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ALTERNATIVE METHODS OF SULFUR DIOXIDE USED IN WINE PRODUCTION

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ABSTRACT

Sulfur dioxide (SO_2) is the most common additive used in winemaking for many years. This compound is important for wine producers and consumers due to its antiseptic and antioxidant properties. However, excessive sulfites caused some symptoms such as a headache, nausea, stomach irritation and respiratory distress in asthmatic patients. Additionally, excessive SO_2 in winemaking process leads to organoleptic changes of final product. For these reasons, the maximum SO_2 concentrations allowed in wines were gradually reduced. In the wine industry it is essential to reduce or even eliminate SO_2 especially in the production of organic wines. These obstacles lead to requirements of new healthier and safety strategies for reduction of SO_2. Up to know have been discussed the priorities of SO_2 used in wine making. Recently some authors evaluated studies with chemical and non-thermal alternatives of SO_2. Some other authors reviewed the side effects of SO_2 used in wines, but none of them have comprised the effects of new techniques in grape, must, wine and pomace. This review discussed effects of different alternatives (thermal, non-thermal, chemical and natural additives) techniques demonstrated in grape, must, wine and pomace as possible alternative to SO_2 in comprehensive manner. The antioxidant, antimicrobial and sensory properties of tannin, oak and vine shoot extracts are also discussed as new alternatives of SO_2. The studies demonstrated that SO_2 could be lowered and even changed by using the development of new methods.

Keywords: sulfur dioxide (SO_2), natural alternatives, plant extracts, physical treatments

INTRODUCTION

SO_2 is a chemical preservative that has been used in the preservation of dry fruits and vegetables, canned fruits and vegetables, tomato paste and jam production in the food industry for many years (**Taylor *et al.*, 1986**). Although the historical process of usage SO_2 dates back to ancient times, it is thought that the first use of SO_2 in the food industry was primarily begun in the early 18th century (**Pasteur, 1866**). Subsequently, the use of this preservative has been followed by foods, especially with low pH such as fruit juices and fermented products. (**Ribereau-Gayon *et al.*, 2006**).
Phenolic compounds in red and white wines are primary substrates for oxidation. During wine aging, there is a gradual loss of phenolic compounds due to their participation in a number of chemical reactions such as oxidation (with polysaccharides and tannins) and formation of other stable anthocyanin-derived pigments. All these reactions could result in marked changes in the color, mouth feel and flavor properties of red wines (**Fulcrand *et al*, 2006**). SO_2 is the most common additive in winemaking, because of its multifunctional properties; inhibition of unwanted microorganisms, preventing oxidation and inhibition of enzymatic and non-enzymatic browning reactions and contribution to wine quality (**Cabaroglu and Canbas, 1993; Bakker *et al.*, 1998**). Even these advantages, negative effects of SO_2 on human health have been subject to researches for many years (**Vally *et al.*, 2009**). Excessive SO_2 leads to organoleptic changes in the final product as well as resulting in toxic effects on human health. With increasing health concerns and narrowing legal limits on chemical protectors, consumers are increasingly demanding to consume foods that contain non-chemical additives. As a result, there is an increasing tendency to reduce the use of SO_2 in winemaking and to use it in combination with additional alternative methodologies. For these reasons, current studies focused on compared effects of SO_2 against its new alternatives in wine production. A number of studies have been conducted as an alternative of SO_2. Some of them included non-thermal processes; some of them proposed using of new chemicals. Recently, the uses of natural preservatives, which may be an alternative to SO_2 and the effects on the final product, have been currently tested.
Many alternatives have been proposed as promising tools for replacement of SO_2 in wine but nowadays it is still do not completely possible to find a wine without preservative in the global market stores. However, there is a need for further review in which are discussed in a more comprehensive manner with current existing alternative techniques available to fully or considerably replace of SO_2 in wine making.
Until today, the authors have discussed various techniques that have the potential to be used in the wine production for preservation as an alternative method of SO_2 (**Crapisi *et al.*, 1988; Santos *et al.*, 2012; Morata *et al.*, 2017; Lisanti *et al.*, 2019**). Most of the studies have focused on chemical or non-thermal methods able to replace the SO_2. Despite the number of literature reviews as alternative methods to SO_2 in wine industry, it is surprising that there has been scarcely absence of a fully comprehensive overview of the current state-of-the-art about deeply discussed their influence, contribution and advantages/ disadvantages on the wine quality. In this review article various groups of chemical, physical and natural alternative methods have been discussed for this purpose: non-thermal treatments such as high hydrostatic pressure, high power ultrasound, ultraviolet, pulsed electric field and low electric application; chemical treatments such as dimethyl dicarbonate, lysozyme, chitosan, colloidal silver complex; treatments with bacteriocins and killer toxins; application of natural plant extracts such as grape based phenolic extracts, wood and grapevine shoot extracts, olive-based extracts and other plant extracts. In this sense, it is also important to remark thermal treatments' effects on wine quality. The latest investigation of thermal processing techniques on grape, must and wine focusing on the transformation of polyphenols and the fluctuation of antioxidative activities upon various processing. This technique currently reported to reduce the addition of SO_2 to wine. However, the effect of the thermal treatments on the composition and sensory content of wines were also reported. Therefore, we reviewed provides hints for the future processing of grapes and wines to reduce the use of SO_2.
In addition this review will thus help the reader to identify and evaluation of these new techniques reported up to now for wine industry and specifically focus on: (i) discussed the potential applications of methods underlying their ability to control unwanted microorganisms, (ii) understanding the effect of their antioxidant activity, their mechanism of action and (iii) their stability and contribution to sensory properties of wines.

MECHANISM OF ACTION AND IMPORTANCE OF SO_2 IN WINE INDUSTRY

Nowadays, SO_2 is known as the most effective chemical additive in winemaking. In case of development of new alternative methodologies to SO_2, the effects of this additive on wine properties and the mechanism of action in wine should be well understood (**Guerrero and Cantos-Villar, 2015**).

In general, SO_2 is mostly liquid (5% v/v diluted solution) form during wine production. Other common sulfur-containing salts are sodium metabisulfite ($Na_2S_2O_5$) or potassium metabisulfite ($K_2S_2O_5$), potassium bisulfite ($KHSO_3$), sodium bisulfite ($NaHSO_3$), calcium bisulfite ($CaHSO_3$), and sodium sulfite (Na_2SO_3) (**Aktan and Yıldırım, 2014**). After addition to the wine, sulfide-containing compounds are dissolved into their sulfide ions (HSO_3^-, SO_3^-) and sulfurous acid (H_2SO_3). These ions react directly with the oxygen before the wine polyphenols and are converted into sulfate (SO_4^{-2}) and sulfuric acid (H_2SO_4) forms (**Ribéreau-Gayon *et al.*, 2000; Clarke and Bakker, 2004**). These reactions could be presented by following reaction (Reaction 1) (**Navarre, 1988; Cabaroğlu and Canbaş, 1993**).

$$2HSO_3^- + O_2 \leftrightarrow 2H^+ + 2SO_4^{-2} \text{ (Reaction 1)}$$

In a different view in explaining the mechanism of action of SO_2, it was concluded that SO_2 acts as an antioxidant by direct oxygen scavenging, by reacting with hydrogen peroxide produced by oxidation of polyphenols in wine and by reducing the quinones formed during the oxidation process back to their phenol form (**Boulton *et al.*, 1996; Oliveira *et al.*, 2011**). The sulfurous acid (H_2SO_3) which is the one SO_2 form, combines with acetaldehyde (CH_3CHO) to form aldehyde sulfurous acid ($CH_3CH(OH).CH_3H$). Herewith, SO_2 competes for hydrogen peroxide to prevent the formation of aldehyde and prevent unwanted acetaldehyde flavor in the wine (**Elias and Waterhouse, 2010**).

Besides its antioxidant effect, SO_2 plays an important role as an antimicrobial additive against unwanted microorganisms such as wild yeast and acetic acid bacteria in wine (**Yıldırım and Altındisli, 2015; Şener and Yıldırım, 2013**). Especially yeasts are very sensitive to SO_2. Bacteria become inactive in the amount of 40-50 mg/L SO_2, while wine yeasts are resistant to 150-400 mg/L of SO_2 (**Erich, 1977; Gomez-Plaza and Bautista-Ortin, 2019**).

