Non-Thermal Technologies for Wine Production

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In winemaking, sulfur dioxide (SO2) is often used at different stages in the production process (e.g., after harvesting the fruit, after crushing, added to the must before fermentation, before maturation, before bottling). SO2 has the ability to control oxidative processes including polyphenol oxidase and to inhibit Maillard reactions. If left untreated, oxidation can lead to a decrease in the sensorial and nutritional quality of wine.

The use of non-thermal or cold pasteurization technologies for wine preservation was reviewed. The effect of pulsed electric fields (PEF), high pressure processing (HPP), power ultrasound (US), ultraviolet irradiation (UV), high pressure homogenization (HPH), filtration and low electric current (LEC) on wine quality and microbial inactivation was explored and the technologies were compared

Keywords: sulfur dioxide ; pasteurization ; pulsed electric field ; high pressure processing ; microbial inactivation

1. Introduction

Oxidation and the undesirable activity of specific microorganisms have a negative effect on wine quality and shelf life. Sensory quality is the most important factor for wine consumers. Most sensory related attributes are largely dependent on wine's phenolic composition which determines color, bitterness and astringency [1]. The loss of wine quality during storage is often accelerated due to exposure to sunlight, high temperatures, oxygen, vibration, pH, contaminants from the storage environment surrounding the wine or cork, microbial spoilage and the failure of bottle closures. The storage of wine for ageing and maturation depends on chemical composition and equilibria, with specific flavors and characteristics, which increase wine quality, developing during this period. On the contrary, the quality of white wines typically does not improve during storage, so they can be sold and consumed straight after production within the first 1 to 2 years. On average, red wines have a longer shelf life than white wines due to their higher phenolic concentration, which reduces their susceptibility to oxidation.

In winemaking, sulfur dioxide (SO 2) is often used at different stages in the production process (e.g., after harvesting the fruit, after crushing, added to the must before fermentation, before maturation, before bottling). SO 2 has the ability to control oxidative processes including polyphenol oxidase and to inhibit Maillard reactions. If left untreated, oxidation can lead to a decrease in the sensorial and nutritional quality of wine [2][3]. In addition to antioxidant action, SO 2 also exhibits antimicrobial capacity against spoilage microorganisms, inhibiting the growth of molds in the must during the early stages of wine production, as well as undesirable bacteria and yeasts during fermentation, preventing unwanted secondary fermentation and the formation of yeast haze [4], and thus avoiding microbial spoilage during wine production and storage. The addition of SO 2 to wine before bottling leads to an increased shelf life, with less likelihood of the formation of off-odors. SO 2 exists in a bound and free form, the latter being the active form of the compound. The amount of each form present depends on the pH of the wine [3][5]. As wine pH increases, antimicrobial capacity decreases. The addition of SO 2 can also increase the extraction of phenolic [2][3].

The excessive use of SO 2 can have a detrimental effect on wine quality including the neutralization of wine aroma, the formation of hydrogen sulfite, unwanted aromas and flavors and cloudiness after bottling [3][6]. Moreover, SO 2 can have adverse effects in humans including allergic reactions, headaches, asthma, dermatitis, abdominal pain, diarrhea and bronchoconstriction. As SO 2 is a commonly used preservative in the wine industry, it is also important to consider the cumulative effect it has on the consumer [4]. This led to the establishment of strict regulations and limits governing SO 2 application in wineries. The SO 2 regulatory limits for wine preservation are constantly being reviewed and reduced [2][3]. Currently, the International Organization of the Vine and Wine (OIV) recommends 150 mg/L total SO 2 for red wines, the European Union limits the total use of SO 2 to 160 mg/L for red wines and 210 mg/L for white and rosé wines and Australia permits 350 mg/L total SO 2 for all wines [3]. The use of fungal-source chitosan for the inactivation of *Brettanomyces* has also been authorized by the OIV and European Union (Regulation (EC) No 606/2009) [7]. Although SO 2 free wines are considered healthier, more natural and sustainable, it is a challenge to produce wines without the addition

