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CHEMICAL AND BIOCHEMICAL MECHANISMS OF PRESERVATIVES USED IN WINE: A REVIEW

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Abstract

Wine stability is a very important step when it comes to marketing. Antioxidant and antiseptic protection of wines can be achieved by using preservatives, a procedure that is regulated by the European laws. Wines are biochemically complex systems and because of this winemakers have to take informed decisions to manage with precision the preservation of their wines. Wine laboratories can ease the work of the winemaker offering at least the minimum routine analyses, such as pH, alcohol concentration and free sulphur dioxide. The results of these analyses can be used in some mathematical calculus to obtain more useful and precise results, such as the content of molecular sulphur dioxide. Molecular sulphur dioxide is the part of the free sulphur dioxide that confers microbiological stability to wines. Also, based on pH and alcohol concentration determinations, the dosage of potassium sorbate can be precisely calculated to preserve wines with residual sugar and prevent a second fermentation in the bottle. In addition, the usage of the combination ascorbic acid and sulphur dioxide in white dry wines can be risky if it is not properly managed. In the presence of sulphur dioxide the ascorbic acid is a strong reductive agent, therefore it can react faster than sulphur dioxide with oxygen. On the other hand, in the absence of sulphur dioxide, the addition of ascorbic acid it can be very unsafe for wines, because it reacts with dissolved oxygen and releases hydrogen peroxide, a more powerful oxidizing agent. Therefore, only proper management of dissolved oxygen and reductive state preservation can lead to quality wines. This paper reviews the main preservation methods and presents a new perspective in the use of preservatives in wine.

Keywords: *antioxidants, antimicrobials, mechanisms, doses, wine*

INTRODUCTION

Antimicrobials are added into wines to inhibit the growth of microorganisms, enzyme-catalysed reactions, non-enzymic browning, and to act as antioxidants and reducing agents. Sulphur dioxide appears to date back to the end of the 18th century in winemaking. It is used for its properties as antiseptic, antioxidant, antioxidasic, and for ethanal binding [1, 41].

Sorbates are used exclusively in wines with residual sugar, only in the presence of sulphur dioxide. Winemakers use sorbates to avoid a second fermentation in sweet wines. The maximum legally accepted concentration, in sorbic acid used in winemaking is 200 mg/l. The necessary dosage can be lower in wines with high alcohol concentration and low pH. Generally, instead of sorbic acid, potassium sorbate is used for its high solubility in water. Potassium sorbate solution, must be introduced slowly into wine, and mixed properly to avoid the insolubilization of the acid. Is used only before filtration and after that wine underwent tartaric stabilization [41].

Ascorbic acid (vitamin C), is an antioxidant used in winemaking for its effectiveness in reactions with oxygen. It can protect the sensitive oxidisable wine components such as phenolic and flavour compounds and has the capacity to reduce the oxidised phenolic compounds (Fig. 4.) [6, 15, 33, 46].

However, ascorbic acid could not be used as a complete replacement for sulphur dioxide, because it does not have antimicrobial properties, will not bind to residual aldehydes in wine, has no impact on oxidative enzymes and the reaction of ascorbic acid with oxygen produces oxidative radicals and molecules, such as hydrogen peroxide. However, in the presence of sulphur dioxide, hydrogen peroxide is scavenged with the formation of sulphuric acid [31, 41].

MATERIALS AND METHODS

Sulphur dioxide (SO_2) behaves like an antioxidant and inhibit the microorganisms as shown in figure 1. In grape juices and wines it is present in molecular form, inorganic and organic combinations.

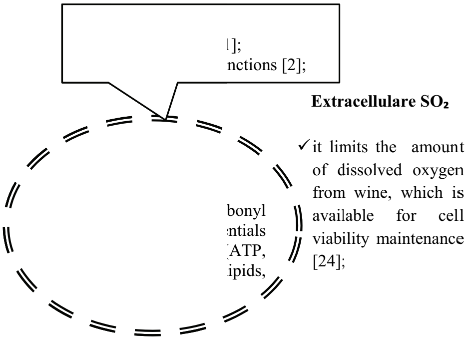


Fig 1. Different modes of action of SO_2 on microorganisms (adapted [8, 42])

Total sulphur dioxide represent an assemble of different forms of sulphur dioxide (mineral and organics) present in grape juices and wine in free state and bound. It results by adding up of free and bound sulphur dioxide [3, 41].

