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REVIEW PAPER

Chemical and physical methodologies for the replacement/reduction of sulfur dioxide use during winemaking: review of their potentialities and limitations

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Abstract Sulfur dioxide (SO_2) is probably one of the most versatile and efficient additives used in winemaking due to its antiseptic and antioxidant properties. This compound is also important for minimizing phenolic polymerization rate and color loss during wine aging. However, allergies caused by SO_2-derived compounds, namely the sulfites, are becoming more frequent, causing symptoms such as headaches, nausea, gastric irritation, and breathing difficulties in asthma patients. Consequently, the legislated maximum concentration of SO_2 allowed in wines has been gradually reduced. For this reason, it is crucial in a competitive global winemaking market strategy, to reduce or even eliminate the use of SO_2 as a preservative and to search for new healthier and safe strategies. This work gives an overview of the main methodologies that have been proposed so far and that have potential to be used in winemaking as an alternative to SO_2. The addition of compounds such as dimethyl dicarbonate, bacteriocins, phenolic compounds, and lysozyme, and the use of physical methods, namely pulsed electric fields, ultrasound, ultraviolet radiation, and high pressure are discussed and critically evaluated.

Keywords Antimicrobial activity · Wine must · SO_2-free wines · Preservation · Antioxidant capacity

Introduction

The consumer demands for foods with high nutritional quality, natural characteristics, microbiologically safe, and minimally processed have increased, leading companies to adopt new techniques of food conservation as alternatives to the traditional ones.

The general use of SO_2 for conservation dates back to the end of the 18th century. It is used nowadays in various food industries, especially in low pH foods, such as fruit juices and fermentable drinks [1]. SO_2 is probably one of the most versatile and efficient additives used in winemaking due to its antiseptic and antioxidant properties. This compound inhibits the development of all types of microorganisms, such as yeasts, lactic acid bacteria (LAB) and, to a lesser extent, acetic acid bacteria. Its action prevents yeast haze formation, undesirable secondary fermentation, *Brettanomyces* growth, the development of mycodermic yeasts, and various types of bacterial spoilage [1–3]. Besides its antiseptic properties, SO_2 in wine plays an important role against oxidation. As antioxidant, SO_2 can act in three different ways: by direct oxygen scavenging; by reacting with hydrogen peroxide; and by reducing the quinones formed during the oxidation process back to their phenol form [4, 5]. In addition, it prevents the wine browning by inactivation of enzymes such as polyphenoloxidade (PPO), peroxidase (POD), and proteases, and also by inhibition of the Maillard reaction [1, 6–8]. Once in wine, SO_2 may react with several constituents, namely acetaldehyde, pyruvic acid, and 2-oxoglutaric acid. In a lesser extent, anthocyanins, cinnamic acids, and reducing sugars may also react with SO_2, contributing for the modulation of the wine properties [5, 6]. The reaction with these compounds reduces the rate of phenolic polymerization and, consequently, the color loss usually observed during wine aging. In addition, SO_2 has also been described to protect the wine aroma [2].

In spite of all the advantages of the SO_2, the sulfites resulting from the addition of SO_2 to wine have been related to allergic reactions in some consumers [9–12].

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Most sulfite-sensitive individuals react to ingested sulfite in quantities ranging from 20 to 50 mg and may experience a range of symptoms, including dermatitis, urticaria, angioedema, abdominal pain, diarrhea, bronchoconstriction, and anaphylaxis [9–11]. Nevertheless, reactions manifesting in the skin, and particularly in the respiratory tract, account for the majority of cases of sulfite sensitivity. However, adverse reactions to sulfites in non-asthmatics are extremely rare [11]. Asthmatics who are steroid-dependent or who have a higher degree of airway hyper-reactivity may be at greater risk of experiencing a reaction to sulfite-containing foods. In this population, sulfite sensitivity reactions could be severe, since SO_2 derivatives can cause the activation of proto-oncogenes, inactivation of tumor suppressor genes, and even can play a role in the pathogenesis of SO_2-associated lung cancer [13].

Due to the health-related problems that have been associated with SO_2 use, the International Organization of Vine and Wine (OIV) has been progressively reducing the maximum concentration authorized in wines [14], which is nowadays 150 mg/L for red wines and 200 mg/L for white wines (Regulation (EC) No 607/2009). However, the use of SO_2 in winemaking is a complex subject. The use of SO_2 excessive doses must be avoided not only for health reasons but also because, from an enological point of view, it can cause organoleptic alterations in the final product [1], neutralize the aroma and even produce characteristic aroma defects. Conversely, an insufficient concentration does not ensure the adequate stability of the wine against an excessive oxidation or microbial development, which can compromise its quality. Due to the complex chemical equilibrium of SO_2 in wine, resulting in several SO_2-combined compounds, the establishment of the precise quantity of SO_2 required for an adequate treatment of the wine, with a final safe level of free SO_2 (10–20 mg/L), is difficult to assess [6]. Thus, there is a great interest in the search for other preservatives and innovative technologies, harmless to health, that can replace or at least complement the action of SO_2, making possible to reduce its levels in wines. In this work, a review of the most important practices that have been so far studied to substitute or reduce the use of sulfur dioxide in winemaking is described, and their potentialities and limitations are pointed out. These methodologies, such as addition of compounds, mainly natural compounds, and physical methods, are summarized in Table 1.

