RESOLUTION OIV-OENO 631-2020

REVIEW OF PRACTICES FOR THE REDUCTION OF SO_2 DOSES USED IN WINEMAKING

THE GENERAL ASSEMBLY,

IN VIEW OF THE ARTICLE 2, paragraph 2 b) ii of the Agreement of 3rd April 2001 establishing the International Organisation of Vine and Wine,

AT THE PROPOSAL of the "Microbiology" and the "Technology" Expert Groups,

CONSIDERING the interest of the wine sector in producing wines with low SO_2 levels,

DECIDES to adopt the following document as OIV guidelines for the reduction of SO_2 doses used in winemaking:

Review of practices for the reduction of SO_2 doses used in winemaking

Background

The production of wines of high organoleptic quality is one of the priority goals for the world oenology industry. In parallel, in the last few years consumer demand for products free from chemical additives has grown. In this context, the OIV made the safety of consumers and their expectations one of the strategic axes of the Organisation's five-year Strategic Plan 2015-2019 (Axis 4: "Contribute to the safety of the consumers and consider their expectations").

One of the issues of contemporary oenology is the use of sulphur dioxide (SO_2), the most frequently used chemical additive in winemaking. In the present document the term "SO_2" refers to the main forms of sulphur dioxide in equilibrium in wine (molecular SO_2, bisulphite ions-HSO_3^- and bound SO_2). SO_2 is a tool of choice to preserve wine quality, thanks to its antiseptic, antioxidant, and of enzymatic inhibition properties (Ribéreau-Gayon *et al.*, 2006). During the phases preceding alcoholic fermentation, SO_2 makes it possible to limit oxidations and to reduce the total microbial load of must, favouring the selection of microorganisms most suitable for alcoholic fermentation such as *Saccharomyces Cerevisiae*, thus reducing the risk of fermentation defects. In finished wine, after malolactic fermentation if any, the use of SO_2 is aimed to eliminate spoilage microorganisms, such as lactic acid bacteria, acetic acid bacteria and yeasts of the *Brettanomyces/Dekkera* genera, which are responsible for the off-odour related to the production of volatile phenols. Oxygen in must and wine comes from the air, and it is dissolved during winemaking, ageing and storage operations. Depending on the wine composition and the level of oxygen exposure during winemaking, oxidation could have both a positive and negative impact on wine quality. SO_2 is effective in preventing the appearance of oxidative off-flavours and degradation of numerous aromas and of the colour. An the form of HSO_3^- is able to inhibit oxidation enzymes in must and to prevent the oxidative browning (Du Toit et al., 2006; Waterhouse et al., 2016). Among oxidase enzymes, laccase from *Botrytis* spp. is less sensitive than grape tyrosinase, so musts produced from grapes affected by *Botrytis cinerea* require higher sulphiting levels (Du Toit et al., 2006). In wine, where chemical oxidation is predominant, sulphites react with hydrogen peroxide, and inhibit the Fenton reaction responsible for the oxidation of ethanol and other organic compounds by hydroxyl radical formation (Danilewicz, 2007). The reaction between sulphites and hydrogen peroxide also occurs in musts, although in these the chemical oxidation occurs to a much lesser extent. Another effect of sulphur dioxide is its ability to react with quinones formed during the initial stages of oxidative chain reactions, reducing them to phenols (Danilewicz et al., 2008; Waterhouse & Laurie, 2006). Moreover, it binds aldehydes (e.g., acetaldehyde), thus playing a role in reducing the perception of typical oxidative off-flavours (Waterhouse & Laurie, 2006). Concerns have recently arisen regarding the safety of sulphites in food. Sulphur dioxide and sulphites have well-known toxic effects, both acute and chronic in sensitive subjects. They exacerbate respiratory, dermatological, cardiovascular and gastrointestinal symptoms, manifested mainly as asthmatic-like reactions in "sulphite sensitive" individuals, while severe allergic-like reactions (anaphylaxis) are uncommon (Papazian, 1996; Vally & Thompson, 2003). Taking this into account, the WHO established an ADI (Acceptable Daily Intake) of 0.7 mg/kg bw for sulphur dioxide. The European Union imposes labelling with the indication ''contains sulphites'' if the total SO_2 concentration is higher than 10 mg/L (Directive 2003/89/EC). Aside to be added, sulphur dioxide is produced by yeasts during alcoholic fermentation (Suzzi et al., 1985; Wells & Osborne, 2011), in concentrations of up to 100 mg/L depending on the yeast strain and must composition (Thomas & Surdin-Kerjan, 1997). Currently, numerous yeast strains are selected in order to produce low quantities of sulphites, but on the market there is still a significant number of strains capable of producing significant levels of sulphites during alcoholic fermentation.