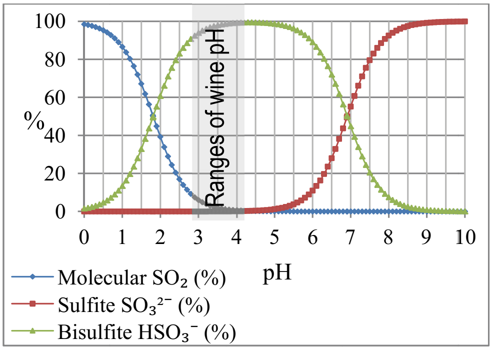


Fig 2. Ionisation forms of sulphur dioxide depending on pH value (adapted to $pKa_1=1.81$ and $pKa_2=6.91$) [3, 41]

Free sulphur dioxide consists of ionisation forms of sulphite and bisulphite which have a proportion of 90-98% and dissolved sulphur dioxide (SO2•H2O), which represent a proportion of 0.5 to 9.5% as shown in figure 2. Dissolved sulphur dioxide is the only part that shows antiseptic and antioxidant proprieties and is also called **molecular sulphur dioxide**. Free sulphur dioxide represent 15-30% of the total sulphur dioxide [3, 14, 41].

Combined sulphur dioxide results from reactions of SO_2 with many organic forms from grape juice and wine. Bounding reactions that occur in wines involve aldehydes, cetonic acids, sugars, uronic acids, oxidation sugar products, phenolic compounds and so on. It can be defined as the difference between total sulphur dioxide and free sulphur dioxide. In grape juice and wine, combined sulphur dioxide represent about 70 to 80% from total sulphur dioxide. This form of sulphur dioxide does not have antiseptic and reducing properties [3, 41]. Binding ratio with acetaldehyde is 1 : 1.46 which means for instance that 30 mg of acetaldehyde binds with 44 mg of sulphur dioxide [41].

The fraction of ionic forms of free sulphur dioxide as shown in the figure 2., can be determined as percentage with the following equations 1-3 derived from Smith C. in 1982 [46]:

$$Molecular\ SO_2(\%) = \frac{100}{[10^{(pH-pKa1)}+1]}$$

(Eq 1)

$$Sulphite\ SO_3^{2-}(\%) = \frac{100}{[10^{(pKa2-pH)}+1]}$$

(Eq 2)

$$Bisulphite\ HSO_3^{-}(\%) = \frac{100}{[10^{(pH-pKa2)}+1]} - SO_3^{2-}$$

(Eq 3)

where:

pH - wine pH value;

pK_{a1} - natural logarithm of the opposite sign of the first acid dissociation constant of sulfur dioxide in wine, K_{a1};

pK_{a2} - natural logarithm of the opposite sign of the second acid dissociation constant of sulfur dioxide in wine, K_{a2};

1 - water value;

100 - value which report the result in percentages.

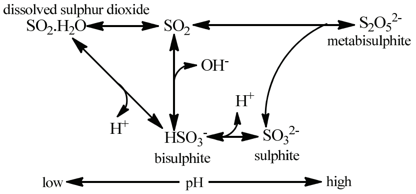


Fig 3. Structural relationships of the sulphur dioxide-generating compounds (adapted form) [14]

To calculate the molecular SO_2 in solutions the following formula [32, 44] can be used:

$$Molecular\ SO_2(mg/l) = \frac{Free\ SO_2(mg/l)}{[10^{(pH-pKa1)}+1]}$$
(Eq 4)

where:
pH – wine pH value;
pKa_1- natural logarithm of the opposite sign of the first acid dissociation constant of sulfur dioxide in wine, K_{a1};
1 – water value;

Often, in practice a pKa1 value of 1.81, seems to be a good compromise, since it appears to have good results for most alcoholic concentrations close to 14% vol./vol. alcohol, at storage temperatures between 13-15°C. In order to obtain more accurate results of molecular SO_2, pKa1 can be calculated with the equation 5.

$$pK_{a1} = 1.9499 + a \times 0.0322 + b \times 0.01971$$
(Eq 5)

where:
a - difference between wine temperature (°C) and 20 value $(T°C - 20)$;
b – difference between alcoholic concentration of wine in volume percentage (% vol./vol.) and 10 value $(c\% - 10)$;

The recommended levels of sulphur dioxide cited from many authors are shown in the Table 1.