Addition of compounds

Dimethyl dicarbonate

Dimethyl dicarbonate (DMDC) is a chemical inhibitor of microorganisms which has been proposed to be used instead of SO_2 in winemaking [15–17]. This compound was recently approved in the European Union for use in wines at a maximum amount of 200 mg/L at bottling for wines with more than 5 g/L of residual sugar (Regulation (EC) No 643/2006). Also, in the USA, it can be used during the storage of wine in regular amounts up to 200 mg/L [18].

DMDC acts by inhibiting some enzymes, notably the alcohol-dehydrogenase and the glyceraldehyde-3-phosphate dehydrogenase, and by methoxycarbonylation of the nucleophilic residues (imidazoles, amines, thiols) resulting in the arrest of cellular growth [19]. The DMDC added to wine is rapidly converted into methanol, as well as low amounts of methyl carbonate and alkyl carbonates are also formed resultant of the reactions of DMDC with polyphenols or organic acids. The concentration of methanol formed from the addition of DMDC has been shown not to produce toxicologically significant levels [15]. Also, very low amounts of methyl carbamate, resulting from reactions of DMDC with ammonium and amino acids are reported to occur [20]. However, as the concentration of the resulting derivatives are very low, they have no contribution to odors or flavors in wine [20].

Its antimicrobial efficiency on wine depends on temperature, ethanol content, pH, and essentially of the species, strains, and initial cell concentration [21]. Several studies reported that the activity of DMDC is more effective against yeasts than against bacteria [15, 20–22]. Also, the DMDC was shown to be more effective against yeast than SO_2 because DMDC kills the yeast cells, whereas the SO_2 only promotes the inhibition of their growth rendering them into a viable but non-culturable (VBNC) state [15].

Delfini et al. [20] reported that concentrations in the range of 250–400 mg/L inhibited a large number of yeasts, like *Saccharomyces cerevisiae*, *Candida guilliermondii*, *Brettanomyces intermedius*, *Pichia membranaefaciens*, *Saccharomyces bayanus*, and *Saccharomyces uvarum*. However, bacteria showed more resistance, since *Acetobacter aceti* and *Lactobacillus* sp. were completely inhibited only for quantities of 1,000 and 500 mg/L of DMDC, respectively. Red wines containing DMDC present an increase in color intensity, due to a possible interaction between DMDC and anthocyanins, which are the substances responsible for wine color [20]. Costa et al. [21] showed that for an initial inocula of 500 CFU/mL, the minimal inhibitory concentration (MIC) for the yeast species *Schizosaccharomyces pombe*, *Dekkera bruxellensis*, *Saccharomyces cerevisiae*, and *Pichia guilliermondii* was 100 mg/L, while for the most sensitive strains (*Zygosaccharomyces bailii*, *Zygoascus hellenicus*, and *Lachancea thermotolerans*), the MIC was 25 mg/L of DMDC. However, for inoculation amounts of about 10^6

Table 1 Effects and disadvantages of wine conservation alternative methodologies

Methodologies		Effects/potential effects		Disadvantages
		In wine	In other food matrixes	
Addition of compounds	Dimethyl dicarbonate (DMDC)	Inhibits microorganisms growth [15, 20, 21]	Inhibits microorganism growth in apple ciders [22]	Less effective against bacteria when compared with yeast [15, 20–22]
	Bacteriocins	Inhibits bacterial growth [23, 24, 26, 28]; control of malolactic fermentation [28]	–	Do not affect yeast growth [27]
	Phenolic compounds	Inhibits microorganisms growth [33–37]; expresses antioxidant activity [1, 4, 5, 29]; does not affect the consumption of nitrogen compounds [30]; can provide better sensory sensations [29]	–	Can provide negative changes in color and aroma [31]
	Lysozyme	Inhibits bacterial growth [40–42, 88]; control of malolactic fermentation [42]	–	Low activity against gram-negative bacteria and inactive against yeasts [43]; binds with polyphenolic components of red wine [39, 40, 43]; leads to the formation of wine haze [40]
Physical methods	Pulsed electric fields (PEF)	Eliminates pathogenic microorganisms [51, 60]; reduces maceration time [64, 65]; increases phenolic compounds extraction [60, 62, 63], accelerates wine aging [67]	Antimicrobial activity in melon and watermelon juices [53], apple cider [54] and grape juice [55]; inhibits PPO in peach [56] and strawberry juice [57]; inhibits POD in orange juice [58]	–
	Ultrasounds	Accelerates wine maturation [80]; increases phenolic compounds extraction [80]	Antimicrobial activity in apple cider [75, 76] and orange juice [73]; inhibits PPO in fruit juices [69]	–
	Ultraviolet	Eliminates pathogenic microorganisms [83]; production of stilbene-enriched wine [89, 90]	Antimicrobial activity in apple juices [82], mango nectar [84] an chill brines [85]; inhibits PPO in apple juice [82] and mango nectar [84]	Less effective in red wine when compared with white wine [83]
	High pressure	Eliminates pathogenic microorganisms [106–108, 110]	Antimicrobial activity in pineapple juice [95] and grape juice [105]; inhibits PPO and POD in strawberry pulps [92]; inhibits PPO in grapes [102, 103]	Depending of the treatment, may activate some enzymes, leading to a decrease in antioxidant activity and anthocyanins content [105, 109]

