recently, HPU bath treatment was applied during the fermentation process with the aim of enhancing the quality of fortified sweet red wine [32]. The study showed an increasing effect of ultrasound (200 W/20 min or 400 W/40 min) on total phenols, anthocyanins, and aroma compounds in fresh fortified wine, thereby improving sensory characteristics. In addition, a positive HPU effect was found on the color stability in aged fortified wines, along with no promoting effect on the increase in harmful ethyl carbamate.

To conclude this section, the literature shows that ultrasound technology can be used at different stages of winemaking to improve the extraction of valuable bioactive compounds, prevent protein precipitation, minimize requirements for SO_2 and fining agents, reduce alcohol content, and improve wine quality and sensory characteristics. In addition, HPU is also considered the most successful technology for the microbial stabilization of wine, with varying degrees of effectiveness in inactivating yeasts and bacteria, and accelerating the aging process. A critical review paper was recently published by Zhang et al. [7], highlighting that studies are moving towards combining ultrasound with other physical and chemical treatments to reduce processing time and nutrient losses and achieve better inactivation results. Despite the associated benefits, there may be potential degradation of phenolic and aroma compounds during HPU treatment, probably as a result of the accompanying thermal effect (increased temperature) of ultrasound. Therefore, it is necessary to precisely optimize and model the HPU conditions in order to achieve the highest treatment efficiency and maintain or even improve the overall wine quality. It should also be emphasized that a comparison of reported data are difficult, as differences in ultrasound systems, process parameters, and grape varieties can significantly influence the final result. Finally, with Resolution OENO 616-2019 [77], the OIV has recognized and supported the use of ultrasound in the processing of crushed grapes (must), particularly with the aim of enhancing grape compound extraction during pre-fermentative maceration after de-stemming and crushing. However, the OIV resolution still does not propose the application of HPU for finished wines.

Table 2. Summary on recent research outcomes for High-Power Ultrasound (HPU).

Sample	Processing Conditions	Key Outcomes	References
Cabernet Sauvignon red wine/Graševina white wine	Ultrasonic bath (40, 60, 100% amplitude; 37 and 80 kHz frequency; 20, 40, 60 °C bath temperature; 20, 50, 65, 90 min); Ultrasonic probe (diameter 12.7, 19.1, 25.4 mm; 25, 50, 75, 100% amplitude; 3, 6, 9 min; immersion depth 2 cm)	*Red wine:* • Mild ultrasound conditions showed a more beneficial effect on wine chemical composition. • Decreased phenolic content, lightness, and fatty acids in aged wines. • No effect on tannins and greater part of color and aroma in aged wines. • Reduction of SO_2 requirement. *White wine:* • No significant effect on color. • Degradation of aroma compounds with higher bath temperature. • Beneficial effect on phenolic and aroma composition with larger probe diameter and higher amplitude. • Reduction in addition of SO_2.	[24,25]
Cabernet Sauvignon red wine	Ultrasonic bath (45, 80, 100 kHz; 120–300 W; 20–60 °C; 20–80 min)	• Increased particle size distribution and rheological characteristics. • Modification of wine sensory characteristics. • Improved Tyndall effect of wine.	[33]

Table 2. *Cont.*

Sample	Processing Conditions	Key Outcomes	References
Cabernet Sauvignon red wine	Ultrasonic bath (25, 40, 59 kHz frequencies; 150–450 W powers; 20–45 °C temperatures; 10–50 min)	• Reduced content of higher alcohols. • Decrease affected by wine physicochemical parameters.	[30]
Red wine	Flat tip ultrasonic probe (diameter 13 mm; 20 kHz frequency; 41 and 81% amplitude; 1, 3, 5 min; immersion depth 2 cm)	• No degradation of phenolics. • Preservation of chromatic characteristics. • Decrease in tannins with increase in amplitude.	[27]
White wine	Flat tip ultrasonic probe (diameter 13 mm; 20 kHz frequency; 30, 60, 90% amplitude; 5 and 10 min; immersion depth 2–2.5 cm)	• Increase in protein stability. • Positive conformational changes in wine proteins. • Prevention of protein precipitation. • Reduction in quantity of fining agents.	[26]
Sauvignon Blanc and Pinot Gris white wines Syrah and Pinot Noir red wines Rosé wine	Ultrasonic probe (diameter 14 mm; max. amplitude of 125 µm; 24 kHz; continuous mode with a flow rate of 0.73 mL/s; residence time of 20.5 s; specific acoustic power 10.8 W/mL)	• No significant effect on pH. • Decrease in antioxidant activity and total phenolic content. • Increase in color density.	[29]
Monastrell red grapes	Pilot-scale power ultrasonic system (400 kg/h capacity; 2500 W power; 8 W/cm^2 power density; 20 and 28 kHz frequency)	• Reduced alcohol content. • Increase in phenolic extraction. • Improved wine chromatic characteristics. • No significant differences in wine physicochemical characteristics and aroma composition. • Modification of physical characteristics of grape skin. / • Increase in free varietal aroma compounds (C6 alcohols, terpenes, and norisoprenoids) in must and wine.	[19,20] / [22]
Cabernet Sauvignon red wine	Ultrasonic cleaning bath (45–100 kHz; 25–45 °C; 180–300 W; 10–50 min); Ultrasonic multi frequency cleaning bath (25–59 kHz; 20–45 °C; 150–450 W; 10–50 min) Horn ultrasonic cell crusher (diameter 6 mm; 20 and 25 kHz; 100–700 W; 18 °C; 30 min)	• Reducing effect on higher alcohols. • Better efficiency of cleaning bath compared to probe. • Changes in wine color parameters. • Slight effect on phenolic compounds. • Accelerating effect on wine aging.	[34]
Cabernet Sauvignon red wine	Ultrasonic probe (diameter 22 mm; 24 kHz frequency; 400 W; 50, 75, 100% amplitude; 60, 90, 180 s)	• Increased content of polyphenols. • Reduced content of tannins. • Reduced astringency.	[31]
Cabernet Sauvignon red wine	Flat tip ultrasonic probe (diameter 13 mm; 26 kHz frequency; 30, 60, 90% amplitude; 2, 6, 10 min; immersion depth 2 cm)	• No effect on phenolic composition. • Increase in HCl index. • Decrease in astringency index and particle size.	[28]

Table 2. *Cont.*

Sample	Processing Conditions	Key Outcomes	References
Monastrell red grapes	Semi-industrial-scale ultrasonic system (30 kHz frequency; 2500 W power; 8 W/cm^2 power density)	• Increase in color intensity and phenolics. • Enhanced extraction of varietal volatile compounds in musts. • Increased content of certain aroma compounds in wines. • Improved sensory characteristics of wines.	[17]
Merlot red grape must	Ultrasonic bath (120, 240, 400 W; 30 min) combined with low temperature pretreatment (4 °C/12 h)	• Increase in phenolics. • Increased antioxidant capacity. • Significant change in color. • Improved aroma and aftertaste.	[21]
Merlot red grapes	Ultrasonic probe (diameter 13 mm; 20 kHz frequency; 50, 70, 90% amplitude; 3, 4, 5 min)	• Increased extraction of phenolic compounds. • Increased antioxidant activity.	[18]
Cabernet Sauvignon fortified sweet red wine	Ultrasonic bath (20 kHz; 200, 300, 400 W; 20, 40, 80 min)	• Increased content of phenolic and aroma compounds. • Reduced degradation of anthocyanins. • Improved color stability and sensory characteristics. • Decreased content of ethyl carbamate after aging.	[32]
Sauvignon Blanc white grape must	Ultrasonic probe (diameter 13 mm; 20 kHz; 3 and 5 min; 153 µm amplitude; 200 W	• Increase in phenols and conductivity. • Increased extraction of free thiols.	[23]