Oxidation of sulphur dioxide. In order to choose the optimal required dosage of molecular SO_2, the winemaker must take into account the amount of oxygen from wine.

$$SO_2 + \frac{1}{2}O_2 \xrightarrow{slow} SO_3$$

Table 1. Optimal molecular SO_2 levels (mg/l) for different situations

Situation	Molecular SO_2 (mg/l)
Storage[20, 26]	0.50-0.80
Market [20]	0.40-0.60
Fungicide & bactericide[20, 26]	0.60-0.82
Bacteriostatic[11]	0.40
Inhibition of Brettanomyces[1]	0.30
Biocidal effect on *Brettanomyces*[1, 11]	0.50-0.8
Biocidal effect on acetic bacteria[41]	0.90
Biocidal effect on lactic bacteria[41]	0.60
Mutage[41]	1.2
Bottling dry white wines[22]	0.4-0.8
Bottling dry red wines [22]	0.3-0.6
Bottling sweet wines[22]	0.8-1.2
Olfactory perception[22]	2.0

Experimentally, it was demonstrated that one molecule of oxygen consumes two molecules of sulphur dioxide, which means for instance that 1 mg/l of dissolved oxygen, consumes 4 mg/l of free sulphur dioxide [15, 16].

Ascorbic acid (AA) does not have antimicrobial properties, but is used for its contributing to the antioxidant properties of sulphur dioxide. In most countries it is used up to a concentration of 150 mg/l, always in combination with sulphur dioxide [41].

Ascorbic acid is a more rapid and effective oxygen scavenger than sulphur dioxide and reacting preferentially with oxygen [4].

Ascorbic acid has the ability to react with oxygen more rapidly than wine phenolic compounds and hence increases the rate of hydrogen peroxide production, which in turn should be scavenged by sulfur dioxide [4].

Consequently it protects from oxidation the sensitive oxidisable wine components, including phenolic and flavour compounds [4, 15, 28].

Ascorbic acid has the role of an antioxidant and behaves as an weak acid due to its enediol functional group of C3 and C4 as shown in figure 4 [4].

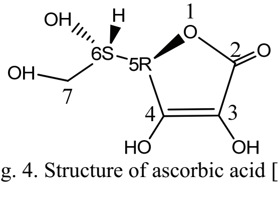
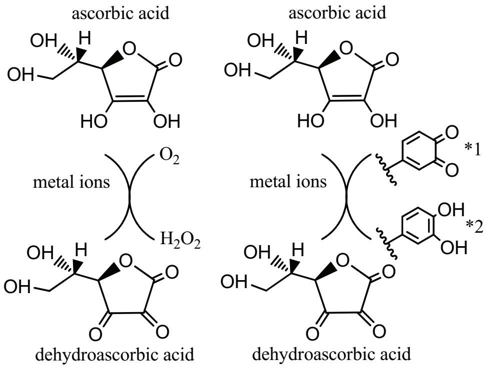


Fig. 4. Structure of ascorbic acid [4]



a. b.

*1 - ortho-quinone; 2-ortho-dihydroxybenzene;

Fig. 5. The antioxidant action of ascorbic acid [4]
a. overall reaction of ascorbic acid with molecular oxygen; b. reduction of *ortho*-quinone compounds by ascorbic acid.

In acidic medium (like wine), an ionisation of ascorbic acid occurs, primarily via the C4 hydroxyl group, due to a pKa value of 4.25, while the C3 hydroxyl group is only weakly acidic (pKa = 11.8) [9].