CFU/mL, the maximum dose of DMDC legally authorized (200 mg/L) was not effective against the most resistant species.

DMDC appears to be a promising inhibitor of yeast growth and consequently a good additive to stop the alcoholic fermentation. However, it is important to refer that due to the complete conversion in few hours of DMDC into methanol [15], its effect is ephemeral and so the use of this compound during wine storage should not be recommended. Besides, the inability of DMDC to inhibit several bacteria growth, using the maximum dose of DMDC legally authorized, and to protect the wine from oxidation makes its use alone in winemaking not sufficient to fully substitute SO_2.

Bacteriocins

Bacteriocins, such as nisin, pediocin, and plantaricin, produced by specific LAB are small polypeptides that are inhibitory to other bacterial species [16]. In addition to this, some are food additives legally permitted. These compounds have been reported to act primarily upon the cytoplasmic membrane of gram-positive bacteria, rendering the cell permeable to small ionic components, prompting cell lysis [23]. However, the effectiveness against gram-negative bacteria is reduced, depending on the species [23, 24]. Bacteriocins are considered the ideal preservatives against gram-positive bacteria because they have no color or smell and are non-toxic [25].

Nisin is the only bacteriocin that can be obtained commercially, and it has been shown to be effective to inhibit the growth of spoilage bacteria in wines [16, 23, 24]. Rojo-Bezares et al. [24] have studied the effect of nisin on growth of lactic and acetic acid bacteria and yeast, showing that nisin is an efficient antimicrobial agent against wine LAB. *Oenococcus oeni* demonstrated a much higher sensitivity to nisin, with a minimum inhibitory concentration (MIC) of 0.024 μg/mL, whereas other LAB species have a MIC of 12.5 μg/mL. Concerning yeasts, nisin demonstrated to have a very poor effect on the tested strains, with a MIC value higher than 400 μg/mL.

Pediocin and plantaricin are active against a number of LAB, including malolactic strains of *Lactobacillus*, *Leuconostoc*, and *Oenococcus* spp [26, 27]. Nel et al. [26] have shown that pediocin PD-1, when compared with nisin and plantaricin 423, is most effective in removal of an established biofilm of *O. oeni* from stainless steel surfaces in Chardonnay must. Also, as nisin, pediocin does not affect yeasts growth [27].

Yurdugül and Bozoglu [28] reported that bacteriocin-like inhibitory substances isolated from *Leuconostoc mesenteroides* subspp *cremoris* show effectiveness in the control of malolactic fermentation (MLF), inhibiting wine *Lactobacillus* species.

Since bacteriocins are very specific and only affect some group of microorganisms, the combined use of these compounds with metabisulfite has been proposed to control the growth of spoilage bacteria in wine and therefore allowing a decrease in the levels of sulfur dioxide currently used by the wine industry [24]. Also, taking into account the effectiveness of bacteriocins against bacteria and DMDC against yeasts, a combination of these two compounds seems promising to preserve the wine against microbial spoilage and, consequently, substitute SO_2 addition. However, it is important to note that the addition of bacteriocins to wine is not yet authorized. Besides, its antioxidant capacity and effect on wine organoleptic properties are still unknown.

Phenolic compounds

Phenolic compounds are very important in wine since they are responsible for several organoleptic properties, namely color and astringency. The major phenolic compounds present in wines are: phenolic acids, flavonoids, stilbenes, and tannins. Also, for red wines, anthocyanins constitute an important group of phenolic compounds responsible for the red wine characteristic color. Wine phenolic compounds are also associated with the beneficial effects related with moderate wine consumption, especially in relation to cardiovascular and degenerative diseases, due to the antioxidant capacity of polyphenols. Their structures enable them to scavenge and neutralize free radicals [1]. These compounds are oxidized sequentially to semiquinones and quinones, while oxygen is reduced to hydroperoxyl radicals and hydrogen peroxide. This process is catalyzed by the redox cycle Fe^{3+}/Fe^{2+}. Hydrogen peroxide is then reduced by Fe^{2+}, by the Fenton reaction, to hydroxyl radicals, which oxidize hydroxyl groups of saturated compounds. Fe^{3+} can also react with hydrogen peroxide recovering the Fe^{2+}. Radical intermediates can react with oxygen to form an additional pathway to their reduction. [1, 4, 5].