of SO 2. Consequently, the wine industry is interested in finding alternative strategies to reduce or eliminate SO 2 in wine production, while maintaining wine quality. To be successful, the alternative must provide the same level of microbial stability and antioxidant activity while also safeguarding the quality of the wine produced, and be less harmful to humans [3]. The use of thermal technologies is unacceptable for the wine industry because of their detrimental effects on the delicate organoleptic characteristics of wine (e.g., flavor, aroma and color) [8]. Thus, the application of non-thermal technologies to produce, age and preserve wine is an area of great interest. Ideally, these technologies will allow the reduction in the use of SO 2 additive in wine production, while keeping or improving the original characteristics of the produced wine [4][9][10][11][12]. Van Wyk et al. (2018) compared sensory, microbiology and other quality parameters in wine subjected to SO 2 addition, HPP and PEF treatments during one year storage [13]. No sensory differences were detected between HPP and PEF treated wines and the untreated wines after being stored for one year [13]. The inactivation of polyphenoloxidase enzyme by US, PEF and HPP has been demonstrated [14], and this could be another way to control the undesirable change in the polyphenol profile of wines.

In this investigation, a review of the application of the following non-thermal technologies for wine pasteurization was carried out: pulsed electric fields (PEF), high pressure processing (HPP), ultrasound (US), high pressure homogenization (HPH), low electric current (LEC), ultraviolet irradiation and filtration. The specific objectives were: (i) to present the fundaments of the non-thermal technologies mentioned and their benefits in terms of wine quality; (ii) to review and introduce the main microorganisms of concern that can potentially spoil wine; (iii) to investigate the effect of non-thermal technologies on microbial inactivation and compare the technologies in terms of the efficiency of key microbes' inactivation in wine; (iv) to discuss the commercial viability of using non-thermal technologies to reduce or eliminate the use of sulfur dioxide in the wine industry.

2. Microbial Wine Spoilage

The microbes are the main targets of the preservation technologies presented in the following Section (Section 3 —Effect of PEF, HPP and other non-thermal technologies on microbial inactivation in wine). This section is based on a previous publication by Van Wyk and Silva (2019) [4]. Yeasts and bacteria are common types of wine spoilage microorganisms which can have negative effects on wine quality and shelf life, leading to detrimental economic losses. As the yeast *Saccharomyces cerevisiae* is generally more tolerant to high ethanol concentrations compared to other microorganisms [15], it is widely employed for several industrial fermentation processes, including the production of alcoholic beverages. With respect to wine, *S. cerevisiae* is the most abundant microorganism found in the final wine at the end of fermentation, converting the must sugars into alcohol and generating important compounds (e.g., aroma), which are vital for the final wine properties. However, it is important to control the activity of this oenological yeast after fermentation to keep the wine desirable properties and stability during storage. This fermenting yeast can be controlled in wine by SO 2 additions or inactivation with non-thermal processes, as investigated by a number of authors.

Brettanomyces yeasts are infamous for causing mousy off-flavors, also known as 'Brett character'. The off-odors produced are characterized as being 'barnyard-like', 'medicinal', 'Band-aid®' or 'horsey'. The chemical compounds responsible for the off-flavors and -odors are 4-ethylguaiacol (4-EG) and 4-ethylphenol (4-EP). *Brettanomyces* is the only known microorganism to cause the formation of these compounds in wines. *Zygosaccharomyces bailii* is another yeast that can cause cloudiness in bottled wine through the formation of flocculants and granular deposits. It can also produce acetic acid and metabolize malic acid resulting in off-odors and pH increase. Due to *Z . bailii* 's high resistance to yeast inhibitors including sulfur dioxide and tolerance to high ethanol (18%) environments, it can be difficult to control this yeast in wine [2][16]. Film-like growths can form on the surface of wines due to the presence of *Saccharomyces cerevisiae, Saccharomyces bayanus, Zygosaccharomyces fermentati* and species of *Candida, Pichia* and *Hansenula* [16].