Today, following consumers demand, the tendency is to decrease the use of sulphite in winemaking. Different other additives and innovative physical methods, such as pulsed electric fields, have been proposed in order to achieve the various objective of the use of sulphur dioxide (Lisanti *et al.*, 2019). Some alternative additives are already authorised in winemaking, while most of additives and innovative physical methods have been tested only on an experimental scale. However, based on current knowledge, none of these alternatives showed to be able to totally replace SO_2, which remains a useful, even indispensable additive in some cases.

These guidelines contain recommendations for the management of the entire winemaking process – from the vine to the bottle – to reduce the use of SO_2 without compromising wine quality in terms of organoleptic characteristics and microbiological stability.

General principles

A small and modulated oxygen intake could be necessary to enhance wine colour and aroma, especially in red wine production, always guaranteeing microbiological stability.

The **factors** to be considered are:

- the must and wine microbial load,

- the presence of oxidase enzymes derived from fungi in musts,

- the concentration of SO_2-binding substances in must and wine,

- pH,

- wine temperature,

- oxygen exposure,

- presence and concentration in wine of endogenous antioxidant compounds.

Some viti-vinicultural practices are able to influence one or more of these factors, thus modifying the SO_2 requirement, by increasing or decreasing it.

The **phases** in which the addition of SO_2 is advised in order to prevent oxidation and microbial spoilage are, as a minimum:

- at harvesting, in the case of mechanical harvesting, in the production of white and rosé wines,
- pre-fermentative phases (grapes and must), especially for white and rosé wines,
- at the end of alcoholic fermentation (or malolactic fermentation, if applicable),
- at conditioning or disgorgement for sparkling wines (final corking).

Especially in the case of white winemaking, it is necessary to use SO_2, and possibly additives that complement its action (e.g. ascorbic acid), potentially in combination with pure or mixed inert gases (e.g. argon or nitrogen), with the aim to protect against oxidation and to preserve aroma.

For the treatment of microbiological accidents, sulphitation through the addition of SO_2 in gaseous form (released from liquid SO_2) should be preferred for its greater effectiveness.

With the same total SO_2 concentration, the balance between its different forms (free, molecular and bound) depends on the chemical-physical characteristics of wine, in terms of pH, alcohol content, compounds capable of binding SO_2, as well as temperature. The fraction of SO_2 bound to acetaldehyde is very poorly available for wine protection, as this combination is very stable, therefore it should be minimised by reducing the formation of acetaldehyde in wine, both from chemical and from microbiological origin (Waterhouse et al., 2016; Capece et al., 2020).
In order to guarantee effective protection, reference values of 20-40 mg / L of free SO_2 can be considered for antioxidant protection, while to prevent microbial alterations in the finished wine 0.6 mg / L of molecular SO_2 in dry wines and at least 0.8 mg / L of molecular SO_2 in sweet wines are reported as reference values (Waterhouse, 2016). The reduction of the total SO_2 levels should be carried out by always guaranteeing adequate levels of protection, based on the characteristics of the wine, the storage conditions and the expected commercial life.

In the present document the entire winemaking process is analysed by identifying the Points of Intervention (PI) that are useful for the reduction of total SO_2 doses and giving indications for their proper management.