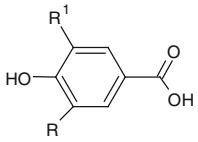
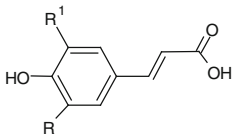
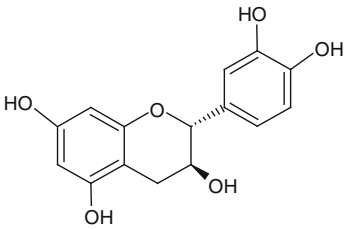
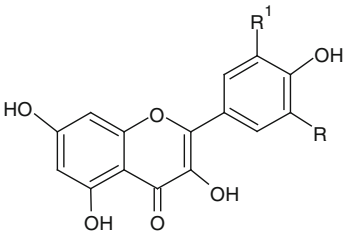
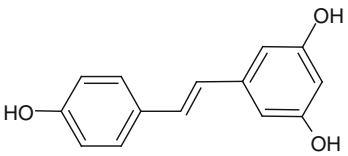
In the last years, the addition of phenolic compounds during winemaking, to replace SO_2, has been studied due to their antioxidant and antimicrobial properties. It was reported that the addition of enological tannins can affect the oxidative phenomena of musts and wines, probably as a consequence of a double mechanism of enzyme inhibition and radical-scavenging activity [29]. The addition of tannins replacing the addition of SO_2 was shown not to affect the fermentative process [30], providing even a better sensory perception when compared with the wine with SO_2 [29]. However, Bautista-Ortin et al. [31] showed that the use of two different enological tannins (gallotannins and procyanidins) did not provide any improvement in the chromatic and sensory characteristics of red wines, since they caused a higher yellow color, resulting in lower ratings of color and aroma sensory characteristics.

Phenolic extracts obtained from enological products showed antimicrobial activity against the strains of *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* [16, 32]. Also, phenolic compounds, such as phenolic acids and flavonoids, can act effectively against pathogenic bacteria [14, 16, 33–37]. A list of the different wine phenolic compounds that were tested as wine additives presenting antimicrobial activity is shown in Table 2.

The antimicrobial effect of phenolic compounds is due to their capacity to increase cytoplasmic membrane permeability, resulting in a leakage of bacterial cell constituents [34]. The different effects of the phenolic compounds have been related to the differences in their structure, lipophilic character, and particularly to the concentration added [33, 34]. Recently, Garcia-Ruiz et al. [37] reported a comparative study of the inhibitory potential of some phenolic acids, stilbenes, and flavonoids on different LAB strains isolated from wine. IC_{50} values of most phenolic compounds were higher than those of SO_2. Nevertheless, flavonols and stilbenes showed the greatest inhibitory effects (lowest IC_{50} values); phenolic acids and their esters exhibited medium inhibitory effects, and the flavan-3-ols showed the lowest effect on the growth of the LAB strains studied.

The exploitation of wine production waste, such as grape cane and pomace, in order to extract phenolic compounds to incorporate in wine during the winemaking,

Table 2 Wine phenolic compounds tested to control the microorganisms growth in wine

Group	Chemical structure	Name	References
Hydroxybenzoic acids		R^1 = R=H—*ρ*-Hydroxbenzoic acid	[34]
		R^1 = H; R = OH—Protocatechuic acid	[33, 34]
		R^1 = R = OH—Gallic acid	[33–35]
		R^1 = H; R = OCH_3—Vanillic acid	[33, 34]
		R^1 = R=OCH_3—Syringic acid	[34]
Hydroxycinnamic acids		R^1 = R=H—*ρ*-Coumaric acid	[34–36]
		R^1 = H; R = OH—Caffeic acid	[33, 35, 36]
		R^1 = H; R = OCH_3—Ferulic acid	[34–36]
Flavanols		(+)—Catechin	[33, 35]
Flavonols		R^1 = OH; R = H—Quercetin	[33]
		R^1 = R = H—Kaempferol	[35]
		R^1 = R = OH—Myricetin	[35]
Stilbenes		*trans*-Resveratrol	[35]

can lead this wine conservation methodology to an economically viable stage. However, despite the promising results, the antimicrobial effect of phenolic compounds appears to occur at higher doses than those usually found in wines. Therefore, it should be considered that the application of phenolic compounds as antimicrobial agents in wines would be conditioned by possible changes that effective concentrations of these compounds would produce in the physico-chemical (viscosity) and organoleptic properties (color and aroma) of the wine [14]. Taking into account the antioxidant activity of the phenolic compounds that can be added during winemaking with the effectiveness of the conjugated effect of bacteriocins and DMDC, a combination of these three approaches can be promising to confer antioxidant and antimicrobial activity suitable for wine preservation with no need to use SO_2.