Preservation of oxidation is great importance both for taste and color of wines. Polyphenols are oxidized by polyphenol oxidase (tyrosinase) and laccase enzymes that cause changes of color and flavor of wine (**Aktan and Yıldırım, 2012,2014**). SO_2 inhibits enzymes such as polyphenol oxidase, peroxidase, protease and inhibits Maillard reactions that lead to browning of wine (**Garde-Cerdan *et al.*, 2008; Mayen *et al.*, 1996; Ribéreau-Gayon *et al.*, 2006**).

ADVERSE EFFECTS OF SO_2 AND LEGAL REGULATIONS

In addition to all the positive effects of SO_2, the negative effects on human health have been the subject of research for many years. Consumers observed different adverse effect level against SO_2 (most of the sulfide-sensitive individuals are affected in amounts of SO_2 ranging from 20 to 50 mg), which are associated with the many health risk such as angioedema, diarrhea, abdominal pain, bronchoconstriction and anaphylaxis (**Guerrero and Cantos-Villar, 2015**). Some other disorders are associated with SO_2 such as asthma, allergic reactions, headache, fatigue, itching (**Vally and Thompson, 2001,2003**). SO_2 and its derivatives may cause activation of proto-oncogenes and inactivation of tumor suppressor genes. It may even play a role in the pathogenesis of SO_2-related lung cancer (**Qin and Meng, 2009; OIV 2016**)

As a result of various studies, the daily intake of sulfite was assumed to be 43 mg / g on average for an individual weighing 60 kg (**Taylor *et al.*, 1986**). The World Health Organization (WHO) has set limits on daily intake of sulfite as 0.7 mg/kg body weight (**WHO, 2009**). Considering this value, for an individual consumer weighing 60-80 kg, the acceptable daily dose is between 42 and 56 mg per liter/day. It should be kept in mind that a consumer who drinks only half a liter of wine can easily overcome this value.

With the understanding of the adverse effects of SO_2, legal regulations and standards have been introduced in national / international legislation related to SO_2 used in wines. In the European Union legislation, the manufacturers required to specify in the label that "contains sulfites", in the case of sulfite content higher than 10 mg/L in foods (**Regulation EC No 203/2012**). According to International Organization of Vine and Wine (OIV), these limits are 150 mg/L for wines with sugar content <5 g / L; 200 mg/L for wines with a sugar content ≥ 5 g/L (**OIV, 2017**). OIV and European Union regulations have gradually reduced the use of SO_2 to 100 ppm for "Organic Wines" (Table 1). According to U.S. Department of Agriculture (USDA), this limit is 100 ppm for wines labeled as "produced organic grapes" (**USDA, 2019**). SO_2 maximum limits may be higher based on the type of wine and sugar content in the regulations.

Table 1 Maximum limits of SO_2 according to sugar content of wines

Wine types	SO_2 limits for conventional wine Categories as in Regulation EC No 606/2009	SO_2 limits for conventional wine as in Canada (**CFIA, 2011**) and in USA (27 CFR 4.22(b)(1)) (**USDA, 2019**)	SO_2 limits for organic wine as in Regulation EC No 203/2012 (**IFOAM, 2013)**	SO_2 limits for organic wine as in Canada and USA (**CFIA, 2011; USDA, 2019**)
Red wines residual sugar < 5g/L	150 mg/L	350 ppm	100 mg/L residual sugar <2g/L 120 mg/L residual sugar >2g/L and < 5g/L	100 ppm
Red wines residual sugar ≥ 5g/L	200 mg/L		170 mg/L	
White & rosé wines residual sugar < 5g/L	200 mg/L		150 mg/L residual sugar <2g/L 170 mg/L residual sugar >2g/L and < 5g/L	
White & rosé wines residual sugar ≥ 5g/L	250 mg/L		220 mg/L	

ALTERNATIVE METHODS OF SO_2 USED IN WINE PRODUCTION

Many different methods have been tried to be an alternative of SO_2 used in wine production. Among these methods are chemical preservatives such as sorbic acid, ascorbic acid, dimethyl dicarbonate (DMDC). According to studied methods are bacteriocins (lacticin and nisin) (**Bauer *et al.*, 2003; Rojo-Bezares *et al.*, 2007**). In addition to these methods, non-thermal processes such as high hydrostatic pressure (HHP), pulsed electric field (PEF), ultraviolet irradiation (UV), high power ultrasound (HPU) and low electric current (LEC) have been studied as a potential alternatives of SO_2 to be used in winemaking (**Fredericks and Krügel, 2011; Morata *et al.*, 2015; Delsart *et al.*, 2015a,b; Costantini *et al.*, 2015; Gracin *et al.*, 2016; Briones-Labarca *et al.*, 2017**). In addition to all these alternatives, natural alternatives continue to be tested currently such as eucalyptus and almond skin extracts (**Garcia-Ruiz *et al.*, 2013b**), stilbenes extracts (**Raposo *et al.*, 2016a, 2018**), thyme essential oil (**Freidman *et al.*, 2017**), grape and wood tannins (**Sonni *et al.*, 2009; Alamo-Sanza et al., 2019; Sánchez-Palomo *et al.*, 2017**), hydroxytyrosol and oleuropein (**Raposo *et al.*, 2016b,c**), glutathione (**Hosry *et al.*, 2009**). All these tested methodologies are listed in Table 2.

The key proposed alternatives for development of alternatives of SO_2 during wine production should meet some topics summarized as follows;

- Human health should not adversely affected;
- It should be easily available and cheap;
- Must have antimicrobial and antioxidant properties;
- Substances or techniques should not cause very large changes in quality of wine;

Experimental studies in laboratory scale should also be applicable in the industry;

THERMAL PROCESSES

Thermal treatments such as pasteurization and sterilization are effective to inactivate undesirable microorganisms and enzyme, and thus are commonly used by the food industry. Thermal treatments in the wine-making process are very important for the final quality of the wine. Even if it cannot replace all functions it can complement the effect of sulfur dioxide (SO_2) with combination with other effective alternative techniques (**Lambri *et al.*, 2015**). These technologies individually or in combination have shown great potential not just for sterilization also for extraction of anthocyanins and other polyphenols from grape to wine at fermentation step (**El Darra *et al.*, 2013a; Corrales *et al.*, 2009**). Moreover, heat treatment prevents browning due to inhibit oxidizing enzymes, such as laccase and polyphenol oxidase (**Clarke and Bakker, 2011**). This chapter summarizes the recent advances of thermal processing technologies in winery including heating and freezing grapes, must and wines. Contributions of these methods to wine about their content and compositional change during processing were highlighted, as well as the primarily studies of the underlying heat conditions. The advantage and limitation of these technologies are also discussed along with the perspective insight of their future development.

Table 2 Some alternatives of SO_2 used during wine production

A. Chemical Additives	B. Non-thermal Applications	C. Phenolic Compounds and Plant Extracts	D. Killer Toxins and Bacteriocins	E. Combine Methods
DMDC (**Costa and Loureiro, 2008**)	High Hydrostatic Pressure (HHP) (**Briones-Labarca *et al*., 2017**)	Eucalyptus and almond skin extracts (**Garcia-Ruiz *et al*., 2013b**)	*Kluyveromyces phaffii* DBCG 6076 (**Ciani *et al*., 2001**)	Glutathione and cafeic acid or gallic acid (**Roussis and Sergianitis, 2008**)
Lysozyme (**Azzolini *et al*., 2010**)	Pulsed Electric Field (PEF) (**Delsart *et al*., 2015a,b**)	Stilbens extracts (**Raposo *et al*., 2016b**)	Pediocin PA-1 (**Diez *et al*., 2012**)	Glutathione and/ or elligatannis (**Panero *et al*., 2015**)
Ascorbic acid (**Sonni *et al*., 2011**)	Ultraviolet Irradiation (UV-C) (**Fredericks and Krügel 2011**)	Thyme essential oil (**Freidman *et al*., 2017**)	Nisin (**Rojo-bezares *et al*., 2007**)	Glutathione and ascorbic acid(**Comuzzo *et al*., 2015**)
Ethanethiol (**Dias *et al*., 2013**)	Ultrasound (HPU) (**Jiranek *et al*., 2008; Gracin *et al*., 2016**)	Grape and wood tannins (**Sonni *et al*., 2009; Alamo-Sanza *et al*., 2019;Sánchez-Palomo *et al*., 2017**)	Killer toxins CpKT1 andCpKT2 (**Mehlomakulu *et al*., 2014**)	Lysozyme and polyphenols (**Chen *et al*., 2015**)
NaOCl (sodium hypochlorite) (**Yoo *et al*., 2011**)	Dry Ice Application (**Costantini *et al*., 2015**)	Hydroxytyrosol and oleuropein (**Raposo *et al*., 2016a,b**)	Lacticin 3147 (**Garcia-Ruiz *et al*., 2013a**)	Lysozyme and tannins (**Sonni *et al*. ,2009**)
Chitosan (**Chinnici *et al*., 2014; Elmacı *et al*., 2015**)		Glutathione (**Hosry *et al*.,2009a,b**)		
Collaidal silver complex (CAgS) (**Izquierdo-cañas *et al*., 2012; Garcia-Ruiz *et al*., 2015**)				