3.2. High Hydrostatic Pressure (HHP) Effects on Wine Phenolics, Color, and Aroma

High hydrostatic pressure is one of the most studied non-thermal processing technologies used to preserve or modify food. HHP broadly encompasses the application of pressure (between 100 and 800 MPa) to food placed in a chamber, with or without packaging [75]. In HHP processing, the pressure is applied uniformly and instantaneously to the food from all directions according to Le Chatelier's principle (Figure 5) [78]. Regarding winemaking, HHP showed great potential in several areas, resulting in a reduction in microorganisms and requirements for SO_2, as well as in accelerated aging and improved wine quality characteristics [10]. In addition to that, HHP has proven to be excellent in enhancing the extraction of phenolic compounds into the must/wine by affecting the integrity of membranes and cell walls at higher pressures [79,80]. The latest HHP applications and their effects on wine are outlined in Table 3.

Santos et al. [36] examined the effect of HHP (500 MPa for 5 min) on the phenolic composition of red wine and compared it to the conventional wine aging methods (oak barrels, oak chips, and microoxygenation with oak chips). The results revealed that HHP affected wine phenolic composition, reducing monomeric anthocyanins, phenolic acids, and flavonols. Moreover, HHP treatment did not cause major modifications in the taste and color of wines. However, wines treated with HHP and those treated with microoxygenation and oak chips were characterized by a similar rate of tannin polymerization, pyranoanthocyanins, and percentage of prodelphinidins, indicating that HHP can lead to the production of wines with more aged-like characteristics. Recently, Valdés et al. [37] found that HHP treatments (400 MPa for 5 and 30 min) in combination with holm oak chips could produce wines with physicochemical and sensory characteristics similar to those from oak chips during classic maceration in tanks for 45 days. The impact of accelerated aging was observed in the white wine variety, while in the red Tempranillo variety, only minor sensory changes occurred, indicating the importance of the optimization of HHP processing conditions (pressure and holding time) for each wine variety, along with differences in the concentration and composition of wood chips.

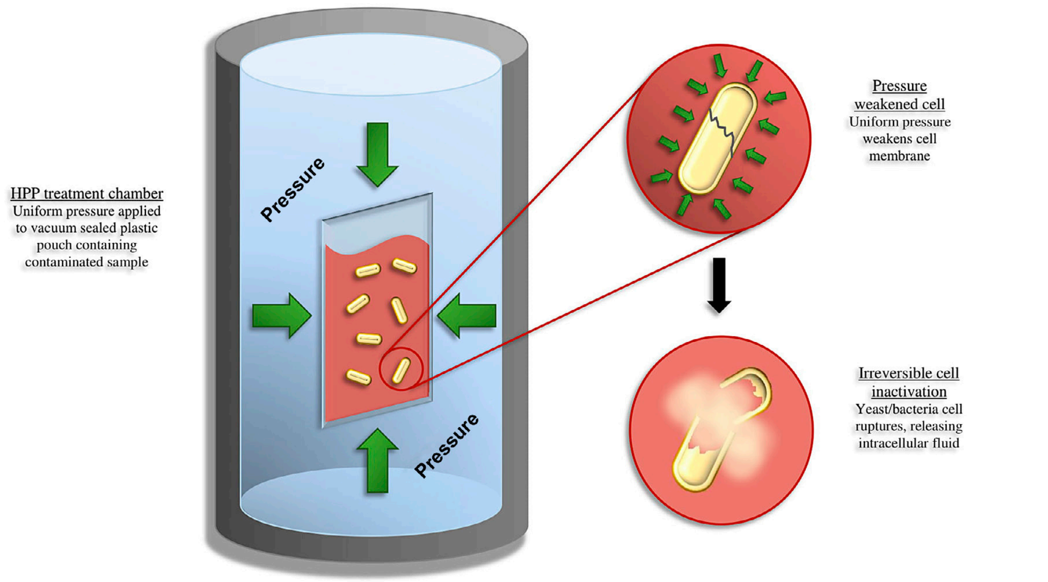

Figure 5. High hydrostatic pressure (HHP) mechanism of microbial inactivation in wine. This Figure was published in Preservatives and preservation approaches in beverages, Vol 15, Sanelle van Wyk and Filipa V.M. Silva, Nonthermal preservation of wine, 203–235, Copyright Elsevier (2019) [75].

In a study by Christofi et al. [39], HHP was applied to limit the use of SO_2 in wine production. Red wines made with the addition of SO_2 (0–100 mg/L) were pressurized at 350 MPa for 10 min, and the impact on the wine's chemical composition was investigated during 12 months of storage. The results showed that HHP did not affect the chemical composition of wine right after processing. However, after 6 months, HHP-treated wines (<60 mg/L SO_2) presented increased hue value and decreased concentration of monomeric anthocyanins and flavanols, as well as antioxidant activity. In addition, those wines were also characterized by scarce aromaticity, higher oxidation, and dried fruity notes compared to untreated wines. Nevertheless, after 12 months, no great difference was found in the chemical composition of pressurized wines containing over 60 mg/L SO_2 and unpressurized ones, suggesting that HHP could be used to complement the action mechanisms of SO_2.

Recently, Lukić et al. [41] treated young red and white low-SO_2 wines (free SO_2 25 mg/L for both) with different pressures (200–600 MPa) and treatment times (5–25 min) to evaluate the HHP effects on wine chemical composition. Aside from that, the authors investigated the combined effect of HHP (200 MPa for 5 min) and the addition of antioxidants (SO_2 and glutathione) during 12 months of aging. In general, HHP treatments led to a slight reduction in total and individual phenols in both wines immediately after processing, while an increase in some free phenolic acids (white wine) and color was observed. These changes were more conditioned by applied pressure than by treatment time. After 12 months, the pressurized red wines had a lower concentration of phenols, anthocyanins, and flavanols, while the color and aroma composition remained the same. In contrast, HHP affected the aroma composition of white wines after 12 months without major changes in most phenols and color. Finally, pressurized wines with standard SO_2 (free SO_2 25 mg/L for reds; free SO_2 45 mg/L for whites) and low SO_2 and glutathione (free SO_2 10 mg/L + 20 mg/L GSH for reds; free SO_2 25 mg/L + 20 mg/L GSH for whites) were very similar in aroma composition (red wine) and in phenolic and color composition (white wine). This aging experiment indicated that combining the addition of HHP and glutathione along with lower SO_2 doses may be a promising approach for preserving wine. In addition, Christofi et al. [40] also combined HHP with the addition of glutathione and SO_2. Red wine was firstly subjected to different HHP treatments (200–600 MPa, 0–15 min), after which optimal HHP parameters (400 MPa for 5 min) were selected based on acceptable changes in quality characteristics, precisely on the lower reduction in polyphenols, anthocyanins, and proanthocyanidins, and the lower increase in volatile acidity and acetic acid values. Then, red wines containing glutathione (10 mg/L) and SO_2 (0–100 mg/L) were treated with selected HHP conditions. After 12 months of storage, HHP-treated wines were sensory characterized as "mature" with higher content of aldehydes and ketones, and lower content of phenols and esters. Furthermore, pressurized wines with intermediate content of SO_2 (40 and 60 mg/L) were considered to be better in body, balance, and overall quality, followed by lower astringency and bitterness, suggesting that these SO_2 doses in combination with HHP and glutathione could ensure adequate wine stability.