Lysozyme

Lysozyme is a 129-amino acid protein isolated from egg albumen and has been shown to be an effective antimicrobial agent in many foods [38, 39]. Its use in winemaking has been increasingly explored due to its maximum stability and activity at pH values in the range of 2.8–4.2 [39]. Lysozyme has recently been found to be useful to inhibit bacterial growth in wines, controlling spontaneous LAB growth that often causes spoilage or stuck fermentation [23, 38, 40–42]. The efficacy of lysozyme to inhibit undesirable LAB differs according to species and winemaking conditions [38, 39, 41, 43]. Bacterial sensitivity to lysozyme depends on the peptidoglycan structure constituent of the cell wall, since it is highly active against gram-positive bacteria, has low activity against gram-negative bacteria, and is inactive against the eukaryotic cell walls

[43]. Delfini et al. [39] reported that several strains of wine bacteria belonging to *Oenococcus* spp. proved to be sensitive to lysozyme at low concentrations. Nevertheless, *Lactobacillus* and *Pediococcus* strains survived at higher lysozyme concentrations.

Some studies demonstrated that lysozyme is more active in white wines than in red wines [38, 40, 43]. This could be due to the large amount of polyphenolic components present in red wine that can bind lysozyme [39, 40, 43]. Bartowsky et al. [40] studied the impact of lysozyme on the chemical and sensorial properties of commercial red and white wines. In this study, lysozyme retained 75–80% activity in the white wine after 6 months, but no detectable activity remained in red wines after 2 days. Upon addition of lysozyme to the red wines, a rapid initial decrease (up to 17%) in red wine color density and phenolic content occurred in association with the formation of a light precipitate. Despite lysozyme has greater activity in white wine, its action can led to the formation of wine haze.

Wines treated with lysozyme showed no important change in aroma [40] and lower volatile acidity and biogenic amine content [43]. Nevertheless, Lopez et al. [43] showed that the use of lysozyme in different stages of vinification is important to maintain low histamine levels in wine, namely before the settling of the musts, at the beginning of the alcoholic fermentation, and also during the stabilization and conservation processes.

The combination of lysozyme with other compounds, namely nisin [23] and hydrolysable tannins [29, 30], to reduce or replace the use of SO_2 in winemaking has also been studied. Chang and Hancock [23] showed that a mixture of lysozyme with nisin improved MIC values when compared with the individual compounds for food spoilage *Lactobacillus sake*, *Lactobacillus curvtus*, *Brochothrix thermoshpacta*, *Pediococcus acidilactici*, and *Leuconostoc mesenteriodes*. Also, the use of lysozyme and tannins on musts prevents the development of undesirable bacterial fermentations and influences the volatile compounds of wine, resulting in wines with better sensory impact [29].

Although the use of lysozyme was approved by the OIV more than a decade ago, its use involves significant additional costs for winemakers (enzyme purchase, clarification, and fining procedures) [38]. In addition, its use in wine production could present a risk for consumers allergic to hen's egg. Due to its allergenic character, the presence of lysozyme should be mentioned in the wine bottle label, even if used as a processing aid (maximum limit added of 500 mg/L, Regulation (EC) No 607/2009) [44].

Recently, the use of other antimicrobial enzymes to control LAB in wine has also been investigated. A lytic cocktail of *Streptomyces* spp. was described as a valid alternative to lysozyme because of its higher activity against resistant strains, lysing nearly all wine-relevant strains of LAB and gram-negative acetic acid bacteria [45]. Also, the use of beta-glucanases has been shown as an efficient way to control wine spoilages yeast [46, 47] with no effects in wine enological parameters [47].

Physical methods

Pulsed electric fields

Pulsed electric fields (PEF) technology constitutes a fast, non-thermal, and highly effective technique for the inactivation of pathogenic microorganisms in foods without modifying food quality [48, 49]. This technology involves the application of short pulses (μs) of high electric field strengths (up to 70 kV/cm) to products placed between 2 electrodes [49–51]. The short duration and high intensity field strengths cause electroporation of cell membranes and an increase in their permeability [51]. Namely, electric high-voltage impulses generate a transmembrane potential across the cell membrane which overlays the natural membrane potential. If the difference between outer and inner membrane potential rises above a critical value of about 1 V, polarization and, in the end, breakdown of the membrane is induced. At sufficient high field-strength and duration of the pulses, vegetative microorganisms in liquid media are inactivated due to irreversible membrane destruction [52].

Several investigations in juice fruits have shown the efficiency of PEF treatments on the inhibition of bacteria and yeast growth [53–55]. However, lethality of the PEF treatments depends of the food matrix, microorganisms, and treatment conditions. It has also been reported that PEF treatments decrease the activities of enzymes, such as PPO [56, 57] and POD [58] due to changes in their secondary structure [59].