Classification of the Points of Intervention (PI)

In this document, PIs are coded as follows:

PIa) point of intervention to limit the presence and the activity of oxidase enzymes;

PIb) point of intervention to prevent must and wine microbial spoilage;

PIc) point of intervention to limit dissolution and consumption of oxygen in musts (c1: enzymatic oxidation) and oxidation reactions (c2 chemical oxidation);

PId) point of intervention to enhance SO_2 efficacy (d1: increasing of molecular SO_2; d2: minimising the formation of compounds binding SO_2, including those derived from grapes affected by fungal diseases);

PIe) point of intervention to avoid excessive or unwanted addition of SO_2;

PIf) point of intervention to ensure the efficacy of SO_2 protection.

All the oenological practices should be applied according to the OIV *International Code of Oenological Practices*. The present document should be updated following the admission of new oenological practices.

POINTS OF INTERVENTION:

A. IN ALL WINEMAKING PHASES

The following is recommended:

- to ensure suitable hygiene practices are applied throughout the whole process (PIb),

- to ensure that the grape transport and winemaking equipment do not release metal ions (iron, copper and manganese) into must and wine (PIc2),

- to ensure that the grapes do not contain or contain low concentrations of metal ions from phytosanitary treatments such as copper (Bordeaux mixture) (PIc2),

- to ensure that pipes and the connections between pipes do not have any cracks or holes, and to periodically replace the tubes (PIc),

- to ensure that the internal surfaces of tanks and vats are perfectly intact and do not have any cracks (PIb),

- when choosing pumps to transfer must and wine, to consider that different types have different oxygen uptakes (e.g. centrifugal pumps are particularly disadvantageous if cavitation of the liquid which occurs during the transfer is not prevented) (PIc),

- to consider the oxygen porosity of the materials that make up the winemaking or storage vessels (PIc),

- to limit the oxygen uptake during the dynamic phases (turbulence due to the start / stop of pumps, Venturi effect due to poorly tight connections, transport in open compartments) and static phases (through holes and porous materials) (PIc),

- if SO_2 is supplemented in the form of potassium metabisulphite or solutions, to ensure that the product is not expired, that it has been properly stored and that the solutions have not crystallised (PIf),

Important note: It should be considered that SO_2 binding substances are generated by fungi from the vineyard as well as during alcoholic fermentation, and also in wine following contamination by aerobic yeasts and bacteria or following chemical oxidation reactions catalysed by metals such as iron and / or copper.

B. VINEYARD MANAGEMENT AND HARVEST

- The selection of cultivation site and grape variety should be aimed at producing healthy grapes that have an adequate level of acidity at maturity, so that they have a suitable level of acidity upon reaching maturity. Any viticultural practice ensuring the sanitary state of grapes and/or low pH of the must should be applied in the vineyard (PIa, PIb, PId).

- As copper is a catalyst of chemical oxidation, its use in the vineyard should be limited as much as possible, but adapted to the protection of grapes from fungi development (PIc2).

- Viticultural practices capable of promoting the aeration of fruit and reducing the risks of fungal infections (e.g. defoliation, nitrogen nutrition, etc.) should be performed, especially in the case of varieties with very compact bunches (PIa, PIb).

- Harvest time should be chosen with precision in order to obtain musts that have adequate acidity and sugar content, which at the same time allow the completion of fermentations and the satisfaction of sensory expectations (PIb, PId1).

- Especially for certain grape varieties and in hot climates, harvesting and processing a portion of the crop before maturity makes it possible to obtain a more acidic wine, which can be useful for increasing the acidity of the wine obtained from the remaining fraction harvested at full maturity ("double harvest"). As an alternative, it is possible to use a blend of different grape varieties at different stages of maturity. To do so, it is necessary to monitor the chemical parameters linked to technological maturity (soluble solids, total acidity, malic acid, tartaric acid, potassium and pH) (PId1).

- Harvesting should be selective in order to vinify separately bunches damaged by fungi in order to limit the presence of oxidase enzymes and spoilage microorganisms (PIa, PIb).

- In the case of mechanical harvesting, ensure that grape integrity is preserved as much as possible. Where possible, manual harvesting allows to limit the mechanical damage to the grapes (PIb).