Thermal treatment is often used for the processing of grapes to eliminate future bacterial contamination (**Li *et al*., 2017**). **Boban *et al.* (2010)** were indicated that thermally treated red wine at 75 and 125 ºC for 45 min effective against two common foodborn pathogens, *Salmonella enterica* serovar Enteritidis and *Escherichia coli*. Beyond the microbiological impact, the heat application on grapes or must has been proposed as pre-fermentative treatments to reduce the enzymatic activity and enrich composition of wines (**Ševcech *et al*., 2015**; **El Darra *et al*., 2013a**; **Corrales *et al*.,** 2009; **Clarke and Bakker, 2011**). Thermal treatment at 70°C, 10 or 20 minute allows the extraction of phenolic compounds in aqueous phase, mainly anthocyanins and aromatic compounds but a lesser extent tannins in the grape mash (**Girard *et al*., 1997**; **Ševcech *et al*., 2015**). However, heating young wine together with grape mash to 35–40°C causes an increase of tannin content and released of colour pigments with disrupted by the action of heating grape berry cells (**Ševcech *et al*., 2015**). However long periods over 80 ºC could result in cooked flavour in winemaking (**Rankine, 1973**).Even though, thermal processing is the most widely used process to inhibit microorganisms in the food industry, application of excessive heat to achieve lethality against specific food borne pathogens also degrades the quality and sensory attributes of products (**Li *et al*., 2014**; Wu, et al., 2014). However, they might have adverse effect on heat-sensitive polyphenols and other bioactive components (**Može Bornšek *et al*., 2015; Rodríguez *et al*., 2016**). Thus, wine components may be significantly affected by heating and phenolic compounds are subject to thermal degradation with corresponding significant changes in their antioxidant activity by application of excessive heat (**Larrauri *et al*., 1998; Pinelo *et al*., 2004, 2005; Sadilova *et al*., 2007; Volf *et al*., 2014**). For higher temperatures, the treatments are shorter because the release of phenolics in the must and wine is faster (**El Darra *et al*., 2013b**).

Freezing temperature is another choice to inactivated enzyme activity and inhibited pathogenic microorganisms. Cold soak and freezing (i.e. using dry ice) of grape mash were reported as a method for this purpose (**Ortega-Heras *et al*., 2012; Li *et al*., 2017**). Freezing have been reported to contribution to total poly-phenol extractability due to break tannin-containing cells of the seeds while cold soak have a low effect on the phenolic composition compared to freezing the mash (**Peinado *et al*., 2004; Sacchi *et al*., 2005**). Freezing of grape mash has been also reported to release anthocyanins due to break cell walls (**Ševcech *et al*., 2015; Sacchi *et al*., 2005**). For these reasons, applying this technique on grapes or must could be eliminate some microbiological degradation of wines and thus combination with other techniques help to reduce SO_2 concentration in winemaking.

NON-THERMAL PROCESSES

Especially in the last two decades, the importance of non-thermal processes has increased in the food industry and even in winemaking; these technologies have been tried by many researchers. The common characteristics of these technologies, in contrast to thermal processes don't cause the greatest changes in the colour, smell, taste, quality of the wine by reducing the effect of temperature. In the winery, non-thermal processes such as high hydrostatic pressure, pulsed electric field, ultrasound and ultraviolet radiation were mainly applied. These technologies have been observed that have positive effects on wine quality. Below, non-thermal processes which can be used in winemaking are mentioned.

High hydrostatic pressure (HHP)

HHP is a method of inhibition of microorganisms and inactivation of enzymes in packaged or unpackaged food by applying pressure with different parameters (about 200-800 MPa). Over the past decade, the use of HHP for food preservation and modification has increased significantly. This technology has been proposed for pasteurization of grape juice (**Buzrul, 2012**). HHP is the most offered technology in must and wine as non-thermal process. Recently, researchers have been demonstrated that pressure treatments affect the physicochemical and sensory properties of red wine. For this reason, the use of HHP technology to in wine industry could be used for non-aged wines. Although this practice has been suggested to be used with wine, it has not yet been applicable in wine industry.

Current studies demonstrated the effect of HHP application and how it changes aroma compounds as well as the sensory and quality characteristics of young wines (**Briones-Labarca *et al*., 2017**). Some researchers claimed that very high pressure (≥650 MPa) applied over a long period time (≥2 hours) could affect the red wine colour and reduce the amount of phenolic material (**Tao *et al*., 2012**). In the mentioned conditions, the HHP application could significantly affected the chromatic properties and phenolic composition of the *Nero D'avola Syrah* red wine ($p<0.05$). Meanwhile, sensory analyses demonstrated that the processing of HHP for 2 hours significantly reduced the severity of wine sour and fruity smell. On the other hand, **Santos *et al.* (2013a)** reported that HHP applied to red wine without SO_2 had better sensory properties than the SO_2 added wine. Same author showed that colour, taste and other sensory characteristics changes were not observed in red wine after application of HHP method some changes after 6 months storage of wine might occur (**Santos *et al.* 2013a**). In a different study, HHP technology applied on red wines at pressures of 500 and 600 MPa (5 and 20 minutes) and results showed a lower content of monomeric anthocyanins (13 to 14%), phenolic acids (8 to 11%), and flavonols (14 to 19%) when compared to the unpressurized wine, respectively (**Santos *et al.* 2016**). During 5 mount of storage, these HHP wines showed better sensory characteristics with less bending, higher cooked fruit flavour and lower density of fruity notes compared to unpressurized wine (**Santos *et al.* 2016**). Unlikely, **Briones-Labarca *et al.* (2017**) showed that, HHP treatment did not affect the physicochemical parameters of white wine, total phenols and flavonoid content after. Also, sensory properties such as taste, smell and overall quality were not affected by the HHP process at 300 MPa. **Santos *et al.* (2013b**) reported that HHP applied in white wine without SO_2 leaded to formation of brownish colour and had fewer phenolic substances with processing between 425 and 500 MPa pressures (5 minute) than the SO_2 added and untreated white wines after one year of storage in the bottle. According to these studies, HHP application is accelerating the Maillard reaction in white wine.

There are many reports that HHP application can be used to inactivate unwanted microorganisms in wine and must such as acetic acid bacteria, lactic acid bacteria, molds and yeast while providing positive contributions to wine quality (**Puig *et al*., 2003; Tonello *et al*., 1998; Buzrul, 2012; Mok *et al*., 2006; Delfini *et al*., 1995**). **Buzrul *et al* (2008)** reported that HHP treatment inactivated *E. coli* and *L. innocua* in kiwifruit and pineapple juices at lower pressure values at room temperature than the conditions used in commercial applications (>400 MPa). Studies have shown that HHP application in white wine able to decrease yeast count 3 $\log_{10}$ with 250 MPa pressure and 20 minute holding parameters; when these parameters were tested with higher pressure (300-400 MPa and 15-20 minute), the yeasts were completely inactivated. HHP treatments were also tested for red and rose wines and results showed that pressure application to the wines between 300-600 MPa (3-6 minute) had a strong antimicrobial effect on different unwanted microorganisms (such as molds, yeasts, acetic and lactic acid bacteria) (**Tonello *et al*., 1996a,b, 1998**).