The potential application of PEF technology to improve wine safety and quality has been exploited in the last decade. Puértolas et al. [51] investigated the PEF resistance of different wine spoilage microorganisms such as *Dekkera anomala*, *Dekkera bruxellensis*, *Lactobacillus hilgardii*, and *Lactobacillus plantarum*. It was observed that in both must and wine, yeasts were more PEF-sensitive than bacteria, and a treatment of 186 kJ/kg at 29 kV/cm could reduce 99.9% of the spoilage flora of the genera *Brettanomyces* and *Lactobacillus*, limiting the risk of wine deterioration by these microorganisms. This inactivation in must or wine could be enough to avoid the contamination of the processing contact surfaces and to control the development of modifications during the wine aging in barrels and storage in bottles [60].

Garde-Cerdán et al. [8] have shown that when the must is treated by PEF, the SO_2 concentration could be reduced,

or even eliminated without any important effect on the content of volatile compounds of the final product. Besides, the absence of SO_2 in these conditions has no negative impact on the sensory characteristics of wine. Also, the PEF treatment of musts does not affect the contents of nitrogen compounds, fatty acids, or nutritive compounds for yeasts growth [61].

Other vantages that this technology can bring to winemaking are the reduction of maceration time and the increase in phenolic compounds extraction to the liquid part of the must [60, 62, 63]. The process is based on the fact that applications of external electric fields induce also the electroporation of grape cell membranes, increasing the diffusion of solutes [62]. Some studies have shown that the maceration time can be reduced 48 h with PEF application on musts [64]. Beyond that, the wine produced presented higher color intensity, anthocyanin content, and total polyphenols index, than the wine produced without PEF treatment [65]. Puértolas et al. [66] showed also that a better chromatic characteristics and higher phenolic content can be obtained by a PEF treatment after the fermentation process. These color characteristics were shown to remain or even increase during aging under oxidative conditions, at least if American oak barrels are used and the resultant wine is stored in bottles. However, these differences in color and bouquet were not detected after 8 months of aging in bottle when triangular sensory tests were performed.

The decrease of wine aging time seems also to be possible with PEF treatments. Chen et al. [67] demonstrated that PEF treatments on wine change significantly the content of proanthocyanidins, catechin, and epicatechin, and the trends were close to the evolution of wine during natural aging.

The capability of PEF to inactivate microorganisms without causing any deleterious effect on flavor, color, or nutrient value of must and wine, to improve the extraction of phenolic compounds, along with the low energy consumption and the short processing times required [60], makes this technology a good alternative to reduce SO_2 in wine conservation. However, it should be noticed that the wines obtained without addition of SO_2 should have less complex flavor than the wines produced with SO_2 due to the vast complexity of reactions of SO_2 with wine components [68].

Ultrasounds

In the last decade, ultrasounds have emerged as an alternative processing option to conventional thermal treatments for pasteurization and sterilization of food products [69]. The inactivation of pathogenic and spoilage microorganisms or enzymes by sonication is mainly caused by physical (cavitation and other mechanical effects) and/or chemical (formation of free radicals due to sonochemical reactions) principles [52, 70, 71]. When high power ultrasound propagates in a liquid, cavitation bubbles are generated due to pressure changes. These micro bubbles collapse violently in the succeeding compression cycles of a propagated ultrasonic wave. This results in regions of high localized temperatures, exceeding 5,500 °C and pressures of up to 50 MPa, resulting in high shearing effects [69, 71]. Consequently, intense local energy and high pressure bring about a localized pasteurization effect without causing a significant rise in macrotemperature [72].

Power ultrasound has been reported to be sufficient to meet the mandatory 5 log reduction of food borne pathogens in fruit juices [69]. However, the effectiveness of an ultrasound treatment is dependent on the type and number of bacteria being treated and on the frequency of the ultrasonic wave. Spores are relatively resistant to the ultrasound treatments, thus extended periods of ultrasonication and conjunction of ultrasound with others technologies or preservatives would be required to render a product free from spores [71]. According to Valero et al. [73], to completely avoid microorganisms growth in orange juice, it is necessary to combine ultrasound with other processing methods with greater antimicrobial effect, as well as to attain a very low initial concentration of bacteria, yeasts, and/or molds in the juice. Ultrasound alone or in combination with mild temperature is reported to be effective against *Escherichia coli* in model solutions [74] and apple cider [75], and also against *Listeria monocytogenes* in apple cider [76]. Tsukamoto et al. [77] showed that the rate of inactivation of *Saccharomyces cerevisiae* yeast cells by ultrasound irradiation depends on the wave frequency and initial number of cells. The highest effect is observed at higher frequencies and lower initial cell number. Confirming these observations, Borthwick et al. [78] reported that yeast cell disruption was greater in a novel compact 267 kHz sonicator than in a lower frequency 20 kHz probe sonicator at the same exposure time.