In wine, ascorbic acid exists mostly in the non-ionised form. In fact, at pH 3.2, only 5% of ascorbic acid is in the mono-anionic form, and even if the pH is raised to pH 3.5, which is relatively high for white wine conditions, the mono-anionic form will only accounts 15% of the total ascorbic acid [4].

Ascorbic acid has been claimed to have the capacity to reduce the oxidised phenolic compounds and brings them back to their original state as shown in figure 5 [6, 15, 33].

Oxidation of ascorbic acid. Assuming a 1 : 1 ratio in the reaction between oxygen and ascorbic acid, in a sulphur dioxide – ascorbic acid – dissolved oxygen system, the consumption mole ratio would be theoretically 1.7 : 1 : 1. Theese ratios are not confirmed due to the absence of oxygen mesurements [7].

Barril Célia demonstrated in his PhD thesis in 2011 that the combination of sulphur dioxide and ascorbic acid significantly inhibited the production of pigmented phenolic compounds by a ratio of 2 : 1 [4].

Moreover, a synergic relationship between sulphur dioxide and ascorbic acid has been confirmed [4].

To minimise pigmentation development in white wine, the ratio of sulphur dioxide: ascorbic acid should be at least 2 : 1 [4].

In brief, we can say that ascorbic acid is used in wines due to its capacity to react faster with dissolved oxygen than other substances.

Experimentally, one molecule of oxygen consumes one molecule of ascorbic acid and two molecules of sulphur dioxide, in other words 1 mg/l of dissolved oxygen consumes 5,5 mg/l ascorbic acid and 4 mg/l of free sulphur dioxide [4, 15, 16].

Sorbates are used under the form of sorbic acid and potassium sorbate to prevent a second fermentation of sweet wines. Sorbic acid is authorized in many countries at maximum concentration of 200 mg/l. Concentration of sorbic acid from potassium sorbate is given by the following formula [20, 41]:

$$KS = SA \times \frac{150.22}{112.13}$$

(Eq 6)

where:
KS – potassium sorbate (mg/l);
SA – sorbic acid (mg/l);
150.22 – molar mass of potassium sorbate (g/mol);
112.13 - molar mass of sorbic acid (g/mol);

Sorbic acid is relatively insoluble in water (1.5 g/l at room temperature), due to this inconvenient, in winemaking is usually used as a potassium salt, which is readily soluble (58.2 g/l) [20].

The required amount of potassium sorbate should be first hydrated in cold water prior to addition to wine [20]. The solution should be prepared prior wine administration to prevent its oxidation. The adition should be done slowly in wine to prevent the insolubilisation of sorbic acid due to wine acidic pH. If its concentration at a given moment is too high, it will precipitate. Due to its concentration in potassium ions, it is recommended to be used only prior to tartrate stabilisation treatment of wine. Also, the dosage is lower in wines with good limpidity or filtered and a number of yeast cells under 5000 cells/ml, preferably under 1000 cells/ml [41].

It is known that the antiseptic proprieties are conferred by the non-dissociated free acid molecule. Due to its pK of 4.76 it is known that in the pH range of wines, the non-dissociated free acid state varies between 90 to 98% [2].

Potassium sorbate dosages are mainly influenced by alcoholic concentrations and pH. At high levels of pH, the lower the alcohol concentration, the more potassium sorbate is used.

According to Henderson - Hasselbalch equation [10, 18], effective and non-effective forms of sorbic acid can be calculated as follows:

$$E\% = \frac{100}{10^{(pH-pKa)} + 1}$$

(Eq 7)

where:

E% - effectiveness, expressed as percentage of non-dissociated from of sorbic acid;

pH – wine pH value;

pKa – acid dissociation constant of sorbic acid which is 4.76 at 20°C;

1 – value of water;

Non-effective form of sorbic acid can be calculated as follows:

$$N\% = \frac{100}{10^{(pKa-pH)} + 1}$$

(Eq 8)

where:

N% - non-effectiveness, expressed as percentage of dissociated from of sorbic acid;

pH - wine pH value;

pKa - acid dissociation constant of sorbic acid which is 4.76 at 20°C;

1 – value of water;