- To avoid crushing of grapes and microbial proliferation, favoured by the release of the juice, whole healthy grapes should be transported in clean and well aerated containers, in order to avoid high humidity and mould growth. However, if grapes are damaged, it is preferable to vinify them separately, by using inerted containers to limit oxidation of the must and proliferation of aerobic microorganisms (Pia, PIb).

C. GRAPES AND MUST

- If musts from microbiologically altered grapes are obtained, these musts should be treated separately, from the reception of the grapes to the processing in the cellar, as they will need greater protection by the SO_2, therefore the use of higher doses (PIa-PIb).

- All pre-fermentative operations should be carried out as rapidly as possible, in order to limit oxygen exposure (PIc), except where hyperoxygenation of the must, followed by clarification, is used as a practice to eliminate oxidisable compounds before vinification.

- Pre-fermentative operations of destemming, crushing, pressing (or combinations of these) should be conducted as delicately as possible, in order to limit the extraction of potassium cations, thus preserving the must acidity (PId1), and reduce the extraction of oxidase enzymes (PIa).

- If necessary, acidification of musts should be performed. Acidification should be carried out as soon as possible (PId).

-The addition of SO_2 prior to alcoholic fermentation should be limited as much as possible and adapted to must pH, to avoid the SO_2 bounding with carbonyl compounds, most of all acetaldehyde, produced during the subsequent alcoholic fermentation (PId2).

- Early inoculation with selected microorganisms (yeasts and/or bacteria), even during the pre-fermentative phase, could be useful in preventing the development of undesirable indigenous microbial flora.

Specifically for white or rosé winemaking:

- Antioxidant protection should be ensured during or soon after pressing. The addition of SO_2 to the mass should be perfectly homogeneous (PIc).

- To complement the action of SO_2, the addition of antioxidants (such as ascorbic acid, inactivated yeasts rich in glutathione, tannins) and antimicrobials (such as lysozyme, chitosan) may be considered (PIc, PIc).

- The addition of ascorbic acid, if any, must follow the addition of SO_2 (PIc2).

- Inert gas may be used in the more critical phases in oxidation terms, such as the transfer after destemming and / or crushing, pressing and must clarification (i.e. static settling with or without enzymes or processing aids, flotation, filtration or centrifugation) (PIc).

- The temperature of the musts in the pre-fermenting phase must be controlled so as to be compatible on the one hand with the chosen clarification process and on the other with the limitation of the risk of development of a spontaneous microbial flora that would hinder the clarification process. Although low temperatures slow down oxidative processes, the effect of low temperatures on the increase in oxygen solubility should be considered. As an indication, a temperature of about 15 °C in flotation, less than 10 °C during static clarification, of 20 °C in filtration or centrifugation can be recommended if the musts are treated continuously after their production and much lower the longer the time between mashing and treatment (PIc).

D. ALCOHOLIC FERMENTATION (AF)

- Inoculation with a starter yeast is advised; yeasts with good fermentative activity, high dominance, and low SO_2, H_2S, SO_2 binding compounds, and acetaldehyde production should be chosen. Yeasts should be properly stored. The preparation of yeast inoculums from active dry yeast or in other forms should be conducted according to manufacturer's instructions and should lead to an initial population sufficient to ensure a quick start to AF (approx. 1-2 x 10^6 CFU/mL), in order (PIb, PId, PIe).

- Co-inoculation with selected starter yeasts and lactic acid bacteria may favour the reduction of the lag period between the end of alcoholic fermentation and the start of malolactic fermentation, and may reduce the development of undesirable bacteria and of *Brettanomyces* spp. (Resolution OIV-OENO 462-2014) (PIb). In the case of co-inoculation, avoid SO_2 addition to the must. Addition of SO_2 during AF should be avoided. There is no need for SO_2 during AF if it takes place quickly, as oxygen dissolution is limited and the microbial population is dominated by fermenting yeasts, involving a very low risk of undesirable microbial development. SO_2 added in this phase would bind with carbonyl compounds, thus increasing the total SO_2 uselessly, at equal quantities to the free SO_2 present (PId2). Furthermore, the SO_2 added during AF is metabolised directly by fermentative yeasts with a proven risk of producing compounds responsible for reduction defects (H_2S).