The number of bacteria found on grapes varies depending on their condition, with healthy fruit typically having considerably less than damaged grapes. Spoilage caused by lactic acid bacteria typically occurs in warm environments with a pH higher than 3.5 and insufficient sulfur dioxide. *Lactobacillus brevis* and *Oenococcus oeni* cause the transformation of tartaric acid to lactic acid, which leads to a rise in pH, a dull red-brown color in red wines, an increase in carbon dioxide, cloudiness, and the formation of viscous deposits and mousy off-odors. *L. brevis* and *Lactobacillus buchneri* can also cause bitterness. Furthermore, *O. oeni* and *Pediococcus* can cause ropiness, characterized by the flotation of silky threads in spoiled wines [2][16]. Since the 19th century, acetic acid bacteria, including strains of *Gluconobactera* and *Acetobacter*, have been known to cause the oxidation of ethanol to undesirable acetic acid and the oxidation of polyols to ketones. Due to the ability of these acetic acid bacteria to survive anaerobic conditions, they are able to grow in barreled and bottled wines. Bacteria of the genus *Bacillus* cause the formation of sediment and earthy, musty off-odors [16]. Bacteria can be kept under control in wine by maintaining a low pH and temperature environment, minimizing the concentration of oxygen and adding sulfur dioxide [16].

Molds including *Aspergillus, Penicillium, Alternaria, Botrytis, Cladosporium, Mucor, Oidium, Plasmopara, Rhizopus* and *Uncinula* are known to infect grapes. These can enter in the process in the crushing stage, decreasing the juice yield and increasing the grape pressing time. Molds deteriorate the wine quality by altering its composition, producing off-flavors, and encouraging the undesirable growth of spoilage yeasts and bacteria. The resistance of molds to HPP is very variable, depending on the species [17]. Molds can easily be controlled in wine as they are unable to survive due to their susceptibility to alcohol concentration of ≥ 3% and SO 2 [2].

3. Effect of PEF, HPP and Other Non-Thermal Technologies on Microbial Inactivation in Wine

Table 1 shows a summary of *Brett* inactivation expressed in terms of log reductions for different non-thermal PEF, HPP and US conditions. An electric field strength of 20 kV/cm applied to red wine for 6000 µs, led to more than 4.8 log reductions of *Brettanomyces bruxellensis* [19]. Puértolas et al. (2009) [17] achieved 5.2 log reductions of *Dekkera bruxellensis* and 5.8 log reductions of *Dekkera anomala* in red wine using 100 pulses at 31 kV/cm. These results suggest that *D. bruxellensis* in more resistant to PEF inactivation than *D. anomala*. Van Wyk et al. (2019) [12] could reduce the treatment time to as low as 39 µs by increasing the electric field intensity to 50 kV/cm, to obtain 3.0 log reductions in *B. bruxellensis.*

Table 1. Inactivation of *Brettanomyces bruxellensis* yeast in wine by non-thermal PEF, HPP and US technologies*.

Pasteurization Technology	Wine	Alcohol Content (% v/v)	Processing Conditions	Treatment Time	Log Reduction	Reference
PEF	Red	13.0	31 kV/cm, 1 Hz, 100 pulses, batch, T < 30 °C	−	5.2	[17]
PEF	Red	nr	20 kV/cm, 0.5 Hz, 10 µs pulse width, T ≤ 37 °C	6000 µs	> 4.8	[19]
PEF	Red	13.5	50 kV/cm, 100 Hz, 1.7 µs pulse width, T < 40 °C	39 µs	3.0	[12]
HPP	Red Cabernet Sauvignon	13.4	400 MPa	5 s	> 7.0	[11]

HPP	White Chardonnay	13.0	200 MPa	15 s	> 7.0	[10]
HPP	Rosé	12.5	200 MPa	120 s	> 6.0	[10]
HPP	Red Pinot Noir	13.0	200 MPa	180 s	6.0	[10]
HPP	Red & white	nr	500 MPa	300 s	6.0	[9]
HPP	Red Cabernet Sauvignon	13.5	200 MPa	180 s	5.8	[10]
HPP	Red Syrah	12.5	200 MPa	180 s	5.0	[10]
HPP	Red SO_2-free Cabernet Merlot	13.7	200 MPa	180 s	3.8	[10]
HPP	Red Dolcetto Syrah	10.5 14.0	200 MPa	180 s	3.0 4.2	[10]
US	Red	14.0	24 kHz, 0.2 W/mL, T ≤ 25 °C	20 min	0.24	[53]

* HPP was carried out at room temperature, maintaining nonthermal conditions; PEF—pulsed electric fields; HPP—high pressure processing; US—power ultrasound, nr—not reported.