In 1984 Emile Peynaud has tested in laboratory the various dosages of sorbic acid against *Saccharomyces bayanus*, in order to prevent a second fermentation of sweet wines. Laboratory tests consisted of inoculation of *Saccharomyces bayanus* to a 5 x 10^{-3} cells/ml. According to Henderson - Hasselbalch equation and the results of Peynaud (1984), it was developed the following equation given by statistical software Statistica 10.0, using repeated polynomial regressions [41, 10, 18, 38, 40]:

$$SA = (k1) \times c\%\ vol./vol.alc + (k2)$$

(Eq 9)

where:

SA – sorbic acid dosage in mg/l;

$k1 = -73.254 \times pH^2 + 148.09 \times pH - 137.44$

$k2 = 21.972 \times pH^4 - 259.72 \times pH^3 + 1170.2 \times pH^2 - 2365.5 \times pH + 2196$

$c\%\ vol./vol.alc$ – alcohol concentration from wine in volume percentages;

Interactions between preservatives. In wines that have minimum two types of preservatives interactions occur between them.

SO_2 with AA. Sulphur dioxide inhibits ascorbic acid oxidation and is therefore frequently used in white and rose wines, in order to preserve their aromas. They can react
under anaerobic or aerobic conditions [1, 41].

Hydrolysis of the ascorbic acid lactone ring, under anaerobic condition, could be catalysed by the highly nucleophilic sulphite ion [1, 17].

Decarboxylation and dehydration reactions can slowly form 3,4-Dideoxypentosulos-3-ene [1, 17].

Dominant form of SO_2 under wine pH is bisulfite ion (HSO_3^-), readily adds across the double bond to yield 3,4-dideoxy-4-sulphopentosulose as shown in the figure 6. [1, 4, 17].

Under aerobic conditions, dehydroascorbic acid, oxydised form of ascorbic acid, can form with bisulphite ion (HSO_3^-), monohydroxysulphonate as shown in the figure 6. [1, 4]

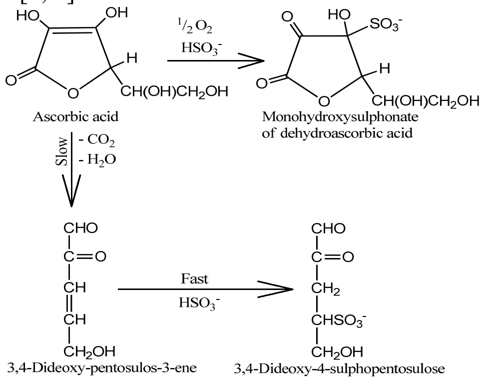


Fig. 6. The reaction of sulphur dioxide with ascorbic acid degradation products [1, 4]

SO_2 with sorbic acid. Sorbic acid is used in combination with sulphur dioxide in sweet wines in order to prevent fermentations. Under neutral conditions, bisulphite reacts with sorbic acid, apparently by 1,2-addition across the diene bound as shown in Fig. 7 [29]. Oxygen interferes with the reaction, hypothetically through sulphite-mediated oxidation in which free radicals such as -OH and -O oxidise sorbic acid. [23]

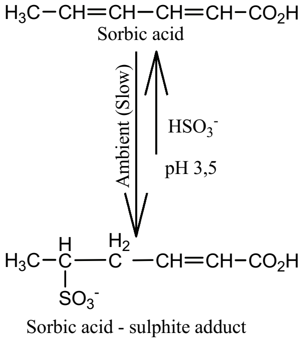


Fig. 7. The reaction of sulphur dioxide with sorbic acid [29]

Oxygen management. Solubility of O_2 in wines is highly dependent on its partial pressure and the temperature. For instance, at atmospheric pressure and room temperature, oxygen saturation with air is reached at approximately 8.5 mg/l. This solubility increases by 10% if the temperature is lowered to 5°C [30].

Literature shows indicative amounts of dissolved oxygen during the entire process of winemaking as shown in the following tables 2, 3 and 4.