- Oenological practices aimed at ensuring that AF runs normally and properly, and lowering the production of carbonyl compounds (among them thiamine addition, supplementation with nitrogen nutrient and growth factors, use of yeast hulls to detoxify the matrix, where necessary) and controlling the temperature should be implemented (PIb, PId2). Nitrogen can be added in mineral or organic form (sources such as yeast autolysate or inactivated yeasts).

- Fermentation kinetics should be monitored daily to verify that AF is running normally and properly. Stuck or sluggish fermentations should be detected as soon as possible. The use of automated temperature control systems is advised (PIb, PId2).

- In the case of stuck or sluggish fermentation, oenological practices to restart alcoholic fermentation must be applied as soon as possible. A proper addition of SO_2 is recommended in order to inhibit bacterial development prior to restarting AF. Detoxifying the matrix (elimination of fatty acids) using yeast hulls is also recommended. The volatile acidity should be monitored (PIb, PId2).

- The total transformation of fermenting sugars is advised for dry wines (reducing sugars < 2 g/L) (PIb).

E. POST-ALCOHOLIC FERMENTATION AND MALOLACTIC FERMENTATION

- In white and rosé winemaking, running off should be carried out while protecting the wine from the air. The receiving tank as well as the pipes and connections should be inerted with carbon dioxide, nitrogen or argon (or a mix of these) prior to wine input (PIc2).

- In red winemaking, soft pressing of pomace allows to reduce the extraction of potassium cations and then to limit pH increase (PId1).

- If malolactic fermentation is performed, it should be rapidly induced (PIb). The co-inoculation with yeasts and lactic acid bacteria or early inoculation with selected bacteria before or just after inoculation with yeasts could be considered useful to reduce the lag time between alcoholic fermentation and malolactic fermentation if conditions are suitable (PIb). Bacteria starter cultures should be prepared in accordance with the supplier's recommendations (PIb).

- It should be ensured that all of the parameters critical for malolactic fermentation to run properly (pH, nutrients, temperature) are controlled and that the volatile acidity, lactic acid and malic acid are monitored until its completion (PIb).

- To avoid the development of malolactic fermentation, the addition of an antimicrobial agent as chitosan complementing SO_2 action may be considered (Plb).

- Starting from the end of alcoholic fermentation, the concentration of molecular SO_2 should be monitored in order to ensure effective protection of the wine (PIf).

F. STABILISATION

- At the end of the alcoholic fermentation (or malolactic fermentation, if carried out), the wine should be sulphited. It is advisable to dose free acetaldehyde. The dose of SO_2 will therefore be chosen according to the pH, alcohol content and temperature, in order to guarantee antioxidant and antimicrobial protection (PIb-PIc2).

- In order to ensure effective protection of the wine, it is very useful to monitor, since the end of alcoholic fermentation, the quantity of total and free SO_2 and to calculate the molecular SO_2 concentration (PIf).

Physical treatments

- After alcoholic fermentation or malolactic fermentation, if any, the microbiological stabilisation of wine by physical (i.e. sterile membrane filtration and/or pasteurisation) and/or chemical methods (SO_2 possibly supported by other permitted antimicrobial chemical agents in wine, as chitosan) is recommended. Among these technical possibilities, the use of tangential microfiltration associated with permeate treatment seems to be particularly appropriate. Care should be taken to avoid subsequent recontamination (PIb).

- The initial and final phases of different stabilisation practices such as filtration, micro-filtration, membrane processes and bottling are the most prone to introducing oxygen into wine, therefore the volume of the treated batch is critical and should be maximised. When possible, continuous processes are preferable (PIc2).