Non-thermal HPP treatment at 400 MPa for only 5 s resulted in the complete inactivation (> 7.0 log reductions) of *Brettanomyces bruxellensis* in Cabernet Sauvignon wine [11]. The same study concluded that the strain of *B. bruxellensis* had a significant effect on HPP inactivation. Strain AWRI 1499 proved to be the most resistant, with 3.0 log reductions in red wine after processing at 150 MPa for 10 min. Puig et al. (2003) [3] achieved at least 6.0 log reductions of *B. bruxellensis* using 500 MPa for 5 min (=300 s). Treatment at 100 MPa resulted in no significant *B. bruxellensis* inactivation [11]. This suggests a minimum threshold pressure below which no inactivation occurs. The results confirm the microbial inactivation dependence on HPP pressure and time [11,85,86]. Van Wyk & Silva (2017a) [10] investigated the effect of wine intrinsic properties on the inactivation of *B. bruxellensis*, by performing HPP studies in seven different wines, including red, white and rosé wines. HPP treatments at 200 MPa for 3 min resulted in 3.0, 3.8, 5.0, 5.8 and 6.0 log reductions in Dolcetto Syrah, SO_2-free Cabernet Merlot, Syrah, Cabernet Sauvignon and Pinot Noir, respectively. Complete inactivation (> 6.0 log reductions) was achieved in rosé wine using 200 MPa for 2 min, while only 15 s was required to achieve complete inactivation (> 7.0 log reductions) in the Chardonnay wine [10], showing the effect of wine composition on *Brett* inactivation. Additionally, results showed that alcohol concentrations above 12.0% v/v had a significant effect on *Brett* inactivation with an increase of log reduction from 3.0 for 10.5–12% to 4.2 for 14% red Dolcetto Syrah wines, while wine pH from 3 to 4 in Cabernet Sauvignon wine was found to have no effect on *B. bruxellensis* inactivation [10].

Ultrasound (US) set at a low acoustic power density of 0.2 W/mL was not efficient for *Brett* inactivation, even after a long processing time of 20 min, which only reduced the yeast in 0.24 log in red wine [18]. When using thermo-sonication, the combination of thermal conditions of 50 °C with US treatment for 1 min, Gracin et al. (2016) [19] achieved 3.0 log reductions of *Brettanomyces* bruxellensis yeast in red wine and 2.0 log reductions of lactic acid bacteria. However, high temperature has a negative impact on wine sensory properties and is not recommended.

Table 2 shows a summary of inactivation of different yeasts in wine submitted to different technologies and processing conditions. Abca and Evrendilek (2014) [20] found that 31 kV/cm bipolar pulses resulted in 4.5 log reductions of *Saccharomyces cerevisiae* in red wine. The same electric field strength applied to *Saccharomyces bayanus* in red wine led to significantly higher inactivation of 5.4 log reductions [21]. Abca and Evrendilek (2014) [20] also looked at the inactivation of *Candida lipolytica* and *Hansenula anomala* in red wine and found that 31 kV/cm caused 4.4 and 3.2 log reductions, respectively. Thus *H. anomala* was more resistant to PEF than *C. lipolyitica* and *S. cerevisiae* .

Table 2. Inactivation of different yeasts in wine by non-thermal PEF, HPP and US technologies *.