High power ultrasound (HPU)

Ultrasound application is a method that inhibits the microorganisms in the food products by using the sound waves (>14-16 kHz). Currently, there are many types of researches on the potential of use of HPU technology in wine production. As an alternative of SO_2, HPU can be used to control wine spoilage (**Luo *et al*., 2012**). HPU technology was successfully used for inactivating the yeast *S. cerevisiae* in red grape juice (**Bermúdez-Aguirre and Barbosa-Cánovas, 2012**). The results indicated that HPU application did not cause any changes in the amount of anthocyanin in red grape juice (**Tiwari *et al*., 2010**). **Jiranek *et al.* (2008)** suggested that HPU technology could be used for inactivation of undesirable microorganisms without changing colour and taste of wine. Also, **Masuzawa *et al.* (2000)** reported that HPU process increases some phenolic compounds in red wines.
In some studies have been investigated that the effect of HPU in the continuous flow treatment have an effect on reducing the number of *Brettanomyces* yeasts and lactic acid bacteria (LAB) in wines (**Gracin *et al*., 2016**). **Gracin *et al.* (2016)** screened yeast cells and lactic acid bacteria for susceptibility to HPU application using an ultrasonic processor (400 W, 24 kHz, 100 lm amplitude) at two different wine temperatures (30 and 40 °C). HPU application in continuous flow leads to satisfactory reduction of *Brettanomisces* yeasts (89.1-99.7%) and lactic acid bacteria (71.8-99.3%). More care should be taken to maintain the sensory properties of the wine during HPU application.

Ultraviolet (UV) irradiation

UV irradiation is one of the techniques for the inactivation of microorganisms in liquid food products. This practice can be applied to reduce or even eliminate the use of SO_2 as preservative in wine production by using radiation with a wavelength of 100-400 nM (**Falguera *et al*., 2011,2013**). UV irradiation successfully inhibited microorganisms in red and white grape juice. It also, didn't cause any significant changes in grape juice quality parameters (**Pala and Toklucu, 2013**).
The effect of UV irradiation, as an alternative technology for inactivating microorganisms in grape juice and wine, has been investigated. **Fredericks and Krügel (2011)** studied with white and red wines product from Chardonnay and Pinotage grapes, red and white grape juices product from Shiraz and Chenin Blanc grapes and succeeded to inhibit lactic acid and acetic acid bacteria by using UV technology at 254 nm. UV irradiation has been tested as an innovative technology in white wine production (**Fredericks and Krügel, 2011; Rizzotti et al., 2015**). According to **Falguera *et al.* (2013)** reported that UV irradiation could prevent spoilage of wine at the same rate as SO_2 without altering other quality parameters such as pH, tartaric acid and alcohol content. Moreover, the changes in the wine colour parameters need to be optimized the irradiation process before it was applied. It was observed that microorganisms found in white grapes and wines were more easily inactivated than red grapes and wines by using UV irradiation (**Fredericks and Krügel, 2011**). It was emphasized that the red wines and grapes absorbed more UV light (**Fredericks and Krügel, 2011**).

Pulsed electric field technology (PEF)

PEF is another method that inhibits microorganisms in the food product such as HHP and HPU application. In this method, electrical impulses are applied to the product for a short time (μsec) placed between a series electrode (effect intensity up to 70 kV/cm). PEF has been extensively tested in wine-making compared to HPU and UV technologies. **Garde-Carden *et al.* (2008)** demonstrated that PEF technology could be an alternative for reducing of SO_2 in must and wine. Also, **Abca and Evrendilek (2015)**, demonstrates that the PEF has potential for processing red wine without negatively impacting key features of wines.
The use of PEF technology is an alternative to the microbiological control system in winemaking process. This technology has been tested in must and wine and has succeeded in inactivating yeast and lactic acid bacteria. **Puertolas *et al.* (2009)** demonstrated that the PEF resistance of different wine degradation microorganisms such as *Dekkera anomala*, *Dekkera bruxellensis*, *Lactobacillus hilgardii* and *Lactobacillus plantarum* by applivation of this alternative at intervals of 16 to 31 kV/cm and at a temperature of 10 to 350 kJ/kg at 24 °C. As a result, the optimal was achieved at treatment of 186 kJ/kg at 29 kV/cm. It has been observed that the bacteria are more resistant both in wine and must than the yeast (**Puertolas *et al*., 2009**). Studies indicated that PEF treatment was inactivated *Brettanomyces/Dekkera* in long-term aged wine without sensory deviations (**González-Arenzana *et al*., 2019 a,b**). Moreover, 31, 40 and 50 kV/cm treatments were resulted in *B. bruxellensis D* values of 181.8, 36.1 and 13.0 μs, respectively and at 50 kV/cm, a temperature rise determined of almost 10 °C, doubled inactivation to 3.0 log reductions indicated in red wines (cfu/mL) (**Wyk *et al.*, 2019**).
PEF applications proposed as new techniques for the inactivation of yeasts in the sweet white wine are discussed. The results were compared with high-voltage electrical discharges (HVED) (**Delsart *et al*., 2015a,b**). The maximum yeast inactivations have been obtained with PEF and HVED of 3 and 4 log respectively. However, wine browning was less pronounced for samples treated by PEF compared to HEF and SO_2 treatments. PEF seems to be more suitable alternative technique for sulfide addition.
The advantages of the use of PEF technology in winemaking have been reported as reducing the maceration time and increasing the phenolic compounds (**Puertolas *et al*., 2009; Lopez *et al*., 2009; Puertolas *et al*., 2010a,b,2011**). On the other hand, it was observed that the colour intensity of PEF applied pink wine the amount of anthocyanin and the total polyphenol index were lower than non-applied PEF wine (**Puertolas *et al*., 2009**). The PEF application after the fermentation of red wine showed better colour characteristics with higher phenolic content (**Puertolas *et al*., 2010b**). It was also argued that PEF could affect of the ripening of wines (**Chen *et al*., 2009**). Regarding the positive effects of PEF treatment, studies showed that it was caused an increase in the colour intensity and was not altered the organoleptic wine quality. It is important to remark that, LAB were remained viable in wines six months after treatment (**González-Arenzana *et al.*, 2019b**).

Low electric current (LEC)

LEC is another alternative method as potential to be used for reduction of SO_2 during wine production. Currently, this technique has been successfully applied to grape musts (200 mA, 16 days) (**Lustrato *et al*., 2006**). According to **Lustrato *et al.* (2006)**, although LEC method inhibits the yeast, it does not have an affect over growth of *S. cerevisiae*.
As an alternative to SO_2 in wine storage, LEC method was applied to *Montepulciano d'Abruzzo* red wine (**Lustrato *et al*., 2010**). **Lustrato *et al.* (2010)** studied for inactivation of selected yeast *Dekkera bruxellensis* strain (4481) by using LEC method (200 mA) for 60 days. The results showed that LEC significantly reduced viable living cells and increased the mortality rate of *D. bruxellensis* strains 4481 yeast.

CHEMICAL SUBSTANCES AS AN ALTERNATIVE OF SO_2 USED DURING WINE PRODUCTION

Many chemical preservatives have been identified to reducing the level of SO_2 that uses in wine production. It has recently been suggested that the use of chemical compounds such as dimethyldicarbonate, lysozyme, chitosan, and etc. can prevent the oxidation of wine and inhibit the unwanted microorganisms.