- The use of inert gas is advised for all of the processes mentioned in the previous point. If inert gas cannot be used for the whole duration of the process, consider that the most critical points are: the initial and final phases of the process, the inerting of the empty apparatus (i.e. filters and pipes) and the inerting of feeding and receiving tanks. Consider that different types of filters may enrich wine with oxygen differently: careful attention should be paid to this characteristic when choosing between them (PIc2).

- The Venturi systems used for the addition of fining agents to wine and for their homogenisation may cause an excessive solubilisation of oxygen in the wine (PIc2).

- Cold tartaric stabilisation is a highly critical point, as a high quantity of oxygen may be solubilised due to the low temperature. Static stabilisation is even more critical than dynamic systems. Inert gas should be used, taking great care when inerting the empty apparatus (including the filtration system) before wine input. Protection of wine using SO_2, even at low doses, is also advised at this step (PIc2). Alternative stabilization techniques such as physical treatments (cation exchange resins, electrodialysis) or products (carboxymethylcellulose, potassium polyaspartate, mannoproteins or metatartric acid) have to be considered, depending on the wine profile and constraints (organic production for instance).

G. CONSERVATION AND AGEING

- Tanks where wine will be transferred should be inerted and filled from the bottom to minimise the air that enters (PIc2).

- Implement a regular control plan for wines, based both on standard analytical parameters (pH, alcohol content, titratable acidity, volatile acidity, free and total SO_2), and on microbiological parameters (presence and level of contaminating microorganisms and products of their metabolism).

- Containers (tanks, barrels) should always be kept full (no headspace). Microbiologically stable wine should be used for topping up to avoid greater microbiological contamination. If the presence of headspace is unavoidable for a short period, use inert gas (PIb, PIc2).

- In case of yeast development at the surface of the wine, consider that these produce high quantities of acetaldehyde that binds with SO_2 in a stable way. For this reason, it is useless to try to eliminate these yeasts by adding SO_2 because the majority are extremely tolerant to it. Before re-establishing the free SO_2 content to protect the wine, remove the layer of yeasts by filtration and top up the container (PIb-PIc2-PId2-PIf).

- During wine conservation or ageing, maintain a constant temperature (indicatively, 13-18 °C). An overly low temperature increases oxygen solubility, while an overly high temperature is favourable to oxidation reactions and microbial proliferation (PIb, PIc2).

- Ageing on the lees of yeast and bacteria could be a useful practice in enhancing the antioxidant efficacy of SO_2. This may be due to the consumption of oxygen for lipid oxidation, the bounding of carbonyl compounds and the release of reducing compounds. However, the volatile acidity and microbial load, as well as the free, bound and molecular SO_2, should be carefully monitored because products of yeast autolysis may favour the proliferation of spoilage microorganisms (acetic and lactic acid bacteria, *Brettanomyces* spp.). SO_2 protection should be ensured for its entire duration (PIc2).

- To complement the action of SO_2, the addition of antioxidant (such as tannins and inactivated yeasts rich in glutathione) and antimicrobial agents (such as lysozyme and chitosan, in the case of *Brettanomyces* spp. contamination) may be considered (PIb, PIc).

- The cellar humidity, temperature and ventilation should be controlled to avoid excessive wine loss by evaporation from barrels and the formation of headspace (PIc2).

- Consider that there are differences in oxygen permeability for wood from different botanical species, and for new barrels with respect to used ones, so these should be chosen carefully according to wine characteristics (i.e. polyphenol content, necessity for colour stabilisation, level of astringency) (PIc2).

- During wood ageing, efforts should be made to limit air penetration via the bunghole, or while topping up or opening for tasting. It should be ensured that there are no cracks in the wood (PIc2).

- Consider that the perfect sanitisation of old barrels is quite difficult, especially with respect to contamination by *Brettanomyces* spp*.* Shaving and re-firing make it possible to remove the most contaminated wood layer, thus enhancing the efficacy of sanitisation. Use effective techniques and materials in the deep layers of the wood. Potassium bitartrate crystals should be eliminated prior to sanitisation (PIb).

- Consider that the gaseous SO_2 used for wood container sanitisation may enrich the wine with SO_2 at the time of filling (PIe).