In practice is required to calculate the total life cycle package oxygen from wines (TLCPO) using the following equations [45]:

$$I_{DO} + P_{DO} = F_{DO} \qquad \text{(Eq 10)}$$

where:

I_{DO} - initial dissolved oxygen from wine before filling (mg/l);

P_{DO} - dissolved oxygen pickup during filling (mg/l);

F_{DO} - final dissolved oxygen after filling; (mg/l);

$$HO = \frac{(V_1 \times \%O_2 \times 1.429)}{V_2} \qquad \text{(Eq 11)}$$

where:

HO – headspace oxygen right after filling (mg/l);

V_1 - headspace volume in ml;

$\%O_2$ – the percentage of oxygen from headspace (varies from 0 to 21%);

1.429 – density of oxygen to convert from ml to mg;

V_2 - volume of packaged wine in liters;

Table 2. Estimated quantities of oxygen absorbed during vatting (values expressed in mg/l O_2) [39]

Operation	Maximum	Minimum
Prefermentation skin contact	8	5
Alcoholic fermentation (pumping over)	60	30
Post-fermentation vatting	4	1
Running-off	6	4
Total during vinification	78	40

Table 3. Oxygen dissolved during various operations involved in the aging of red wines [39, 48, 49]

Process	Dissolved oxygen (mg/l)
Operation[48]:	
✓ pumping	2
✓ transfer	4-6
✓ racking without aeration	3
✓ racking with aeration	5
✓ topping up barrels	0.25
✓ earth filtration	7
✓ plate filtration	4
✓ centrifugation	8
✓ bottling	3
Storage[49]:	
✓ age 1 year (Bordeaux 2.25 hl barrel)	0.4
✓ age 2 year (Bordeaux 2.25 hl barrel)	0.2
✓ age 3 year (Bordeaux 2.25 hl barrel)	0.2
✓ stainless steel vat (70 hl)	< 0.1

Table 4. Oxygen exposure of wine during storage and operations

Process/Receptacle	Rate (mg/l/year)	Amount (mg/l)
Bottle closures (white wine)		
Natural cork, inverted[27]	0.85	-
Screw cap (Stelvin), inverted[27]	0.61	-
Natural cork, horizontal[12]	1.18	-
Microoxygenation		
1 month at 10 ml/l/m. then 4 months at 5 ml/l/m.[34]	-	42
0-5 ml/l/m., 3-6 months	-	≤42
30-40 ml/l/m. up to 4 weeks prior to completion MLF[37]	-	42-56
Full saturation		
20°C[36]	-	8.4

*ml/l/m. – ml/l/month

%O2 from headspace, can also be expressed using the following equation [45]:

$\%O_2 = \frac{P_{kPaO_2}}{T_{kPaO_2}}$ (Eq 12)

P_{kPaO_2} - partial pressure (kPa)

T_{kPaO_2} - total pressure, determined with an aphrometer in the case of sparkling wines (kPa);

$\%O_2$ - volume fraction of oxygen component.

Partial pressure of oxygen (kPa). Each gas has a partial pressure which is the pressure which the gas would have if it alone occupied the entire volume. Partial pressure of oxygen can be calculated with the following equation [45]:

$P_{kPaO_2} = T_{kPaO_2} \times \%O_2$ (Eq 13)

where:

P_{kPaO_2} - partial pressure (kPa)

T_{kPaO_2} - total pressure, determined with aphrometer in the case of sparkling wines (kPa);

$\%O_2$ - volume fraction of oxygen component.

For example at T_{kPaO_2}= 101.325 kPa, and 21% O_2, P_{kPaO_2}= 21.28 kPa. A T_{kPaO_2}= 101.325 kPa, would work for still wines. In a sparkling wine bottle with a total pressure of 500 kPa, 15% O_2, the partial pressure would be 75 kPa.

Total pressure (kPa): The total pressure of a gas mixture is the sum of the partial pressures of each individual gas in the mixture. Average sea-level pressure is 101.325 kPa, value that work for still wines [45].