Dimethyl dicarbonate *(*DMDC)

DMDC is a chemical additive that inhibits the development of microorganisms such as SO_2 (**Ough *et al*, 1975; Divol *et al*., 2005**). DMDC also, acts by inhibiting alcohol-dehydrogenase and the glyceraldehydes-3-phosphate dehydrogenase enzymes (**Renouf *et al*., 2008**). The use of this additive up to 200 mg/L in wines has been approved by the European Union (EU) and the United States (USA) (**Santos *et al*., 2012**). In studies on the antimicrobial effect of DMDC have been reported that it is more effective on yeast than SO_2 (**Divol *et al*., 2005; Costa *et al*., 2008**). DMDC added stopped the growth of *B. bruxellensis* at different winemaking stages (**Renouf *et al*., 2008**). The concentration of 250-400 mg/L DMDC inhibits *Saccharomyces cerevisiae*, *Candida guilliermondii*, *Brettanomyces intermedius*, *Pichia membranaefaciens*, *Saccharomyces bayanus*, and *Saccharomyces uvarum* (**Delfini *et al*., 2002**). Morover, **Costa *et al.* (2008)** reported for the inoculums of 500 CFU/ml as the minimal inhibitory concentration (MIC) for the yeast species *Schizosaccharomyces pombe*, *Dekkera bruxellensis*, *Saccharomyces cerevisiae*, and *Pichia guilliermondii* was 100 mg/L of DMDC. The MIC for the most sensitive strains such as *Zygosaccharomyces bailii*, *Zygoascus hellenicus*, and *Lachancea thermotolerans* was 25 mg/L of DMDC (**Renouf *et al*., 2008**).
DMDC is less effective against bacteria when compared to SO_2. For this reason, in the practice of winery, the legally permissible maximum dose of DMDC is an effective preservative to control the low contamination rates of yeasts but is ineffective against lactic acid and acetic acid bacteria in wines (**Renouf *et al*., 2008**). On the other hand, DMDC action is temporary, so it is not recommended to be used for wine storage (**Delfini *et al*., 2002**).
Mixtures of SO_2 in different concentrations (25 and 50 mg /L) with lysozyme and DMDC favoured the formation of volatile compounds and biogenic amines in the wines**. Ancín-Azpilicueta *et al.* (2016**) indicated the effects of lysozyme and dimethyl dicarbonate mixtures on reduction of SO_2 level during wine making and remarked; i) Mixing low concentrations of SO_2 with lysozyme and DMDC reduced the concentration of biogenic amines (histamine, tyramine, putrescine, cadaverine, phenylethylamine + spermidine and spermine); ii) the total concentration of volatile amines (dimethylamine, isopropylamine, isobutylamine, pyrrolidine, ethylamine, diethylamine, amylamine and hexylamine) had been

determined higher in the sample fermented only with SO_2; iii) concentrations of amines with secondary amino groups (dimethylamine, diethylamine, pyrrolidine) have been determined higher in the sample only fermented with SO_2 than those fermented with DMDC and lysozyme or with a mixture of preservatives; iv) lysozyme by itself, and lysozyme mixed with SO_2, both reduced the formation of biogenic amines and the preservative mixture was seemed more advisable.

Lysozyme

Lysozyme is a protein that has been show to have an antimicrobial effects on many foods derived from white egg (**Azzolini *et al*., 2010; Delfini *et al*., 2004**). It is active at pH values in the range of 2.8-4.2 (**Delfini *et al.*, 2004**). Studies reported that lysozyme is effective on many microorganisms in wine, especially on some lactic acid bacteria strains in wines (**Azzolini *et al*., 2010; Delfini *et al*., 2004; Chung and Hancock, 2000; Bartowsky *et al*., 2004; Gao *et al*., 2002; Gerbaux *et al*., 1997**). However, *Lactobacillus* and *Pediococcus* strain survived at higher concentrations of lysozyme (**Delfini et al., 2004**).
Today, practical methods and comparison experiments have been developed and validated to reduce the content of SO_2 during the wine ageing process. Current experiments indicate that a combined antibacterial system with lysozyme can be used to stabilize the wine during the ageing process, to reduce the SO_2 concentration and effectively prevent contamination from the dangerous LAB (**Chen *et al*., 2015; Sonni *et al*., 2011; Cejudo-Bastante *et al*., 2010**). **Sonni *et al.* (2011)** were tested the effects on the volatile composition of white wines during fermentation with lysozyme and tannin for replacement of SO_2. The data suggest that the addition of lysozyme and oenological tannins during alcohol fermentation may represent a promising alternative to the use of SO_2 and the production of wines with low SO_2 content. **Cejudo-Bastonte *et al*. (2010)** added at different doses (25 and 50 mg/L) lysozyme, DMDC and their mixtures with SO_2. In general, the finding that mixture of lysozyme and DMDC and SO_2 are advantageous for the formation of volatile compounds in wines. The wines obtained from mixtures of lysozyme and DMDC with 25 mg/L SO_2 had a better sensory quality than the wines obtained with 50 mg/L as the only preservative used.
Although OIV has allowed lysozyme to be used up to 500 mg/L in wines many years ago, this substance is not highly preferred by wine producers because of the over prays (enzyme use, clarification and fining procedure) (**Azzolini *et al*., 2010**). Also, its use in wine production could present a risk for consumers allergic to hen's egg (**Santos *et al*., 2012; Mainente *et al*., 2017a**). It's necessary to be mention containing lysozyme in the wine bottle labels. However, **Mainente *et al.* (2017a)** were described risk of the accidental presence of lysozyme in alcoholic beverages. In studies were determined traces of hen egg white lysozyme in 12 samples without label declaration. Moreover, **Mainente *et al.* (2017b)** highlighted that, mistranslations and misinterpretations because of the complexity of the regulations and lack of information in the EU Regulations caused the problems in the comprehension of the regulations.

Chitosan

Chitosan is a biopolymer approved by European authorities and by OIV for use as a purification and antimicrobial agent for wines (**Gómez-Rivas *et al*., 2004**). In winemaking, it can be used as helping to prevent bacterial spoilage. Fungal source chitosan has shown an increase reduction of oxidized polyphenolics in juice and wine and control of the spoilage yeast *Brettanomyces* (**Chorniak, 2007**).
The inactivation of acetic acid bacteria has been investigated in artificially contaminated wines. **Valera *et al.* (2017)** compared chitosan and SO_2 effects and both molecules reduced the metabolic activity of acetic acid bacteria strains treated in wines.
In addition, **Chinnici *et al.* (2014)** have been investigated protective effects of sulfides and chitosan additives against oxidative degradation of varietal thiols. Thiol oxidation had been significantly reduced by chitosan. **Chinnici *et al.* (2014)** suggested that this additive may contribute to preserving the diverse character of wines from aromatic grapes and reduced sulfide levels.
Chitosan-genipin films were used to produce white wines without addition of sulfur dioxide as a preservative (**Nunes *et al*., 2016**). It was observed that these wines were less susceptible to browning than organoleptic properties prepared using sulfur dioxide. In addition, the formation of iron-tartrate-chitosan complexes had been shown to inhibit oxidation reactions as well as microbial growth, reducing oxidation reactions by reducing the availability of iron and other metals. The use of chitosan-genipin films in wine production was proposed as an environmentally friendly and easy technique that can be preserved wines with required organoleptic qualities.

Colloidal silver complex (CSC)

Silver, which is widely used for water purification and medicine, has been used for many years due to its antimicrobial properties (**Silver *et al*., 2006; Pradeep and Anshup, 2009**). Current studies have shown that silver nonmaterial play role as an antimicrobial agent against a large scale of Gram-negative (Gr-) and Gram-positive (Gr+) bacteria (**Marambio-Jones and Hoek, 2010**). In the last decade, researchers examine the effects of replacing sulfur dioxide with a colloidal silver complex (CSC) during the production of wines. The legal limits established by the OIV for silver content in the final CSC wines were demonstrated as 100 mg/L (**OIV, 145/2009**).
CSC has been tested alone and in combination with small amounts of SO_2 in wines. **Garde-Carden *et al.* (2014)** determined that the red wines treatment with colloidal silver had similar physicochemical, aromatic and sensory properties to the control group but contained lower alcohol. As a result of this study, although the colour intensity of the wines produced with colloidal silver that stored 4 months was found to be higher, both anthocyanins and total polyphenol concentrations were lower. In addition, it was found that the concentration of bioamine and similar aromatic components were higher than the control group (**Garde-Carden *et al*., 2014**). Furthermore, **Izquierdo-Canas *et al*. (2012)** investigated the effectiveness of a CSC as an antimicrobial agent instead of SO_2 in both white and red wine. The CSC at 1 g/kg grape dose was shown to be an effective antimicrobial treatment to control the growth of acetic acid and lactic acid bacteria. Moreover, although red and white wines produced with CSC displayed chemical and sensory characteristics that were very similar to those obtained using the SO_2, the white wines were significantly affected by oxidation compared to those produced with SO_2. The silver concentration of white and red wines, 18.4 mg/L and 6.5 mg/L, respectively, were below the legal limits (**Izquierdo-Canas *et al*., 2012**). Therewithal, **Gil-Sanchez *et al.* (2019)** were studied effects of two silver nanoparticles coated with biocompatible materials (polyethylene glycol and reduced glutathione) and reported both silver nanoparticles were effective against the different microbial population present in tested wines. Regarding their in vitro digestion, the size and shape of the nanoparticles were determined almost unaltered in the case of silver nanoparticles coated with reduced glutathione, while in coated with polyethylene glycol some particle agglomeration was observed.
These results confirm the potential of CSC to be used in wine production. However, the wine composition was slightly affected with CSC treatment. CSC wines had a lower alcohol grade and acetaldehyde content than SO_2 wines (**Izquierdo-Canas *et al*., 2012**). Furthermore, these results indicate that the use of CSC for white wine production would require more studies of its probable combination with other antioxidant additives, such as ascorbic acid, to evaluate their effects in the final products.