Tomašević et al. [38] reported that HHP (200 MPa for 15 and 25 min) had more beneficial effect on the reduction in *B. bruxellensis* in red wine, as its culturability was not established immediately after processing, while the same HHP conditions were only able to decrease the population of *S. cerevisiae* yeasts in sweet white wine. However, during a 90-day storage, the results showed that the population of *B. bruxellensis* fully recovered after initial inactivation by HHP, indicating its ability to transition to a viable but nonculturable state, whereas the *S. cerevisiae* population was completely reduced. Moreover, HHP treatments (100 and 200 MPa, 1–25 min) slightly decreased the content of total anthocyanins in red wine, while minor variations in total phenols and total color difference were not related to applied treatments.

Van Wyk et al. [29] used HHP of 600 MPa for 5 min to treat five different table wines and found that HHP had very small or even no impact on wine quality characteristics (total phenolic content, antioxidant activity, color density, and pH) directly after treatment. After 2 months of storage, no significant differences were observed between total phenols, antioxidant activity (except of lower value in HHP Pinot Gris), and pH of pressurized and unpressurized wines. Additionally, some HHP-treated wines (Pinot Noir, Rosé, and Pinot Gris) showed higher color density compared to the untreated ones. More recently, Cao et al. [35] applied HHP (200–600 MPa for 5 min) on red raspberry wine and observed that HHP greatly affected the aroma and phenolic composition, as well as sensory quality. Among the applied treatments, HHP at 400 MPa resulted in considerably higher content of alcohols, esters, and ellagic acid. However, HHP conditions up to 400 MPa had no effect on the alcohol content of wine, while higher pressures (500–600 MPa) reduced its content, as well as the content of ellagic acid.

In summary, these studies have shown that HHP provides certain benefits to the winemaking process, such as accelerating wine aging, reducing wine spoilage microorganisms, limiting the need for SO_2, and improving some quality characteristics and stability of wines. These reports have also highlighted the trends in a multidisciplinary approach to the HHP processing of wine, which aims to produce high-quality wines with low or no SO_2 by using combined physical and chemical methods. Among the various high pressure technologies, high-pressure treatment with supercritical CO_2 (100 bar, 10% CO_2, 10 min) has recently been proposed as a novel and effective method to reduce the use of SO_2 in white grape must while ensuring microbial safety, preserving the phenolic composition and maintaining the sensory characteristics of the must [81]. In addition, the effect of HHP has been recently reviewed in terms of the extraction of bioactive compounds such as anthocyanins from grape/grape juice and their stability [13–15], indicating that this is also an important area under investigation by scientists, especially in terms of shortening maceration. In addition, HHP was admitted in 2019 by OIV Resolution OENO 594A-2019 [77] for discontinuous HHP processing of grapes and musts (>150 MPa), with the purpose of controlling the microbial population of grapes, reducing SO_2 doses and accelerating maceration in red winemaking.

Table 3. Summary on recent research outcomes for High Hydrostatic Pressure (HHP).

Sample	Processing Conditions	Key Outcomes	References
Red wine	500 MPa for 5 min at 20 °C	• Decrease in phenolic compounds after 5 months of aging. • No significant effect on sensory characteristics. • Promotion of phenolic reactions similar to those observed during aging processes.	[36]
Red wine	350 MPa for 10 min at 8 °C	• No effect on chemical composition at the early aging stage. • Reduced content of phenolics and antioxidant activity in pressurized wines (<60 mg/L SO_2) after 6 months. • No significant difference in chemical composition compared to unpressurized wine (>60 mg/L SO_2) after 12 months. • Changes in wine sensory characteristics. • Complementation of SO_2 action mechanism.	[39]
Cabernet Sauvignon red wine and Graševina white wine	200, 400 and 600 MPa for 5, 15 and 25 min at room temperature	• Greater changes in phenolic composition with higher processing conditions. • Increase in color parameters in red wine and some phenolics in white wine. • No effect on greater part of aroma in red aged wine. • No effect on most of phenolics and color in white aged wine. • No significant changes in sensory characteristics. • Reduction in uses of SO_2.	[41]
Cabernet Sauvignon red wine and Graševina sweet white wine	100 and 200 MPa for 1, 3, 5, 15 and 25 min	• Culturability of *B. bruxellensis* not confirmed right after pressurization in red wine. • Decrease in culturability of *S. cerevisiae* in sweet white wine. • Complete reduction in *S. cerevisiae* during wine storage. • Complete recovery of culturability of *B. Bruxellensis* during storage. • Slight decrease in anthocyanins in red wine. • Microbial stabilization of sweet wines and reduction in SO_2.	[38]
Red wine	200, 400 and 600 MPa for 0, 5 and 15 min	• Significant reduction in phenolic compounds, volatile acidity, and aceti acid content. • Modifications in wine phenolic and aroma compounds after 12 months. • Changes in wine sensory characteristics during 12 months. • Reduction in required doses of SO_2.	[40]
Tempranillo red wine and Cayetana white wine	400 MPa for 5 and 30 min	• Modified quality characteristics of white wine. • No major changes in chemical composition of red wine. • Accelerated aging of white wine.	[37]
Sauvignon Blanc and Pinot Gris white wines Syrah and Pinot Noir red wines Rosé wine	600 MPa for 5 min	• No significant effect on wine quality parameters directly after processing. • Small overall effect on wine quality characteristics after 2 months of storage.	[29]
Red raspberry wine	200, 300, 400, 500 and 600 MPa for 5 min at 20 °C	• Significant changes in wine aroma compounds. • Increase in total ellagic acid content. • Reduction in alcohol content. • Improvement of wine sensory characteristics.	[35]

3.3. Pulsed Electric Field (PEF) Effects on Microbial Populations, Phenolic Extraction, and Wine Quality

Pulsed electric field is relatively novel non-thermal and emerging technology for food preservation that involves the application of short microsecond pulses of high electric field strength (5–50 kV/cm) to liquid or semi-solid foods placed between two electrodes [82]. In wine production, PEF technology has great potential due to its continuous operating mode [5]. Since PEF has the ability to inactivate microorganisms and enhance mass transfer by increasing cell membrane permeability via an electroporation mechanism (Figure 6), it presents a promising method for the reduction in wine spoilage microorganisms, extraction improvement of valuable compounds during winemaking, and acceleration of wine aging [11]. The main factors that can impact PEF efficiency in grape/wine processing can be divided into process parameters, properties of treated medium, and the microorganisms' characteristics [83]. The effects of PEF on grapes, must, and wine are shown in Table 4.