$F_{DO} + HO = TPO$ (Eq 14)

where:

F_{DO} - final dissolved oxygen after filling; (mg/l);

HO – headspace oxygen right after filling (mg/l);

TPO – total package oxygen right after filling (mg/l);

$TPO + I_{O_2} = TLCPO$ (Eq 15)

where:

TPO – total package oxygen right after filling (mg/l);

I_{O_2} - ingress of O_2 in filled package during several months of storage (mg/l);

$TLCPO$- total life cycle package oxygen during several months (mg/l);

$TLCPO - I_{DO} = TOP$ (Eq 16)

where:

$TLCPO$- total life cycle package oxygen during several months (mg/l);

I_{DO} - initial dissolved oxygen from wine before filling (mg/l);

TOP – total oxygen pickup during several months (mg/l);

RESULTS AND DISCUSSIONS

Usage of SO_2 in wines. The sulphur dioxide alone is used only for dry wines in order to prevent oxidation and microbial wine spoilage.

A model dry white wine may have the following parameters:

- ✓ Alcohol concentration % vol./vol.: 12.5;
- ✓ Temperature of wine: 18°C;
- ✓ pH: 3.4;
- ✓ Free sulphur dioxide: 18 mg/l;
- ✓ Initial dissolved oxygen from wine before filling: 0.5 mg/l;
- ✓ Dissolved oxygen pickup during filling: 3 mg/l;
- ✓ Percentage of oxygen from headspace (%O2): 9.2%;
- ✓ Headspace volume: 8.7 ml;
- ✓ Bottle volume: 0.75 l;
- ✓ Rate of oxygen through cork: 0.85 mg/l/year O2;
- ✓ Expected time of delivery: 6 months;

In order to give an accurate dosage of sulphur dioxide in wine, the winemaker should calculate the total life cycle package oxygen during several months (*TLCPO*). In the case of the above mentioned model wine, the *TLCPO* should be calculated for 6 months.

According to Equation 10, the final dissolved oxygen after filling (F_{DO}) is 3.5 mg/l. Then, in accordance to Equation 11, the headspace oxygen immediately after filling (*HO*) is about 1.53 mg/l. By applying equation 14, the resulted total package oxygen immediately after filling (*TPO*) is 3.5 + 1.53 = 5.03 mg/l.

In accordance with equation 15, the total life cycle package oxygen during 6 months (*TLCPO*) is 5.03 + (0.85/2) = 5.455 mg/l O_2

Then, based on Equation 5, pK_{a1} of sulphur dioxide, for given values is: 1,9348. Using the Equation 4, the molecular SO_2 for given values is: 0,60 mg/l.

In compliance with a consumption ratio of oxygen : sulphur dioxide of 1 : 2, knowing that

1 mg/l of *TLCPO* consumes 4 mg/l of sulphur dioxide. Following this, admit that 5.455 mg/l O_2 of *TLCPO* consumes 21.82 mg/l of sulphur dioxide in 6 months.

Therefore, the free SO_2 needs to be increased with 21.82 mg/l in order to achieve the level of 18 + 21.82 = 39.82 mg/l SO_2.

The molecular sulphur dioxide level will be 1,32 mg/l SO_2 at 18°C. After storage, sulphur dioxide will react with *TLCPO* leading to a molecular sulphur dioxide value about 0.6 mg/l at 18°C.

Usage of SO_2-AA in wines. Is used only for dry, semi-dry white and rose wines in order to preserve the aroma. It should be used judiciously, because it may cause oxidation during wine aging.

Using the same parameters from previous studied wine and knowing that 1 mg/l of dissolved oxygen, consume 5.5 mg/l ascorbic acid and 4 mg/l of free sulphur dioxide we can calculate the precise dosage of ascorbic acid and sulphur dioxide.

To reduce 5.455 mg/l O_2 of *TLCPO,* we need 30 mg/l of ascorbic acid and 21.82 mg/l of sulphur dioxide to be consumed in 6 months.

Free SO_2 need to be increased with 21.82 mg/l in order to achive the level of 18 + 21.82 = 39.82 mg/l SO_2.

The molecular sulphur dioxide level will be 1.32 mg/l SO2 at 18°C. After storage, ascorbic acid will react with *TLCPO*, then will release oxidative compounds, which react with free sulphur dioxide.

After 6 months, molecular sulphur dioxide should have a value about 0.6 mg/l. at 18°C.

Usage of SO_2-Sorbate in wines. Is used only for sweet wines in order to prevent a second fermentation in bottles due to the capacity of sorbates to inhibith the metabolism of yeasts.