H. PACKAGING

- An effective hygienisation of the packaging line, including the bottles and the room, should be carried out, in order to avoid wine recontamination. When designing the process, prefer solutions that save energy and resources. If inert gas is used, this should be microfiltered, as well as the water for washing the bottles (PIb).

- Temperature at packaging should be maintained at around 15-20 °C (PIc2).

- Wine to be packed should be microbiologically stabilised. Microbiological control should be implemented prior to packaging, and carried out randomly on packed wine (PIb).

- Physical methods, such as sterile filtration and, in some cases, pasteurisation, ensure the reduction of wine microbial load and could allow for the use of lower doses of SO_2 (PIb).

- In wines that contain fermentable sugars, the use of products that complement the antimicrobial action of SO_2 (such as sorbic acid and dimethyl dicarbonate) may be considered (PIb).

- During packaging, if the wine contains a high quantity of dissolved oxygen (> 0.5 mg/L), it should first be deoxygenated in a proper manner (PIc2-PIb).

- Bottle closures should be stored unopened in their original well-sealed packaging, in a cool and dry place (PIb).

- Considering that the binding of SO_2 and wine molecules is not an instantaneous phenomenon, a few days after the addition of SO_2 to wine, the free SO_2 concentration should be verified, to ensure that it corresponds with the desired concentration to obtain the stabilisation conditions. If necessary, a supplementary addition should be carried out (PIf).

- The addition of SO_2 should take in account losses due to oxidation phenomena (quantity of dissolved O_2) and the expected shelf life of the wine (PIf).

- Ascorbic acid may enhance the antioxidant activity of SO_2, however its addition should be carefully evaluated considering the loss of SO_2 during the expected wine shelf life. SO_2 antioxidant protection should be guaranteed until the expected time of wine consumption. Ascorbic acid should only be added after addition of SO_2 (PIb -PIc2).

- Inerting of the packaging line and of the containers (if possible and necessary) should be applied, paying particular attention to the beginning of the process of packaging and the last phases (PIb -PIc2).

- It should be ensured that the filling volume is properly regulated, also according to the temperature (PIc2).

- It should be ensured that the headspace is properly inerted after filling (PIb -PIc2).

- It would be good practice to measure the Total Package Oxygen (TPO) randomly during different phases of packaging, with a non-destructive optical method. The same method could be used to monitor the oxygen dissolved along the entire packaging line. Reference values should be taken into account to ensure these are not exceeded in wine after packaging (e.g. < 0.5 mg/L). It will be necessary to insert equivalent containers in the line that allow the measurement (transparent and equipped with sensor) (PIc2).

- The use of colourless glass bottles and transparent plastic containers should be avoided, with the latter material being permeable to oxygen. The use of green or brown glass bottles is advised (PIc2).

- It should be ensured that the homogeneity of the diameter of bottle necks is certified by the bottle manufacturer, as deviations from the nominal diameter may cause uncontrolled oxygen ingress (PIc2).

- Closures should be selected considering the oxygen permeability in relation to the wine compositional characteristics, in order to avoid oxidation. If using cork or plastic stoppers, choose products with short closure recovery times after compression (good elasticity). For wines particularly sensible to oxidation, the use of screw caps could be recommended (PIc2).

I. SHIPPING, STORAGE AND DISTRIBUTION

- High temperatures and direct light exposure should be avoided during shipping and storage. For the storage of bottled wines, a temperature of between 15 °C and 20 °C and humidity not higher than 70% are recommended (PIb-PIc2).

- The use of thermally insulated containers is recommended and the cooler seasons of the year should be preferred for shipping of wines, especially for wines which have to be shipped between continents. The use of systems capable of measuring and recording the temperature and humidity during transport should be provided for (PIc2).

- The final wine distributor should be informed about the appropriate storage and distribution of the wine, in light of its low content of SO_2 (PIc2).

- Note: The OIV *Good Practices Guide for Bulk Wine Transportation* should also be taken into account with regard to this section.

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