BACTERIOCINS AND KILLER TOXINS

Bacteriocins are extracellular substances produced by different types of bacteria, including both Gram-positive (Gr+) and Gram-negative (Gr-) species (**Daw and Falkiner, 1996**). Bacteriocins are peptides with antimicrobial activity that prevent bacterial spoilage of foods. The two most commonly used bacteriocins in the food industry are nisin and pediocin produced by the specific LAB. Nisin is the only bacteriocin approved by the US Food and Drug Agency as a food additive (**Cotter *et al*., 2005; Bartowsky, 2009**). Studies are presented showing the effect of bacteriocins alone and in combination with SO_2 to preserve wine during the ageing and storage process. **Rojo-Bezares *et al.* (2007)** have studied the effect of nisin on the growth of 64 lactic acid bacteria, 23 acetic acid bacteria and 20 yeasts. Results demonstrated that nisin is an effective antimicrobial agent against wine LAB. **Fernández-Pérez *et al*. (2018)** also reported the inhibition effect of LAB by the use of nisin in combination with sulphur dioxide and obtained nisin by the natural producer *Lactococcus lactis* LM29 under enological conditions. They demonstrated that *L. lactis* LM29 produced nisin in the presence of 2 % and 4 % ethanol (v/v), while higher concentrations of ethanol fully inhibited the production of nisin. Finally, these results of wine ageing under winery conditions demonstrated that the use of 50 mg/L nisin decreased 4-fold the concentration of sulphur dioxide required to prevent LAB growth in the wines (**Fernández-Pérez *et al*., 2018**). In addition *Oenococcus oeni* demonstrated a much higher sensitivity to nisin, with MIC of the 0.024 g/mL. On the other hand, nisin demonstrated poor effect on the yeast strains, with a MIC value higher than the 400 lg/mL.
Khan *et al.* (2015) tested the antimicrobial activity of nisin with disodium ethylenediaminetetraacetate (Na-EDTA) in a broad pH range against selected gram-negative (*Escherichia coli* and *Salmonella typhimurium*) and gram-positive (*Listeria monocytogenes*) bacteria. Results showed that nisin concentration of 125-150 mg/mL with a Na-EDTA concentration of 20-30 mM and a pH of 5-6 was found to inhibit all the three selected bacteria.
Studies also described the effect of pediocin (another antimicrobial bacteriocin) on the growth of bacteria and yeast. **Diez *et al.* (2012)** observed inhibitory effects of pediocin PA-1 and either sulfur dioxide or ethanol with the combination on LAB growth. *Oenococcus oeni* was to be more sensitive to pediocin PA-1 (IC50=19 ng/ml) than the other LAB species (IC50=312 ng/ml). However, it has been reported that pediocin produced by the LAB is not effective for yeast (**Bauer *et al*., 2003**). No adverse effects related to any possible toxicity of the pediocin have been observed (**Delves-Broughton, 2011**).
Recently researchers have discovered new bacteriocins that have an increasing potential for food industry, namely lacticin 3147 (**Guinane *et al*., 2005; Martínez-Cuesta *et al*., 2001,2010**). In a study, bacteriocin Lacticin 3147

produced by *L. lactis* 1FPL105 and its mutant *L. lactis* 1FPL1053 was evaluated (**Garcia-Ruiz *et al*., 2013**). **Garcia-Ruiz *et al.* (2013)** indicated that lactisin 3147 and its combinations with potassium metabisulfite and eucalyptus extract may ultimately be effective in minimizing the SO_2 during wine production.

Killer toxins could be proposed as fungicidal biocontrol agents in winemaking to control the development of unwanted yeasts such as *Brettanomyces*/ *Dekkera* found in wine. **Oro *et al.* (2016)** investigated antimicrobial activity of Kwkt and Pikt killer toxins, two zymocins produced by *Kluyeromyces wickerhamii* and *Wickerhanomyces anomalus*, against *Brettanomyces*/ *Dekkera* wine spoilage yeast. These data support the potential use of zymosins to be used for reducing of SO_2. Two killer toxins, CpKT1 and CpKT2 from yeast *Candida pyralidae* showed a particularly lethal effect against several strains of *B. bruxellensis* found in grape juice (**Mehlomakulu *et al*., 2014**). Another killer toxin *Kluyveromyce phaffii* DBVPG 6076 demonstrated extensive anti-Hanseniaspora activity against strains isolated from grapes (**Ciani *et al*., 2001**).

NATURAL PLANT EXTRACTS

Today, consumers increasingly demand for foods that contain natural preservatives instead of chemical preservatives (**Amato *et al*., 2017**). One of the most promising natural alternatives to sulfides in wine production are using of natural plant extracts. Anti-oxidative and antimicrobial plant extracts rich in phenolic compounds have recently been proposed as a total or partial alternative to sulfides in wine production. The flavonoids, phenolic compounds and their derivatives, which are found in the structure of these extracts, have been shown to be effective in preventing auto oxidation (**Yıldırım, 2006, 2013; Yıldırım *et al*., 2007a,b**). It is stated that other phytochemicals (terpenes, alkaloids, lactones, etc.) found in the extract may contribute to the ant- oxidative properties of the extracts (**Yıldırım *et al*., 2015**). The mechanisms of action have been described as free radical scavenging, compounding with metal ions, inhibition or reduction of oxygen formation. In addition, these compounds inhibit the free radicals of the nutrients from being oxidized by giving hydrogen in the hydroxyl groups of aromatic rings (**Yıldırım *et al*., 2007a,b**). Current studies indicate that the growth of pathogenic and spoilage microorganisms can be strongly reduced or inhibited by certain plant extracts (**Xia *et al*., 2010; Bubonja-Sonje *et al*., 2011**). High antioxidant activities as well as effective antimicrobial activities make them a natural alternative method, instead of potentially synthetic preservatives. Then a rises the question, what are the effects of these compounds on wine quality properties. Natural preservatives that consumed in everyday life and tested in foods for being a alternative to chemical preservatives in the literature are only a small part of those found in nature. Nowadays, it needs to expand the current list of natural antimicrobial and antioxidant compounds that can be used as food preservatives.

The wines treated with these natural preservatives will be more competitive in the current global market. For this reason current studies are needed about effects of plant extracts on quality and sensory properties on the final product. It was reported that wines treated with these rich phenolic extracts prevent oxidation and cause sensory perception better than SO_2 (**Sonni *et al*., 2009**). Currently, these tests include; the addition of phenolic compounds (such as caffeic acid, catechin, tannins etc.) in wine (**Aleixandre-Tudó *et al*., 2013; Álvarez *et al*., 2009; Bimpilas *et al*., 2016; Canuti *et al*., 2012**), plant- based extracts (such as eucalyptus and almond skin extract) (**González-Rompinelli *et al*., 2013**) or wine-making by-products such as grape pomace (grape seed and skins), grapevine and oak wood extracts (**Cejudo-Bastante *et al*., 2017; Gordillo *et al*., 2014a,b, 2016; Jara-Palacios *et al*., 2014**).

The contribution of the addition of these compounds to the quality of wines and their antimicrobial and antioxidant activities are summarized in Table 3. Also these subjects presented and described in the following topics.