Yeast Species	Pasteurization Process	Wine	Alcohol Content (% *v/v*)	Processing Conditions	Treatment Time	Log Reduction	Reference
Saccharomyces cerevisiae	**PEF**	**Red**	**12.0**	**31 kV/cm, 3 µs square bipolar pulse, 40 mL/min, T ≤ 40 °C**	**–**	**4.5**	[20]
Saccharomyces bayanus	**PEF**	**Red**	**13.0**	**31 kV/cm, 1 Hz, 100 pulses, batch, T < 30 °C**	**–**	**5.4**	[21]
Candida lipolytica	**PEF**	**Red**	**12.0**	**31 kV/cm, 3 µs square bipolar pulse, 40 mL/min, T ≤ 40 °C**	**–**	**4.4**	[20]
Hansenula anomala	**PEF**	**Red**	**12.0**	**31 kV/cm, 3 µs square bipolar pulse, 40 mL/min, T ≤ 40 °C**	**–**	**3.2**	[20]
Saccharomyces cerevisiae	**HPP**	**nr**	**15.0**	**300 MPa**	**360 s**	**>7.0**	[22]
Saccharomyces cerevisiae	**HPP**	**Red & white**	**nr**	**500 MPa**	**300 s**	**6.0**	[9]
Saccharomyces cerevisiae	**HPP**	**White**	**nr**	**400 MPa**	**20 s**	**>3.5**	[23]
Saccharomyces ludwigii	**HPP**	**Rosé**	**nr**	**400 MPa**	**20 s**	**>3.7**	[23]
Saccharomyces cerevisiae	**US**	**Red**	**14.0**	**24 kHz, 0.2 W/mL, T ≤ 25 °C**	**20 min**	**0.30**	[18]
Schizosaccharomyces pombe	**US**	**Red**	**14.0**	**24 kHz, 0.2 W/mL, T ≤ 25 °C**	**20 min**	**0.13**	[18]
Zygosaccharomyces bailii	**US**	**Red**	**14.0**	**24 kHz, 0.2 W/mL, T ≤ 25 °C**	**20 min**	**No inactivation**	[18]
Pichia membranefaciens	**US**	**Red**	**14.0**	**24 kHz, 0.2 W/mL, T ≤ 25 °C**	**20 min**	**0.60**	[18]

* HPP was carried out at room temperature, maintaining nonthermal conditions; PEF—pulsed electric fields; HPP—high pressure processing; US—power ultrasound, nr—not reported.

Residual yeast inactivation (≤0.6 log reductions) was registered in red wine, even after a very long and unrealistic US treatment time of 20 min at 0.2 W/mL [18]. The highest yeast inactivation was a 0.6 log reduction of *Pichia membranefaciens* and the lowest a 0.13 log reduction of *Schizosaccharomyces pombe*.

Table 3 shows the results of bacteria inactivation in wine by non-thermal technologies. Only 2.7 log reductions of *Lactobacillus delbrueckii* ssp. bulgaricus was achieved in red wine using 31 kV/cm bipolar pulses [20]. Puértolas et al. (2009) [21] treated red wine containing *Lactobacillus plantarum* and *Lactobacillus hilgardii* using 100 pulses at 31 kV/cm, resulting in 4.8 and 5.2 log reductions, respectively. The magnitude of bacteria inactivation was similar or slightly lower than with yeasts (5.2 to 5.8 log reductions), using the same process. Lastly, 20 kV/cm applied for 6000 µs led to >1.0 and >5.3 log reductions of *Pediococcus parvulus* and *Oenococcus oeni* in red wine, respectively [24]. Therefore, research has shown that the size of the microorganisms has a significant effect on PEF inactivation, with larger yeast cells being less resistant to inactivation than smaller bacteria cells [21][20].

Table 3. Inactivation of bacteria in wine by non-thermal PEF, HPP and US technologies *.