Besides its antimicrobial effect, ultrasound treatments have been demonstrated promising characteristics for its use in winemaking as a conservation technique instead of SO_2. Namely, ultrasound showed capacity to inhibit enzymes, such as PPO in food [69], and have a minimal effect on the degradation of key quality parameters, such as color, ascorbic acid, and anthocyanin content of fruit juices [72, 73]. Jiranek et al. [79] proposed the possibility of ultrasound use in several stages of winemaking for wine conservation. The use of ultrasound on musts was proposed to reduce the load of spoilage organisms and enhance color and flavor compounds into the wine. These authors also mention that the ultrasound treatment may be used during the fermentation stage to reduce contaminating organisms

prior to inoculation with yeast and/or initiation of the MLF. The use of ultrasounds can stop or delay MLF or, on the contrary, can accelerate yeast autolysis and promote MLF.

Ultrasounds treatments, as well as the PEF treatments, have the capacity to increase the amount of phenolic compounds in red wine and to accelerate its aging [80], increasing the economics advantages of the use of this technology in winemaking. Nevertheless, the works published so far still require a practical evaluation of the feasibility of ultrasound technology to reduce or substitute the use of SO_2 in winemaking.

Ultraviolet

Ultraviolet (UV) irradiation involves the use of radiation from the electromagnetic spectrum from 100 to 400 nm and is categorized as UV-A (320–400 nm), UV-B (280–320 nm), and UV-C (200–280 nm) [81].

In the last decade, UV irradiation has been used as a non-thermal method to disinfect water. This is very effective for microbial decontamination of surfaces and packaging in the food industry [82]. Several advantages associated with UV irradiation have been described: (1) no known toxic or by-products are formed during the treatment, (2) it can be used for destroying organic contaminants, and (3) the treatment requires very little energy when compared with thermal pasteurization processes [81]. UV-C has been used in food processing to inactivate microorganisms (bacteria and yeasts) and enzymes (especially PPO) in many different types of liquid products, such as fruit juices [82, 83] and nectars [84], and chill brines [85] without significantly changing their quality attributes. In this last medium, it has been shown that UV-C irradiation reduces significantly LAB populations [85]. Enzymes inactivation upon UV-C light exposure occurs as a consequence of protein aggregations [86], while inactivation of microorganisms is primarily due to DNA damage, which destroys the reproductive capabilities and other functions of the cell [70]. However, the penetration ability and ultimate efficacy of UV-C irradiation depends, therefore, on appearance and characteristics of the product such as color, absorbance, density, suspended material, and soluble solids which can prevent UV-C light from reaching the microorganisms in the liquid medium [83, 87, 88].

The use of UV irradiation as an alternative technology to wine conservation has been evaluated recently by Fredericks et al. [83]. In this work, yeasts, and lactic and acetic acid bacteria were singly and co-inoculated into white (Chardonnay) and red (Pinotage) wines that were later treated with UV-C irradiation. The treatment showed a wide spectrum of effective inactivation of wine microorganisms such as *Brettanomyces*, *Saccharomyces*, *Acetobacter*, *Lactobacillus*, *Pediococcus*, and *Oenococcus*. However, the degree of microbial inhibition obtained in Pinotage wine was much lower than that found in Chardonnay, which was attributed to the color of the product affecting the efficiency of the treatment. This result might be related to the fact that phenolic compounds in red wine are capable of absorbing radiation in the UV region of the electromagnetic spectrum, thus preventing the transmission of radiation to the microorganisms [83]. This result opens good perspectives for the use of UV treatments for white wine preservation. However, its use in red wines seems to be limited. Also, even for white wines, UV irradiation should be used in the final stages of winemaking, i.e., when wine presents low turbidity. The necessity of a high residence time of exposure of the wine media to the irradiation light and the low volume required, even in continuous, is nowadays a serious limitation of this technology.

Grapes stressed abiotically by postharvest treatment with UV-C reach higher concentrations of stilbenes [89], which can be used in a novel winemaking process to obtain a white wine enriched in resveratrol [89, 90], a compound with a large range of bioactive properties, namely anticancer, anti-inflammatory, blood sugar-lowering, and cardiovascular beneficial effects [91]. Consequently, the alternative use of UV radiation in a very initial stage of winemaking can be useful to increase the content of health benefit compounds in wine.

High pressure

High (hydrostatic) pressure (HP) is a non-thermal processing technique which subjects products to pressures between 100 and 1000 MPa [92] instantly and uniformly, independently of the product size and geometry [92, 93]. HP is considered a green technology, since it uses water as a compression media and is energetically very efficient [94]. In the last decade, HP technology use for the production of microbiological safe foods has increased significantly in the industry. Currently, most HP equipments in industrial plants used for food processing work in a batch process, whereby the product is placed in a high pressure chamber and the vessel is closed, filled with pressure transmitting medium and pressurized by pumping pressure transmission medium into the vessel. This technology is now used in food products for microbial [95, 96] and enzyme [97, 98] inactivation and also to modify the functional properties of some food constituents [99, 100]. Microbial inactivation by HP is probably due to interferences in cellular structures and function, such as membranes, ribosomes, and enzymes [101], leading to cell leakage. Since HP acts by disrupting non-covalent bonds, without affecting the covalent bonds [100], HP-treated foods keep their original freshness, flavor, taste, and color. Smaller molecules such as volatile compounds, pigments,