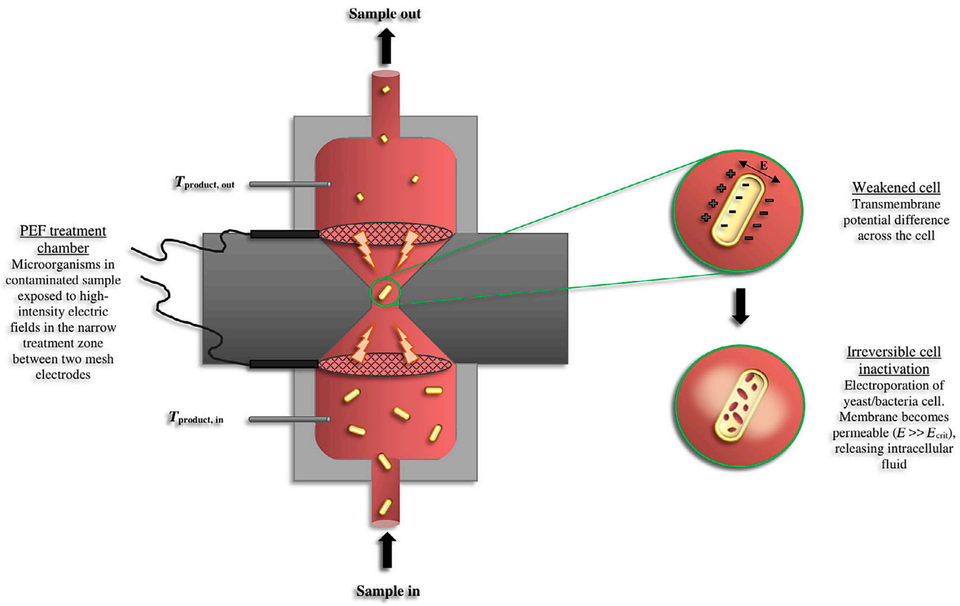

Figure 6. Pulsed electric field (PEF) mechanism of microbial inactivation in wine. T: temperature. This Figure was published in Preservatives and preservation approaches in beverages, Vol 15, Sanelle van Wyk and Filipa V.M. Silva, Nonthermal preservation of wine, 203–235, Copyright Elsevier (2019) [75].

Recently, González-Arenzana et al. [47] investigated the effects of PEF on the microbial population in Tempranillo red wines aged in oak barrels without using additional preservatives. A PEF continuous flow system was used, operating at an electric field strength of 23 kV/cm with a pulse width of 8 µs, specific energy of 95 kJ/kg, and a frequency of 330 Hz. The results showed significant inactivation of must yeasts (*Brettanomyces*, *Dekkera*, *Pichia*) and lactic acid bacteria (*Lactobacillus*, *Oenococcus*, *Pediococcus*) in aged wines right after PEF treatment, which was also accompanied by a reduction in volatile phenols (4-ethylphenol and 4-ethylguaiacol) in wine with the highest population of *Brettanomyces*/*Dekkera*. However, during the sampling period (5–25 months later), the recovery of some microorganisms (*Brettanomyces*/*Dekkera* and *Pediococcus*) in PEF-treated wines was observed. Similar findings were reported in another study by González-Arenzana et al. [48], where PEF treatments (PEF alone and PEF with 15 mg/L SO_2) applied to red wine just after malolactic fermentation resulted in higher inactivation of yeasts, acetic acid, and lactic acid bacteria compared to one achieved only with SO_2 (30 mg/L) four days after treatments. After 6 months, the results showed that all treatments were effective for controlling most of microbial population, with the exception of lactic acid bacteria which was still viable in some of the wines. Moreover, PEF maintained wine sensory quality and provided wines with lower volatile acidity and greater color intensity despite reduced anthocyanin content.

More recently, Delso et al. [46] reported on the lethal effects (up to 4.0 log reductions) of both individual PEF treatments (15–25 kV/cm) and those that are moderate (15 kV/cm) combined with SO_2 (0–30 mg/L) on *S. cerevisiae* and *O. Oeni* in treated red wine after fermentation. After 4 months, PEF-treated wine after alcoholic fermentation had no viable yeasts, while lower than 1×10^2 CFU/mL of *O. Oeni* viable cells were detected in PEF-treated wine with 20 mg/L of SO_2 after malolactic fermentation. Moreover, applied PEF treatments did not affect oenological as well as wine sensory characteristics after the storage period. Similar conclusions were drawn by Delso et al. [44], who reported that PEF treatments did not influence the physicochemical and aroma composition of Chardonnay white wine during 4 months of storage. The authors applied PEF treatments of low (35 kJ/kg, 65 µs) and high (97 kJ/kg, 177 µs) intensity at 15 kV/cm in combination with different doses of SO_2 (0, 5 and 20 mg/L), and found that even the least intense PEF treatment was efficient in obtaining *S. cerevisiae* var. *bayanus* CHP yeast-free wine during the storage period without added SO_2. In addition, the synergistic effect of combined PEF (10–25 kV/cm, 25–1000 µs, 40–170 kJ/kg) and SO_2 (10, 25 and 50 mg/L) treatments on the inactivation of *S. bayanus* and *B. bruxellensis* in red wine was also studied by Delso et al. [45]. The authors reported the significant inactivation (>4.0 log cycles) of both yeast types right after applying the less intense PEF treatment (10 kV/cm, 115 kJ/kg). After 24 h of incubation among the treated yeasts, moderate PEF treatment (15 kV/cm, <100 kJ/kg) alone and the ones combined with SO_2 treatment demonstrated efficient lethal effect on both yeasts.

In a study by van Wyk et al. [50], the authors examined the effects of PEF parameters (electric field strength, outlet temperature, and specific energy), *B. bruxellensis* yeast strain, and alcohol content on *B. bruxellensis* inactivation in Cabernet Sauvignon red wine. Among the processing parameters, the electric field strength had a higher effect on *B. bruxellensis* inactivation than specific energy, resulting in 1.0, 2.1, and 3.0 log reductions when using PEF at 31, 40, and 50 kV/cm. Furthermore, an increase in outlet temperature of 10 °C at 50 kV/cm resulted in the double inactivation of 3.0 log CFU/mL. Other factors such as yeast strain and alcohol content also showed a significant influence on PEF inactivation. Moreover, PEF processing did not lead to the dangerous dissolution of metal ions (Fe, Cr, Ni) in wine. Recently, Akdemir Evrendilek [43] showed that PEF with increased applied energy (2.4–13.2 kJ) resulted in significant inactivation of all yeasts (*S. cerevisiae, H. anomola, C. lipolytica*) and bacteria (*L. delbrueckii* ssp. *bulgaricus*, *E. coli*) in red wine with a greater increase in pH value, conductivity, color parameters (L* and b*), and total phenolic substance content with no remarkable change in the concentration of metal ions. Additionally, 13.2 kJ PEF treatment induced significant changes in the sensory characteristics of wine. On the other hand, van Wyk et al. [29] exposed five different table wines to PEF at an electric field strength of 45 kV/cm and frequency of 800 Hz and found that PEF had no effect on the total phenols, antioxidant activity (except for a decrease in Syrah red wine), and pH value, while it increased the color density in red and white wines directly after processing. After 2 months, authors observed similar patterns in the quality characteristics of most treated wines.