Bacterium Species	Pasteurization Process	Wine	Alcohol Content (% *v/v*)	Processing Conditions	Treatment Time	Log Reduction	Reference
Lactobacillus plantarum	**PEF**	**Red**	**13.0**	**31 kV/cm, 1 Hz, 100 pulses, batch, T < 30 °C**	**–**	**4.8**	**[21]**
Lactobacillus hilgardii	**PEF**	**Red**	**13.0**	**31 kV/cm, 1 Hz, 100 pulses, batch, T < 30 °C**	**–**	**5.2**	**[21]**
Lactobacillus delbrueckii ssp. bulgaricus	**PEF**	**Red**	**12.0**	**31 kV/cm, 3 µs square bipolar pulse, 40 mL/min, T ≤ 40 °C**	**–**	**2.7**	**[20]**
Pediococcus parvulus	**PEF**	**Red**	**nr**	**20 kV/cm, 0.5 Hz, 10 µs pulse width, T ≤ 42 °C**	**6000 µs**	**>1.0**	**[24]**
Oenococcus oeni	**PEF**	**nr**	**nr**	**20 kV/cm, 0.5 Hz, 10 µs pulse width, T ≤ 38 °C**	**6000 µs**	**>5.3**	**[24]**
Lactobacillus plantarum	**HPP**	**Red & white**	**nr**	**500 MPa**	**300 s**	**8.0**	**[9]**
Pediococcus damnosus	**HPP**	**Red**	**nr**	**400 MPa**	**20 s**	**>3.4**	**[23]**
Oenococcus oeni	**HPP**	**Red & white**	**nr**	**500 MPa**	**300 s**	**8.0**	**[9]**
Acetobacter aceti	**HPP**	**Red & white**	**nr**	**500 MPa**	**300 s**	**8.0**	**[9]**
Acetobacter aceti	**HPP**	**Red**	**nr**	**400 MPa**	**20 s**	**>4.2**	**[23]**
Acetobacter pasteurianus	**HPP**	**Red & white**	**nr**	**500 MPa**	**300 s**	**8.0**	**[9]**
Lactobacillus plantarum	**US**	**Red**	**14.0**	**24 kHz, 0.2 W/mL, T ≤ 25 °C**	**20 min**	**0.13**	**[18]**
Pediococcus sp.	**US**	**Red**	**14.0**	**24 kHz, 0.2 W/mL, T ≤ 25 °C**	**20 min**	**0.35**	**[18]**
Oenococcus oeni	**US**	**Red**	**14.0**	**24 kHz, 0.2 W/mL, T ≤ 25 °C**	**20 min**	**0.22**	**[18]**
Acetobacter pasteurianus	**US**	**Red**	**14.0**	**24 kHz, 0.2 W/mL, T ≤ 25 °C**	**20 min**	**0.60**	**[18]**

* HPP was carried out at room temperature, maintaining nonthermal conditions; PEF—pulsed electric fields; HPP—high pressure processing; US—power ultrasound, nr—not reported.

4. Comparison of Technologies and Final Remarks

PEF and HPP proved to be effective wine pasteurization technologies, as they inactivate key wine spoilage yeasts and bacteria in short periods of time, feasible for application in the wine industry. Both technologies have the potential to complement or be used as alternatives to SO 2 addition to must, grape juice and finished wine at different stages of wine production, to control undesirable microbial growth or stop fermentation, and stabilize and preserve the quality of the finished wine until consumption. In fact, PEF is a promising technology for the wine industry as it is a continuous technology, requiring short processing times, in the magnitude of microseconds, for the inactivation of microbes of concern in the wineries. This enables commercial scale production with higher throughput. In addition, the same PEF unit also has the potential to decrease wine maceration time during the early stages of production. HPP and US have been investigated for the acceleration of wine ageing, reducing the required vinification time. US produced insufficient inactivation even after application of unrealistically long processing times.

Despite the encouraging results demonstrating less or no SO 2 addition to wine by using non-thermal technologies such as HPP [25] and PEF [26], more research is needed to determine the extent to which the use of SO 2 can be reduced or eliminated in the production/stabilization of different types of wine. The role of SO 2 in wine is complex and more research is required involving simultaneous assessment of microbial inactivation and wine quality after processing and during storage. Further wine stability studies with SO 2 free wines are needed to compare the quality of the wine produced using non-thermal methods vs the conventional addition of SO 2. More wine stability/quality studies should focus on the combination of a non-thermal method with a reduced amount of added SO 2 preservative.

Another important aspect is the investigation and comparison of costs of non-thermal technologies in terms of capital investment, energy requirement and environmental impact. In addition, although some technologies such as HPP are already used at commercial scale for other beverages and packed foods, others are not. Fortunately, the modern wine consumer’s increasing demand for healthier and preservative free novel wines serves to promote research as well as applications and improvements of non-thermal technologies to the wine industry.

This review shows the potential of both HPP and PEF for wine preservation, as these technologies have minimal effect on overall wine sensory quality (flavor and aroma) and biochemical quality factors such as antioxidant activity, phenolic content and anthocyanins.

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