vitamins, and other compounds, responsible for the sensorial, nutritional, and health-promoting benefits, are largely retained after HP treatment [92]. For example, Cao et al. [92] showed that HP treatments from 400 to 600 MPa at room temperature can inactivate PPO, POD, and β-glucosidase, while retaining monomeric and polymeric anthocyanins, and individual phenolic compounds in strawberry pulp. Ludikhuyze et al. [102] and Castellari et al. [103] reported the inactivation of grape and grape must PPO, respectively, at pressures above 700 MPa. In the enological sector, the use of HP treatments has already been tested to preserve the quality and sustainability of grape juice and must [104, 105], and also to preserve the wine [106–108]. In 1995, Delfini et al. [107] demonstrated that microorganisms added to the wine, such as *Leuconostoc oenos*, *Lactobacillus* spp., *Acetobacter*, and *Botrytis cinerea,* were killed with pressure treatments of 400 MPa during 2 min at 20 °C. Another study reported that the application of 500 MPa during 5 min resulted in a large decrease in the initial microbial wine population, such as *Saccharomyces cerevisiae, Brettanomyces bruxellensis*, and *Oenococcus oeni* without resulting in changes in the physiochemical or organoleptic properties of the wine [108]. Recently, Mok et al. [106] reported the effect of pressure treatments ranging from 100 to 350 MPa until 30 min on microorganism (aerobic bacteria, yeast, and LAB) of wine. They showed that the microbial inactivation increased with the pressure treatment and with time. It was also reported that aerobic bacteria were more susceptible to the HP treatments than yeasts and LAB [106]. However, some studies conducted in muscadine grape juice demonstrated that HP treatments, depending on pressure and time, may activate some enzymes, such as PPO, leading to a decrease in antioxidant capacity and anthocyanins content [105, 109]. Nevertheless, the principal limitation of the HP treatment is the current impossibility to be used in a continuous process. Therefore, the use of HP for wine conservation is only viable in the final stage of winemaking, replacing the addition of SO_2 before bottling, for a pressure treatment after bottling. The requirement of packaging the wine in a resistant and flexible package before the treatment is expected to be a challenge for product presentation.

Recently, high pressure homogenization (HPH) has been reported for a continuous treatment of must and wine [110]. In this technique, a pump is used to force the fluid into the homogenizing valve, through a small orifice between the valve and the valve seat. The fluid leaves the gap in the form of a radial jet that stagnates on an impact ring [111]. This opens a new way for the utilization of high pressure treatments in winemaking, since that HPH can be used for the must and wine preservation. Puig et al. [110] showed that the use of HPH at 200 MPa is capable of decreasing the microbial load of musts without causing significant sensorial changes to the wine. These results suggest that HPH might also be an alternative process for the preservation of wine, which can lead to the production of a wine with lower amounts of SO_2.

The application of high pressure process in winemaking is still at an early stage of development, and the effect on the physical–chemical characteristics of wine is still largely unknown, namely in respect to color, antioxidant activity, phenolic, and volatile compounds composition.

Conclusion

In this review, it was discussed the main methodologies that have potential to be used for wine conservation, as an alternative to SO_2. The replacement or reduction of SO_2 addition in the wine should be made by methodologies that can ensure its microbiological safety while protecting against oxidation and maintaining as much as possible its organoleptic properties. The methodologies presented are not harmful for the health and present promising properties that allow to consider them as alternative methods for wine conservation in substitution of SO_2.

The addition of compounds such as DMDC, bacteriocins, phenolics compounds, and lyzozyme to the musts or wines seems to be more versatile in terms of facility to use in different stages of winemaking when compared with the physical methods (PEF, ultrasound, UV-C, and high pressure). These compounds can be added to the musts to prevent oxidation reactions and eliminate pathogens. Also, after fermentation, their addition allows to control the microbiological growth of harmful species and to act as antioxidant. Before bottling, they can maintain the microbiological safety and foster the longevity of the wine. Beyond the capacity of wine preservation, the physical methodologies described can also be used in several winemaking stages, namely to improve the wine production process, such as reduction of maceration time, to increase the extraction of phenolic compounds, and to accelerate wine aging. In addition, distinct wines with bioactive properties can be obtained.

In spite of the promising results, the conservation effect of SO_2 in wines is quite extensive and up to now no other methodology reported seems to be able, by itself, to replace completely the use of SO_2. The challenge for the academic community and wine industry is the combination of these or other new methodologies in a concerted strategic approach, at different stages of winemaking, for the complete SO_2 replacement in order to produce healthier and novel wines meeting the modern wine consumer demands.

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