Vaquero et al. [57] applied two continuous PEF treatments, first to crushed red grapes (5 kV/cm) to increase the extraction of phenolic compounds and color, and second to the must after 24 h maceration (17.5 kV/cm) to reduce the wild microbial population and improve the implantation of non-*Saccharomyces* yeasts. PEF processing of grapes resulted in increased color intensity, total phenol index, and anthocyanin and tannin content in produced musts, while pH, °Brix, and the total acidity were not affected. In addition, successful reduction in wild yeasts (up to 2 log CFU/mL) was achieved after a second PEF treatment of higher intensity. Consequently, PEF allowed for selected non-*Saccharomyces* yeasts to better implant in obtained red musts and generate more desired metabolites in wines, resulting in more total esters and anthocyanins, especially vitisin A. The overall sensory perception of wines with PEF was described as more complex, with higher intensity of some attributes such as aroma intensity, fruit, and acidity. A similar trend was observed in a study from Ricci et al. [55] where early-harvested Sangiovese red grapes were subjected to pre-fermentative PEF treatments (0.9–3 kV/cm, 10.4–32.5 kJ/kg) to enhance the extraction of phenolic compounds and to maximize the color quality in musts and wines. Applied PEF treatments coupled to maceration processes resulted in musts and wines with increased total polyphenol content (49.0–60.8% and 22.7–37.1%) and color intensity (39–51% and max. 13.8%). Moreover, Leong et al. [58] applied high-intensity PEF pretreatment followed by 4 days of cold maceration to Merlot red grapes, and also found an increase in a wide variety of anthocyanins and color intensity in grape musts. In addition, a similar PEF experiment was conducted on aroma compounds at different stages of winemaking [59]. Except for the increased content of anthocyanins, stilbenes, and hydroxycinnamic and hydroxybenzoic acids, Merlot wines produced with PEF-treated grapes (>40 kV/cm) showed higher blackcurrant flavor and odor. Recently, Garde-Cerdán et al. [54] reported on the enhanced extraction of total stilbenes from the Graciano, Tempranillo, and Grenache grape varieties by PEF (7.4 kV/cm, 400 Hz, 20 µs), while increased content of aroma and nitrogen compounds was only found in PEF-treated Grenache grape must. Similarly, this was found for stilbenes of the Graciano variety in a study by López-Giral et al. [51]. Except for the improved extraction of valuable grape compounds, the benefit of PEF grape pretreatments was in reducing the maceration time from 6 to 3 days [52], or even 24 h [53], for the Grenache variety.

Another study by Morata et al. [49] reported that the initial microbial population in grape musts was reduced by 1 log CFU/mL with continuous PEF treatment (20 kV/cm, 150 pulses, 3 µs). A high viability of inoculated non-*Saccharomyces* yeasts was also maintained at the end of fermentation. Additionally, PEF-treated musts had a higher amount of some yeast metabolites, primarily esters, due to better implantation of the selected yeasts. Furthermore, Aguiar-Macedo et al. [42] conducted a pilot plant-scale study, using PEF (240 L/h, 4 bar) of 15 kV/cm and 35 kJ/kg for inactivation of *B. bruxellensis* and other spoilage yeasts, and to evaluate its impact on the physicochemical and sensory characteristics of red wine. After PEF treatment, *B. bruxellensis* was not detected in wine (detection limit of <150 viable cells/mL), while total yeast count showed a reduction of 80.66%. A slight but significant increase was observed in anthocyanins, total phenols, color intensity, and turbidity, while sensory characteristics of treated wine were not affected. Another pilot-scale study was carried out recently by Silva et al. [56], who applied continuous PEF at two different stages of winemaking from Arinto and Moscatel white grape varieties. Firstly, PEF was used to treat grapes prior to pressing for must extraction (1.2 and 1.6 kV/cm) and later prior to bottling for stabilization/decontamination of produced wines (10 kV/cm). Both extraction and stabilization PEF treatments did not induce significant changes in the sensory characteristics (color, odor, and taste) of both wines. However, CIE color parameters analysis showed a significant increase in b* value (more intense yellow color) of PEF extracted Arinto and Moscatel wines. Regarding physicochemical characteristics, PEF extraction increased pH value and turbidity in both wines, while there was no impact on total acidity. Total phenols also increased with PEF extraction, but only in Arinto wine. In addition, PEF stabilization treatment had no effect on both wine varieties' quality characteristics, besides for a decrease in total phenols in the Moscatel wine. More recently, Toulaki et al. [60] employed PEF (1.1 kV/cm, 10 µs, 20 min) as an alternative wine aging method in four Xinomavro red wines in combination with various wood chips. They concluded that PEF had a minor to no effect on the enrichment of polyphenols, while the addition of wood chips showed a significant effect. However, PEF treatment notably increased the extraction of aroma compounds (esters, alcohols, and aldehydes) from wood chips, resulting in the enhanced overall sensory quality of wines.

In summary, most of the recently available studies deal with the ability of PEF technology to inactivate spoilage microorganisms in wine, but its effectiveness has also been recognized in the field of extraction of valuable components by enhancing mass transfer, shortening maceration time and reducing SO_2 during winemaking while maintaining/improving wine quality and sensory characteristics. Several reviews have already summarized the recent advances and applications of PEF technology to improve the extraction of various phenolic compounds during winemaking [79,84,85]. Many of the conclusions from these reports are confirmed by the new findings presented here. In addition, the working properties of PEF, such as continuous flow, constant temperature, and very short treatment time, are suitable for grape/wine processing. Moreover, the potential of this technology was recognized by the OIV in 2020, which passed a special Resolution OENO 634-2020 [77] on the application of PEF on red and white destemmed and crushed grapes to improve the extraction of phenolic and aroma compounds (precursors), yeast-available nitrogen, and other components contained inside the grape cells, as well as to reduce maceration process. However, despite numerous reports on the effectiveness of PEF in improving the microbial stability of musts and wines, the OIV resolution does not refer to the application of the PEF system for microbial control in wineries. Furthermore, the potential of combining PEF with other new technologies to improve wine quality and safety was emphasized and recommended as a further direction for the development of this technology [9,11,12].

Table 4. Summary on recent research outcomes for Pulsed Electric Field (PEF).

Sample	Processing Conditions	Key Outcomes	References
Tempranillo red wine	23 kV/cm; 330 Hz; 95 kJ/kg; 8 µs pulse width; square bipolar pulses; continuous flow; T < 22 °C	• Inactivation of *Brettanomyces/Dekkera* population in aged wine. • Significant reduction in volatile phenols. • Inactivation of lactic acid bacteria population in young wine.	[47]
Tempranillo Rioja red wine	33 kV/cm; 158 kJ/kg; 105 µs treatment time; 8 µs pulse width; continuous flow; T < 22 °C	• Effective reduction in yeasts and bacteria populations 4 days after treatment. • Positive effect on physicochemical characteristics 6 months after treatment. • No change in organoleptic quality of wine. • Reduction in SO_2.	[48]
Cabernet Sauvignon red wine	31, 40 and 50 kV/cm; frequencies of 100 Hz (up to 84 µs) and 250 Hz (up to 256 µs); 80–132 kJ/kg; continuous flow; T < 40 °C	• Significant effect on *Brettanomyces* inactivation: 3 log CFU/mL reduction. • No significant changes in metal ions. • No significant effect on wine quality including sensory characteristics.	[50]
Caladoc and Grenache red grapes	4 kV/cm; 3.7 pulses of 100 µs width; 2500 kg/h flow rate / 5 kV/cm; 8 and 46 pulses of 40 µs width; 140 kg/h flow rate; 320 and 1840 µs treatment time; 8.8 and 52.9 kJ/kg; T < 25–40 °C	• Enhanced extraction of polyphenols and aroma precursors. • Reduced maceration time from 6 to 3 days. / • Improved phenolic extraction, increased color intensity and total phenol index. • Reduction in maceration time to 24 h.	[52] / [53]
Red grape must	20 kV/cm; 84 kJ/kg; 150 µs (50 pulses of 3 µs); square pulses; continuous flow; T < 30 °C	• Reduction in wild yeasts by 1 log CFU/mL. • Better implantation of selected non-*Saccharomyces* yeasts. • No major changes in enological parameters of musts. • Improved metabolic and sensory profile of wines.	[49]
Sangiovese red grapes	Pilot plant-scale PEF system; 0.9–3 kV/cm; 10.4–32.5 kJ/kg; continuous flow; T < 25°	• Improved extraction of phenolic compounds. • No effect on physicochemical parameters. • Improved color profile and stability in wines.	[55]