Table 3 Summary of natural protective alternatives tested in wines

Treatments	Contribution to wine quality	Antimicrobial activity	Disadvantages
Winemaking by-products (grape skins and seeds) extracts	Enzyme inhibition, Free radical scavenging activity, The fermentation process is not negatively affected, Better organoleptic character (**Sonni *et al*., 2009; Cejudo *et al*., 2010**)	*Bacillus cereus*, *Campylobacter jejuni*, *E. coli*, *L. monocytogenes*, *Salmonella enterica*, *S. aureus*, *Yersinia enterocolitica*, *Pseudomonas* spp., Lactic acid bacteria (**Baydar *et al*., 2006, 2004; García-Ruiz *et al*., 2011; Papdopoulou *et al*., 2005; Bartowsky 2009; Silva *et al*., 2018; Vaquero *et al*., 2007; Campos *et al*., 2009**)	The addition of enological tannins (gallotanen and procyanidin) to show a higher yellow color value in red wines (**Bautista-Ortin *et al*., 2005**).
Oak woods chips and extracts, grapevine shoots extracts	High antioxidant activity, The contribution of volatile components to sensory and aroma profile, High score in color and sensory scores (**Sánchez-Palomo *et al*., 2017; Pérez-Juan and Luque de Castro, 2015; Raposo *et al*., 2018**)	Acetic acid bacteria and pathogenic bacteria (**Alamo-Sanza *et al*., 2019; Alañón *et al*., 2014**)	Less effective in white wines than red wines (**Zhang *et al*., 2018**) The existence of a limited number of studies
Plant extracts (eucalyptus and almond skins, thyme essential oil, hydroxytyrosol)	Prevention of oxidation, Increase in aromatic composition (**Malayoglu, 2010; González-Rompinelli *et al*., 2013; Raposo *et al*., 2016b; Raposo *et al*., 2016c**)	*E. coli* O157:H7, *Salmonella enteriditis*, *L. monocytogenes*, *Salmonella poona*, *Bacillus cereus*, *Saccharomyces cerevisiae* and *Candida albicans*, Lactic acid bacteria (**Freidman *et al*., 2017; Serra *et al*., 2008; González-Rompinelli *et al*., 2013**).	Further studies are needed at different concentrations and longer storage conditions.

Grape-based phenolic extracts

Red wines contain more phenolic compounds than the white wine due to its fermentation with skins and seeds according to the winemaking technique. In addition, anthocyanins are significant group of phenolic compounds in red wines since they are responsible for the color characteristic of wine. Phenolic compounds are naturally found in wine and as well as in wine by-products (grape pomace, skins, seeds and stems). These compounds are very important because of their antioxidant, antimicrobial and anti-inflammatory effects (**Bianchini and Vainio, 2003; Revilla *et al*., 1998,2000; Cheynier, 2012; Parker *et al*., 2007**).

The addition of only dried red and white grape seeds to white wines has been reported to provide approximately 380 mg/L Galic acid Equivalents (GAE) in the polyphenolic index compared to the control group wine (**Pedroza *et al*., 2011**).In studies are demonstrated that dried grape pomace addition to wine could play an important role on the yield of polyphenols in the wine compared to addition of fresh white grape skins (**De Torres *et al*., 2010; 2015; Pedroza *et al*., 2012,2013**). It is stated that, many different wine by-products such as grape / wine by-products can be utilized as food colorant and antifungal additive (**Cappa *et al*., 2015; Han *et al*., 2011; Lavelli *et al*., 2014; Torri *et al*., 2015**).

One of the reasons behind the potential of reutilization of the grape pomace in winery; It is caused by the extraction of a small amount of color pigments, fragrance compounds and phenolic compounds that found in abundance in the fruit, skins and seeds of pomace by fermentation into the wine (**Pinelo *et al*., 2006**). Thus, a significant amount of compound remains in grape waste.

Current studies demonstrated that the addition of grape pomace to the wines during fermentation increased the total phenolic compounds, catechin and dimeric procyanidin levels in the final product. The wine color and sensory scores remains the same or better than the control group wine (**Revilla *et al*., 1998**). Therefore, the addition of grape seed extracts rich in tannins to increase the wine quality has been suggested by many authors (**Harbertson *et al*., 2012; Neves *et al*., 2010**). It was reported that tannins addition to wine prevents from oxidation and caused better sensory properties than SO_2 added wines (**Sonni *et al*., 2009**). Furthermore, it is indicated that tannins can also be used to facilitate the clarification of must and wines (**Jiménez-Martínez *et al.*, 2019**).

There are some studies demonstrating that the phenolic compounds found naturally in grape seed and pomace extracts have a high antimicrobial capacity against pathogenic bacteria that cause numerous deterioration in wine (**Sagdıc *et al*., 2011; Baydar *et al*., 2006,2004**). In addition to the main components such as polyphenols in grape seed extract, other phytochemicals (terpenes, alkaloids, lactones, etc.) found in the extract contribute to its antimicrobial property (**Tsuchiya *et al*., 1996; Cushnie and Lamb, 2005**). Recently, **Garcia-Ruiz *et al.* (2011)** reported a comparative study of the inhibitory potential of some phenolic acids, stilbenes and flavonoids on different LAB strains isolated from wines. IC50 values of most phenolics were higher than those of SO_2. Nevertheless, flavonoids and stilbenes showed the greatest inhibitory effects. Some of the authors indicate that these extracts inhibit the pathogenic microorganizymes such as *Staphylococcus aureus, Escherichia coli, Salmonella enteritidis, Pseudomonas aeruginosa* and *Candida albicans* (**Papdopoulou *et al*., 2005; Silva *et al*., 2018**).

In another study, the antimicrobial effects of a common 54 different phenolic extracts on Merlot wines (produced in Spain in 2009) were evaluated. It has been found that grape seeds from these extracts have inhibitory effects on six different strains of lactic acid bacteria (*Lactobacillus hilgardii*, *Lactobacillus casei*, *Lactobacillus plantarum*, *Pediococcus pentosaceus* and *Oenococcus oeni*) (**Sonni *et al*., 2009; Garcia-Ruiz *et al*., 2011**). It was also found that grape seed extract has a greater inhibitory effect against these bacterial strains than other phenolic plant extracts in the study. Although polyphenols are the main components in grape seed extract, it is stated that other phytochemicals (terpenes, alkaloids, lactones, etc.) found in the extract may contribute to the antimicrobial properties of the extracts. The by-product of the wine demonstrated an antimicrobial effects over same lactic acid and acetic acid bacteria (**Garcia-Ruiz *et al*., 2011**). It was observed that there was a linear correlation between the total phenol contents of the extracts and the oxygen-radical absorbance capacities of these compounds ($r = 0.9173$ and $p < 0.01$). These extracts are largely responsible for antioxidant properties due to their high levels of polyphenols contents (**Salaha *et al*., 2008; Galuska and Makris, 2013; Vaquero *et al*., 2007; Campos *et al*., 2009**).

Wood and grapevine shoot extracts

Red wine is particularly rich in tannins and therefore less lean to oxidation than white or rosé wines. These tannins can be obtained from tannin rich oak woods and grape seed or gallic acid and ellagic acid produced commercially. During the winemaking process, it is possible to enrich the wines with tannins obtained from grapes or oak and to obtain higher quality wines (**Versari *et al*., 2013**). **Pascual *et al*. (2017)** studied model wines to determine the oxygen-consumption capacities of enological tannins (such as ellagitannins) obtained from different sources and demonstrated that tannin addition was a good alternative of SO_2. In addition, condensed tannins are good antimicrobial agents acting by damaging the microorganisms' cell wall and inactivating binding enzymes (**Ya et al., 1988; Chung *et al*., 1998**). Treatment of wines with tannins is an enological practice permitted in many countries, including the EU and the United States.
Wine aging in oak barrels is a common practice for improving the wine quality due to the beneficial effects of wine on flavor, aromatic composition, color stabilization and astringency. Polyphenolic compounds, naturally occurring in the oak barrel, are particaly transferred to the wine during aging. With the diffusion of oxygen from the wood, different reactions occur between the anthocyanins and proanthocyanidins in the wine that stabilize wine color and astringency (**Zamora, 2019**). During this processes significant changes occur in the composition of the wine. With these changes, the final composition of the wine is enriched by flavor and aroma (**Pérez-Juan and Luque de Castro, 2015**). In a study investigating the effect of oak chips on the aroma profile of Verdejo white wines, 7 g/L oak chips were added to young wine during the alcoholic fermentation. As a result, volatile compounds, sensory and aromatic profiles of wine increased by oak chips addition of wine (**Sanchez-Palomo et al., 2017**). **Alamo-Sanza *et al*. (2019)** stated that there is a significant relationship between the phenolic content and antioxidant activity of the wood extract in the red wines aged 10 years with enological oak chips.
Similar to the approach in oak chips, vine shoots are also oenological materials with high potential due to their high antioxidant and antimicrobial properties (**Raposo *et al*. 2018**). Nowadays, this new alternative was tested in wines. **Raposo *et al*. (2016a)** tested addition of vine shoot extract containing 29% (w/v) stilbene compared to the control group (SO_2 added) wine and found that to the Syrah wines produced by using vine shoot extract demonstrated higher scores in color-related parameters and sensory scores than those treated with SO_2. **Cebrián-Tarancón *et al*. (2019)** tested 12g/L vine shoots in model wines and after 35 days of maceration ellagic acid, trans-resveratrol, vanillin and guaiacol values of samples were determined as higher than the normal wines. The results demonstrated to have a positive contribution for the functional properties of wines.