We can assume that a sweet white wine has the following parameters:

- ✓ Alcohol concentration % vol./vol.: 11.2;
- ✓ Temperature of wine: 18°C;
- ✓ pH: 3.54;
- ✓ Free sulphur dioxide: 28 mg/l;
- ✓ Initial dissolved oxygen from wine before filling: 0.5 mg/l;
- ✓ Dissolved oxygen pickup during filling: 3 mg/l;
- ✓ Percentage of oxygen from headspace (%O2): 9.2%;
- ✓ Headspace volume: 8.7 ml;
- ✓ Bottle volume: 0.75 l;
- ✓ Rate of oxygen through cork: 0.85 mg/l/year O2;
- ✓ Expected time of delivery: 1 year;

First of all, for a wine with the parameters given above, we need to calculate the dosage of potassium sorbate needed to prevent a second fermentation in the bottle.

According to Equation 9, the winemaker can calculate the precise dose of sorbic acid that is necessary to prevent a second fermentation. The calculation shows that a concentration of 124.6 mg/l of sorbic acid will prevent a second fermentation. The amount of potassium sorbate, as results from Equation 6, is 167 mg/l. Potassium sorbate additions require clear wines, with yeasts population of no more than 5000 cells/ml. After potassium sorbate treatments, wines should be stabilized by cold or other treatment for tartaric acid stabilization.

According to Equation 10 and 11, the final dissolved oxygen after filling (F_{DO}) is 3.5 mg/l and the headspace oxygen immediately after bottle filling (*HO*) is aprox. 1.53 mg/l, respectively.

According to Equation 14 and 15, total package oxygen immediately after filling (*TPO*) is 3.5 + 1.53 = 5.03 mg/l and total life cycle package oxygen during 6 months (*TLCPO*) is 5.03 + 0.85 = 5.88 mg/l O_2, respectively.

From Equation 5, pK_{a1} of sulphur dioxide, for given values is calculated as 1.9092. Using the Equation 4, molecular SO_2 for given values is: 0.64 mg/l. Therefore, in order to give a good stability of wine, molecular sulphur dioxide should be at least 0.8 mg/l.

In compliance with a consumption ratio of oxygen : sulphur dioxide of 1 : 2, we know that 1 mg/l of *TLCPO* consumes 4 mg/l of sulphur dioxide. Following this, we find that 5.88 mg/l O_2 of *TLCPO* consumes 23.52 mg/l of sulphur dioxide in 1 year.

To ensure a level of 1 mg/l of molecular sulphur dioxide, after the reduction of *TLCPO* which occurs for 1 year, free sulphur dioxide needs to be increased with 15,7 mg/l + 23.52

mg/l = 39.22 mg/l SO_2, in order to achieve the level of 28 + 39.22 = 67.22 mg/l free SO_2.

The actual level of molecular sulphur dioxide will be 1.54 mg/l SO_2 at 18°C. After one year storage, sulphur dioxide will react with *TLCPO* leading to a molecular sulphur dioxide value of about 1 mg/l at 18°C.

CONCLUSIONS

The proper usage of preservatives according *TLCPO*, wines retain their qualities for a longer time and will be microbiologically stable. Also, in this way the levels of sulphur dioxide will be in the moment of the consumption at the recommended levels presented in Table 1. Management of oxygen is decisive for the wine quality and should be constantly monitored.

By finding an equation for potassium sorbate additions, which depends on wine pH and alcohol concentration, the precise dosage required for wine stabilization was achieved.

Also, a solution was found for the establishment of the optimal ratio of ascorbic acid and sulphur dioxide needed to prevent the pigmentation development as a result of incorrect ascorbic acid additions.

Therefore, the determined optimal ratio of ascorbic acid and sulphur dioxide of 1 : 2 (or higher) also confirmed that sulphur dioxide and ascorbic acid act synergistically.

Also, a proper management of sulphur dioxide in the presence of dissolved oxygen is required in order to preserve the quality in wines. With the equations derived by researchers the calculation of molecular sulphur dioxide is easily performed by any winemaker.

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