Table 4. *Cont.*

Sample	Processing Conditions	Key Outcomes	References
Sauvignon Blanc and Pinot Gris white wines Syrah and Pinot Noir red wines Rosé wine	45 kV/cm; 800 Hz frequency; square bipolar pulses; 46 pulses of 70 µs; continuous flow	• No significant changes in total phenolics, antioxidant activity and pH after processing and 2 months of storage. • General increase in color density.	[29]
Red grape must	5 and 17.5 kV/cm; 63.4 kJ/kg; 45 pulses of 40 µs; monopolar rectangular pulses; T < 40 °C	• Improved extraction of phenolic compounds. • No effect on physicochemical parameters. • Antimicrobial effect: up to 2 log CFU/mL reduction. • Improved implantation of non-*Saccharomyces* yeasts. • Improved organoleptic characteristics.	[57]
Red wine	0, 17, 24 and 31 kV/cm; 500 pps of frequency; 0, 163, 325 and 488 µs treatment time; 40 mL/min; 20 µs pulse delay time; three µs pulse duration; square bipolar pulses; 0–13.2 kJ	• Significant increase in total phenolics and physicochemical parameters. • Significant inactivation of yeasts and bacteria: >5 log CFU/mL reduction. • No significant change in metal ions. • Impact on wine sensory characteristics.	[43]
Graciano, Tempranillo and Grenache red grapes	7.4 kV/cm; 300–400 Hz; 10–20 µs pulse width	• Improved aroma composition, enhanced yeast assimilable nitrogen and total amino acids of Grenache variety. • Enhanced total stilbene extraction in all grape varieties. / • PEF induced changes in chemical composition depending on grape variety. • Improved stilbene content and reduced maceration time for Graciano variety.	[51] / [54]
Chardonnay white wine	15, 20 and 25 kV/cm; 10–117 Hz; 35–117 kJ/kg; 0–200 µs treatment time, pulses of 10 µs width; monopolar square pulses; continuous flow; T ≤ 50 °C	• Reduction in yeast population (*S. cerevisiae*): around 4 log cycles. • No effect on wine physicochemical parameters and aroma during 4 months of storage. • Sublethal effect on yeast cells. • Complementation of SO_2 action mechanism and its reduction.	[44]
Red wine	10–25 kV/cm; frequency up to 200 Hz; square pulses of 5 µs width; 25–1000 µs treatment time; 40–170 kJ/kg; continuous flow; T ≤ 60 °C	• Significant inactivation of *Saccharomyces bayanus* and *B. Bruxellensis* (>4 log cycles) right after treatment. • Sublethal effect of moderate PEF treatments: 3–4 log CFU/mL reduction in yeast population. • Synergistic lethal effect of combined PEF and SO_2 treatments. • Reduction of SO_2 levels in wine production.	[45]

Table 4. *Cont.*

Sample	Processing Conditions	Key Outcomes	References
Red wine	15, 20 and 25 kV/cm; 8–80 Hz frequencies; 20–175 µs treatment time; 35–120 kJ/kg; continuous flow; T < 50 °C	• Significant effect on *S. cerevisiae* and *O.oeni* inactivation: up to 4 log cycles. • Successful wine microbial stabilization for at least 4 months of storage. • No significant effect on wine physicochemical and sensory characteristics. • Reduction of SO_2.	[46]
Red wine	Pilot-scale PEF system; 15 kV/cm; 35 kJ/kg; 150 Hz; 10 µs; 1500 V; square bipolar pulses; continuous flow; T = 25.5 °C	• Effective inactivation of *B. Bruxellensis* (<150 viable cells/mL). • Reduction in total yeast population. • No significant effect on wine sensory quality. • Small effect on phenols, turbidity and color intensity.	[42]
Arinto and Moscatel Graúdo white grapes and wines	Grapes: 1.2 and 1.6 kV/cm; 100 Hz frequency; monopolar pulses of 50 µs width Wines: 10 kV/cm; 150 Hz frequency; monopolar pulses of 25 µs width; 60–70 kJ/kg; continuous flow	• Significant increase in color, turbidity and pH by PEF extraction treatment in both wine varieties. • Increase in total phenols in Arinto variety. • No impact of PEF stabilization treatment on wine quality parameters. • Decrease in total phenols in Moscatel wine. • No effect on sensory quality of wines. • Reduction in pre-bottling SO_2 addition.	[56]
Xinomavro red wine	Stationary bench scale PEF system; 1.1 kV/cm; pulse width 10 µs; pulse period 1000 µs; rectangular monopole pulses; total treatment duration 20 min	• Improved extraction of aroma compounds from wood chips. • A slight decrease in total phenols. • No visually detectable color alterations. • Enhancement of wine sensory characteristics. • Accelerated wine aging.	[60]
Merlot red grapes	Continuous PEF system; 25 and 33 kV; 2–25 Hz pulse frequency; 33.1 and 41.5 kV/cm, 4.7–49.4 kJ/L	• Increased extraction of a wide variety of phenolic compounds. • More intense color of grape must. • Higher blackcurrant flavor and odor in finished wines.	[58]/[59]

3.4. Other Non-Thermal Technologies in Winemaking Process

3.4.1. Ultra-High Pressure Homogenization (UHPH) Effects on Microbial Populations and Wine Quality

Ultra-high pressure homogenization has also been recognized as an emerging non-thermal technology that operates in continuous mode at pressures in the range of 200–600 MPa [86]. Principally, the working mechanism consists of pumping a liquid food containing particles lower than 500 µm at high pressure, and later depressurizing when the fluid passes through a special valve [4]. The fluid medium inside the valve is exposed to extreme impact and shear forces, causing submicron nanofragmentation of microorganisms, colloids, and biopolymers (Figure 7) [15]. The application of UHPH in winemaking is limited to processing grape musts without peels, seeds, and other solids which can clog the homogenizer valves [14,85]. In addition, UHPH mechanical action and nanofragmentation of colloidal particles are the main mechanisms affecting the extraction of anthocyanins and other phenolic compounds by enhancing their release from the cells and oxidative stability [15,87]. Due to its effectiveness in the elimination of wild microorganisms, inactivation of oxidative enzymes, and enhancement of extraction process with low impact on sensory and nutritional quality, UHPH is considered to be a valuable tool for the wine industry [14].