Olive-based extracts

Olive oil waste is a rich source of phenolic compounds. Almost half of the phenolic compounds found in olives and olive oils are hydroxytyrosols and its derivatives. Hydroxytyrosol is a low-cost bioactive compound with high antioxidant activity and good antimicrobial properties (**Anand and Sati, 2013**). Current studies focused on determining the potential capacity of hydroxytyrosol to reduce the amount of SO_2 in wine or model solutions. In Syrah wines, the hydroxytyrosol were obtained from olive wastes was proposed as an alternative of SO_2 (**Raposo *et al*. 2016b**). **Raposo *et al*. (2016b)** compared the white wines treated with hydroxytyrosol and with SO_2 during two winemaking stages (after bottled and stored in a bottle for 6 months). They observed that hydroxytyrosol improved colour as well as odours and tastes of the bottled wine. However, after storage for 6 months in the bottle, the hydroxytyrosol treated wines were more oxidized than the SO_2 wines (**Raposo *et al*. 2016c**).
In addition to hydroxrosol, olive wastes are rich sources of quercetin and oleuropein that show high antimicrobial and antioxidant activity (**Serra *et al*., 2008**). It is possible to observe the studies that tested the antimicrobial effect of these compounds (hydroxytyrosol and oleuropein) on many microorganism species (*Escherichia coli*, *Salmonella poona*, *Bacillus cereus*, *Saccharomyces cerevisiae* and *Candida albicans*) (**Serra *et al*., 2008**). Specifically, some phenolic compounds such as resveratrol, hydroxytyrosol, oleuropein, quercetin are reported to inhibit a variety of pathogenic microorganisms (**Aziz *et al*., 1998; Bisignano *et al*., 2010; Papdopoulou *et al*., 2005**). The results show that these extracts may have important applications as natural antimicrobial agents for the wine industry in the future.

Other plant extracts

Especially in recent years, phenolic compounds rich and aromatic plants such as sage, thyme, rosemary and carnation suggested as a natural preservatives in foods (**Malayoğlu, 2010**). Among them, rosemary has been studied extensively and today, this plant is the only commercial product that allowed as an antioxidant and antimicrobial additive in Europe and the US (**Bozin *et al*., 2007**). Current studies have focused on antibacterial, antioxidant and antiviral effects of rosemary. The *Rosmarinus officinalis* L. from Laminacae (Labiatae) family is an important medical and aromatic plant (**Gachkar *et al*., 2007**). In the literature, it is possible to find out that the protective effects of rosemary are widely tested on many foods, but there is no study with the treatment of rosemary extract for replacement of SO_2 in wine making. In a study, the protective effect of the almond shell and eucalyptus leaf extracts which have rich phenolic compounds on the barrel aged Verdejo wines were evaluated (**González-Rompinelli *et al*., 2013**). As a result of this study, it was observed that no significant difference was found in the sensory score and also, the aromatic composition and phenolic compounds changes were observed. In another study, red wines treated with thyme essential oil were determined as high antimicrobial effect on a food borne pathogen (*Escherichia coli* O157: H7) (**Freidman *et al*., 2017**). However, in a same study, low antimicrobial activity was observed in wines treated with powder mixture of apple peel, green tea and olives that is rich in phenolics (**Freidman *et al*., 2017**).
Glutathion (GSH) is another important natural compound tested for protective effects on wines. GSH is a tripeptide composed by glutamic acid, cysteine and glycine, and an important antioxidant that naturally present in many plants, animals, microorganisms and foods (**Meister, 1988; Yıldırım *et al*., 2007a,b**). GSH is naturally presents in wines in low concentrations (**Meister, 1988; Kritzinger *et al*., 2013**). It is known that GSH prevents the browning of white wine and protects against loss of flavour which occur due to oxidation in white wines (**Coetzee and du Toit, 2012; Vaimakis and Roussis, 1996; Roussis *et al*., 2007; Roussis and Sergianitis 2008; Li *et al*., 2008; Rodríguez-Bencomo *et al*., 2014; Hosry *et al*., 2009; Fracassetti *et al*., 2016**). The addition of GSH in must and wine up to a maximum of 20 mg/L was recently included among the oenological practices recommended by the OIV in 2016 (**OIV 2016; Webber et al., 2017**).
Currently, studies about the effects of the addition of GSH to the must or wine are being discussed. **Gambuti *et al*. (2015)** studied with Cabernet Sauvignon wines in a pilot scale for determining the protective effect of GSH and the results showed that anthocyanins were preserved in red wine containing high levels of GSH. However, **Gambuti *et al.* (2015)** indicate that GSH did not prevent colour stabilization in red wines while determined an increase in the degradation of malvidin 3-monoglucoside (**Gambuti *et al*,. 2017**). **Gambuti *et al*. (2017)** also reported that GSH is not effective enough in prevention of anthocyanins loss during red wine aging. **Webber *et al.* (2017)** assessed the effect of GSH addition (10, 20 and 30 mg/L) after storage of sparkling wines. The results indicated that although total GSH concentration gradually decreased during the storage, GSH reduced browning and acetaldehyde formation by up to 12 months. However, the presence of glutathione had little or no effect on the concentration of free SO_2, total phenolics, catechin, epicatechin, caffeic and coumaric acids (**Webber *et al*., 2017**). Researchers also, studied the effect of GSH and/or ellagitannins added to the bottle on the shelf life of a white wine with SO_2 content. **Panero *et al*. (2015)** observed that the addition of GSH and/or ellagitannins at a dose of 20 mg/L did not limit the oxidative evolution of bottled wines.
Further studies should be aimed by using a combination of different oenological methods with GSH for preventing wine oxidation. The effect of GSH for reducing the use of SO_2 in wines as an alternative should be studied with a higher concentration of GSH under low oxygen content bottling conditions. Therefore, in a study evaluating the quality parameters of Tempranillo and Albariño wines enriched with GSH, chitosan, DMDC and different combinations of hydrolyzable and condensed tannins, is stated that the combination of GSH and grape pomace tannins is the most effective method for increasing the sensory scores and shelf life of wines (**Ferrer-Gallego *et al*., 2017**). In another study to compare the antioxidant activity of GSH alone or with ascorbic acid in model wines, it was emphasized that the presence of ascorbic acid, high concentrations of glutathione may delay oxidative degradation of wine (**Sonni *et al*., 2011**).

CONCLUCION

In this review, it was discussed the main techniques that have potential to be used for wine preservation, as an alternative of SO_2. Taking above into account some of these methods could be proposed to use as a possible alternative of SO_2 during wine production. Despite the promising results, the protective effects of these methods and the number of studies with industrial applications are still limited and further studies are needed in order to see the results for longer storage conditions (> 1 year), different varieties and concentrations, or in combination with existing alternatives. The cost of some non-thermal technique equipment should be considered by the wine producers. But it is also, possible to see that commercial wines with different characteristics that appeal to the market and to the demand of the consumer and which use these technologies and/or substances. These studies demonstrate the requirement of new experiments that could confirm the new alternatives of SO_2 to be used in wines.

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