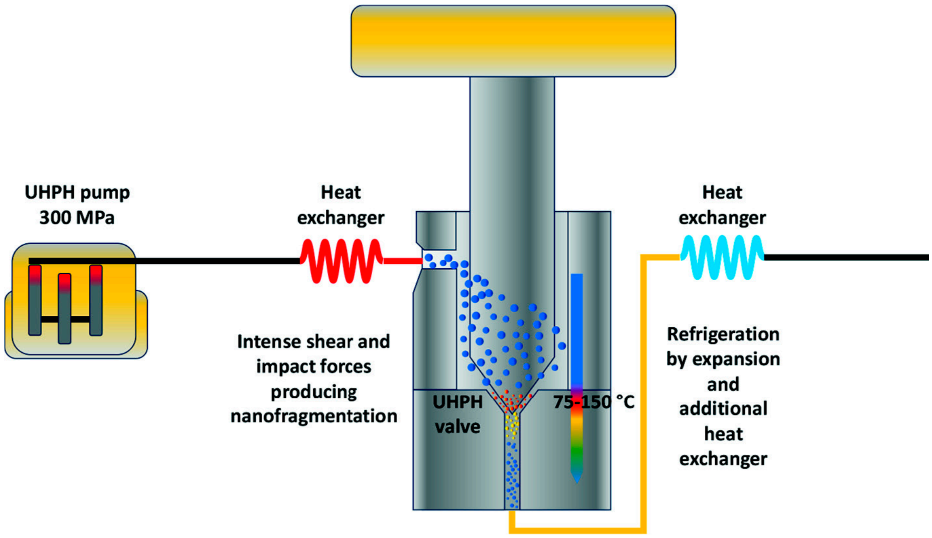

Figure 7. Ultra–high-pressure homogenization (UHPH) working mechanism. © 2024 Antonio Morata, Carlos Escott, Juan Manuel del Fresno, Buenaventura Guamis, Iris Loira, María Antonia Bañuelos, Carmen López, Felipe Palomero, and Carmen González. Originally published in Use of UHPH to Sterilize Grape Juices and to Facilitate the Implantation of Saccharomyces and Other Emerging Fermentation Biotechnologies in Wines under CC BY 4.0 license. Available from: 10.5772/intechopen.1003954 https://www.intechopen.com/chapters/1156686 (accessed on 28 March 2024) [86].

In a research study by Loira et al. [62], authors used UHPH (300 MPa, inlet and outlet temperatures of 20 and 25 °C, in-valve temperature 98 °C for 0.02 s) to treat white must. It was found that UHPH was able to completely eliminate non-*Saccharomyces* wild yeasts and vegetative bacteria populations. Moreover, a high inactivation for polyphenol oxidase activity was also observed. Additionally, wines produced from UHPH treated must were better in global quality, showing more fruity notes and increased aroma complexity. Similar results were obtained in another study by Bañuelos et al. [61], where UHPH treatment (300 MPa, inlet and outlet temperatures of 23–25 °C and 13–15 °C, in-valve temperature 65–78 °C for 0.02 s) of white must resulted in total inactivation of wild microorganisms, and higher antioxidant activity due to inactivation of oxidative enzymes. UHPH musts showed no fermentative activity for longer than 60 days, no thermal damage and no significant changes in terpenes and other aroma compounds. Similar conclusions were also drawn by Vaquero et al. [64], who processed Cabernet Sauvignon red must with UHPH (300 MPa, inlet and outlet temperatures of 4 and 15 °C, in-valve temperature 78 °C for <0.2 s). Among others, authors highlighted the better colloidal stability of UHPH must and the protective effect of UHPH regarding polyphenols and color. Namely, UHPH must showed higher content of anthocyanins, particularly those in acylated forms (+9.3%), resulting in more red–blue color. In order to investigate the influence of UHPH (300 MPa, inlet and outlet temperatures of 4 and 14 °C, in-valve temperature 95 °C for <0.2 s) on wine characteristics, Puig-Pujol et al. [63] carried out alcoholic fermentation with UHPH white musts and compared it to the non-sulfited and sulfited fermentations (60 mg/L total SO_2). This research also confirmed the antimicrobial and antioxidative effect of UHPH with no detrimental changes in quality characteristics, as already mentioned in the above studies. Furthermore, the same authors also used UHPH (300 MPa, inlet and outlet temperatures of 17 and 19 °C, in-valve temperature 94 °C for <0.2 s) to treat aged red wine contaminated by *Brettanomyces*, and found 6.6 log reduction and no increase in volatile phenols after 2 months of storage. Recently, Escott et al. [65] studied the effect of processing Verdejo grape must by UHPH (300 MPa, inlet and outlet temperatures of 8 and 15 °C, in-valve temperature 92 °C) using non-*Saccharomyces* yeasts with no addition of SO_2. The authors reported that UHPH is able to control microbial activity and oxidation in grape must, along with a favoring effect on implantation of yeast starters and a positive impact on wine quality in terms of color and aroma composition.

In general, it can be concluded that UHPH is a useful tool for wine production. Analysis of recent reports in the literature has shown that UHPH technology has a clear potential for the elimination of microorganisms (especially yeast and bacteria) in grape must, the inactivation of oxidative enzymes, the reduction in SO_2, the easier implantation of selected yeast starters, and the improvement of colloidal and color stability, as well as the overall quality of the wine. In addition, UHPH has been approved in 2020 by the OIV (OENO 594B-2020) [77] for the treatment of musts in a continuous operating mode at a pressure of 300–400 MPa, with the aim of reducing/eliminating wild microorganisms while preserving sensory quality, limiting the use of SO_2, reducing/inactivating the activity of oxidative enzymes, improving the stability of the grape must, and obtaining partially fermented musts.

3.4.2. Cold Plasma (CP) Effects on Wine Phenolics and Color Characteristics

Non-thermal plasma, commonly called cold plasma (CP), is an emerging non-thermal technology which has been increasingly investigated in recent years. By definition, plasma can be described as a fully or partially ionized gas with specific electrical, chemical, and physical characteristics that accompany the electromechanical principle of generating electric energy at ambient atmospheric pressure [88]. There are different approaches that can be employed to generate CP, and each of these plasma systems has a wide range of uses. Some of the most often used plasma systems in food processing include dielectric barrier discharge, corona discharge, pulse discharge, high-frequency discharge, microwave discharge, and glow discharge [89]. Recently, Waghmare [16] summarized the latest trends in cold plasma processing of fruit beverages. In this review article, the author emphasized the great potential of CP for inactivation of different types of spoilage microorganisms and various endogenous enzymes while maintaining the quality characteristics of fruit beverages. The shown capabilities of CP on safety and quality control in the beverage industry also offered an opportunity to introduce this technology in the processing of wine. One of the potential applications of this technology in wine, more precisely the high voltage electrical discharge plasma, is shown in Figure 8.

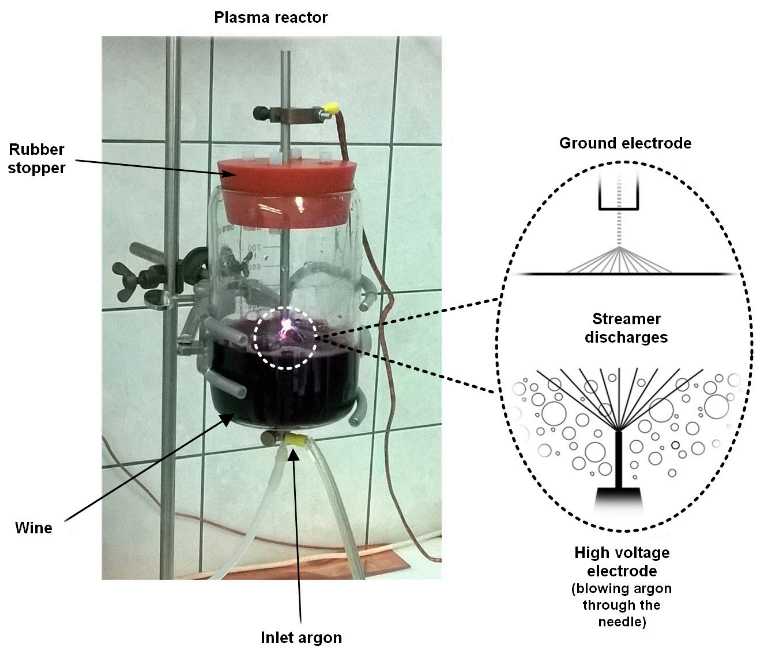

Figure 8. High voltage electrical discharge plasma treatment of wine.

For example, Sainz-García et al. [68] studied the impact of atmospheric pressure cold plasma (APCP) on Tempranillo red wine quality in batch and flow systems. The results showed that argon APCP batch treatments (60 and 90 W at 1, 3, 5, and 10 min) provided wines with lower tonality, higher color intensity, total polyphenols, and anthocyanins. Moreover, the batch treatment with lower power had the most favorable effect. Contrary, continuous flow treatments (1.2 and 2.4. L/min, 60, and 90 W at 25 min) did not result in remarkable improvement of chromatic and phenolic characteristics, nor in the wine spoilage. Another study by Lukić et al. [67] also investigated the effects of CP treatments (60, 90, and 120 Hz at 3, 5, and 10 min) on the phenolic and chromatic composition of red Cabernet Sauvignon and white Graševina wines. Researchers observed that CP affected the phenolic compounds stability in both wines while retaining the color. Among plasma parameters, treatment duration was highlighted as having the most influence. Indeed, the results showed a decrease in total phenolics, anthocyanins, and tannins, while most phenolic acids and flavan-3-ols were slightly increased in white wine, allowing for better oxidative stability. The authors assumed that these various plasma effects were related to the structural diversity of phenolic compounds and consequently different antioxidant properties. Furthermore, Huzum et al. [66] explored white must treated with helium atmospheric pressure plasma jet (He-appj) system (16 kV, 0.8–0.9 mA, 48 kHz, 7–8 W, 200–300 W/cm^2, 4.6–6.6 mJ/cm^2), and observed that selected process conditions provided a cold treatment (max. 40 °C) of must samples as well as a higher quantity of plasma reactive species which supported further oxidation processes. Authors also conducted the characterization of 1- and 2-year-old wines and the correlation of processing parameters (voltage, power, gas temperature, reactive species) with the physicochemical characteristics (pH, Brix, UV-Vis, and FTIR spectroscopy, CIELab and RGB color components) of white must and wine, and supported the possibility of using CP for wine production. Another application of APCP technology with different powers (90 and 500 W) and gasses (air, nitrogen, and argon) was demonstrated for the sanitization of the surface of oak wine barrels [71]. The authors observed complete inactivation of *B. Bruxellensis* yeast after applying air and nitrogen APCP treatments, while a lower inactivation effect was achieved for bacteria *P. Pentosaceus* and *A. Pasteurianu*. Also, there was no change in the morphology of the wood surface after APCP treatments. More recently, Niedźwiedź et al. [69] carried out research on the effect of CP treatments (2, 5, and 10 min, He/O_2 and He/N_2) using a dielectric barrier discharge (DBD) jet system on red wine phenols and biogenic amines. The same authors also compared the impact of CP with the wine preservation effects using potassium metabisulfite (30 and 100 mg/L) and a combined method (CP with 30 mg/L potassium metabisulfite). The results showed that CP-treated wines (5 min, He/N_2) had a 3.1% higher concentration of phenols than that achieved by potassium metabisulfite addition (100 mg/L). Additionally, the application of CP reduced the content of biogenic amines, with a combined CP treatment (10 min, He/O_2, 30 mg/L potassium metabisulfite) causing the highest degree of reduction. Another study by Niedźwiedź et al. [70] with the same methodology for CP treatment was carried out to establish the impact of CP on red wine physicochemical and biological characteristics in contrast to the conventional preservation and combined method effects. The long-term effect of the proposed methods was also considered. The results showed a decrease in phenolic compounds and antioxidant activity after storage, where the mildest changes were observed for methods with potassium metabisulfite and those with a He/N_2 gas mixture. Furthermore, CP treatments did not cause major alterations in wine color, whereas the highest microorganism reduction rate of 4.21 log number was achieved for 10 min by a combined method using He/O_2.

In contrast to other non-thermal technologies, CP is still in the early stages of development and application in winemaking. Despite the scarcity of the literature in the field of oenology, the existing reports provide very promising results on the use of CP in terms of increasing the stability and quality of wine, reducing the amount of SO_2 and sanitizing wine barrels. However, as this technology is product specific and leads to a number of very complex chemical interactions [90], further experiments are needed to explore the chemistry between the generated plasma and the wine components, and to optimize the CP process conditions in order to minimize possible negative effects on the finished wine, and thus allow for the transfer of this technology from the laboratory to the wine industry.

4. Conclusions

In recent years, remarkable progress has been made in the research of non-thermal technologies for winemaking. This review addresses the existing importance of high-power ultrasound, high hydrostatic pressure, and pulsed electric field in the various phases of winemaking such as maceration, fermentation, microbial stabilization, and aging. In general, their beneficial effects have been demonstrated by improving the extraction of bioactive compounds, shortening maceration time, inactivating spoilage microorganisms and oxidative enzymes, reducing SO_2 requirements, accelerating fermentation metabolism and the aging process, and better preserving or even improving wine quality and color. In addition, the latest findings on the application of ultra-high-pressure homogenization and cold plasma in winemaking were discussed. However, it should be highlighted that the technologies presented are at different stages of development. Indeed, most of these technologies have already been applied to other beverages and have been included in the OIV list of recommended oenological practices. Cold plasma, on the other hand, although promising, is one of the least developed technologies in the field of winemaking; therefore, further experimental studies are needed in the future.

Despite these recent advances in the use of non-thermal technologies in winemaking, there are some areas that require further investigation. First of all, it is important to understand the basic mode of action of each technology and its direct or indirect short- and long-term effects on the quality of finished wines. Indeed, there is a particular lack of shelf-life studies for wines produced using these technologies. Secondly, further research with different grape varieties/types of wine and operating conditions is needed to validate the real effectiveness of non-thermal technologies at different stages of winemaking. Therefore, the optimization of treatment parameters stands out as the main goal of future studies. At the same time, more pilot/semi-industrial scale experiments should be conducted to promote faster dissemination and application of these technologies at a commercial level. Thirdly, combining two or more existing non-thermal technologies, as well as combining them with emerging biotechnologies (e.g., the use of selected starter cultures) and other methods (e.g., the addition of antioxidants or oak chips) in conjunction with suitable conditions, could be an efficient way to improve overall processing performance, rather than being limited to stand-alone treatments. Such multidisciplinary approaches will help to achieve many positive effects, including higher wine quality and increased process efficiency. Finally, consumer acceptance of wines produced using non-thermal technologies is still not clear. Further research on the sensory characteristics and acceptance of such wines is therefore necessary to successfully establish these technologies in winemaking.

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