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ENARTIS NEWS

REPLACING SO_2 DURING FERMENTATION

Market demand for low SO_2 or SO_2-free wine is increasing as winemakers attempt to produce wines suitable for consumers suffering from food intolerances. For winemakers, embracing this philosophy means a greater commitment of time and responsibility in avoiding the risk of exposing an unprotected wine to chemical and microbiological modifications. With the recent approval of products such as chitosan and PVI/PVP, it is now easier to replace sulfur dioxide.

SO_2 ACTIVITIES

The three reasons for using SO_2 in the pre-fermentation stage are to:

- Protect juice from oxidation
- Minimize laccase activity in the case of Botrytis infected grapes
- Control the growth of microorganisms that can damage wine quality.

ALTERNATIVES TO SO_2 ANTIOXIDANT AND ANTILACCASE ACTIVITY

To better understand if and how SO_2 can be replaced, it is necessary to know the mechanism behind the oxidation of juice and how SO_2 can interfere with it.

Juice Oxidation Mechanism

Oxidation is considered one of the main problems encountered during vinification as it adversely affects the sensory properties of wine causing browning, loss of flavor and aroma, and increased astringency.

Juice oxidation can be classified into enzymatic (Fig. 1) and non-enzymatic oxidation, with the former dominating on the latter. Many juice and wine compounds are susceptible to oxidation but phenolic compounds are most affected.

Enzymes responsible for phenolic compound oxidation at the juice stage are polyphenol oxidases (PPO): tyrosinase in healthy grapes and laccase in Botrytis infected grapes. PPOs turn phenolic compounds, mainly hydroxycinnamic acids and catechins, into quinones, strong oxidants responsible for juice browning.

This reaction is very fast and occurs within 10-15 minutes beginning from when a grape berry is damaged or crushed.

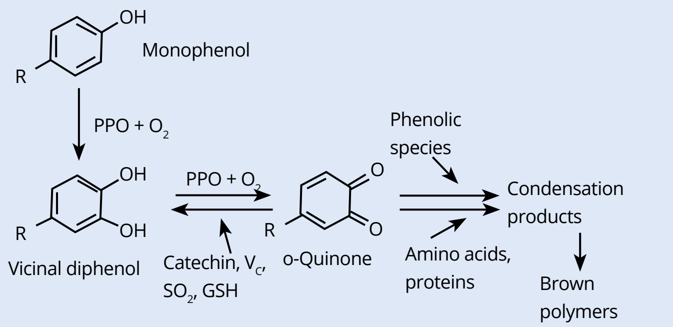

Fig. 1: Enzymatic browning process in grape juice.

SO_2 alternatives for minimizing juice oxidation

For enzymatic oxidation to happen, oxidation enzymes, O_2 and the phenolic substrate must be present. Consequently, the antioxidant strategy can operate on 3 fronts:

Reducing O_2 solubilization in juice

Avoiding exposure to air, thus preventing the solubilization of oxygen in juice is the first step to prevent oxidation. In addition to using inert gas, chemical inertization is possible using compounds that can quickly react with oxygen before it can enter the enzymatic oxidation mechanism.

Contrary to common belief, SO_2 is very slow in reacting with oxygen. **Ascorbic acid** is 170 times faster in reacting with oxygen than SO_2. Its application (always in conjunction with SO_2 and tannins to block oxygen peroxide produced by the oxidation of ascorbic acid), is an effective and cost-effective alternative to inert gas and allows for reduced SO_2 additions.

Gallic and ellagic tannins also have a high capacity for O_2 consumption and can be used to replace SO_2.

Reducing oxidation enzyme activity

SO_2 antioxidant effects depend on its capability of inhibiting juice oxidation enzymes. Up to 90% decrease tyrosinase activity has been observed upon the addition of 50 mg/L SO_2, but higher dosages are necessary to effectively inhibit laccase, resulting in negative consequences for alcoholic and malolactic fermentations and wine quality.

Enological tannins inhibit PPO activity and are more effective than SO_2 in inactivating laccase (Fig. 2).
Recent trials have shown that the application of **PVI/PVP** in white juice settling produces wines with a fresher color, higher content of aromatics and less sensitivity to oxidation. The reason is related to PVI/PVP's ability to remove metals such as copper and iron. During the juice stage, copper is a catalyzer of PPO activity and, similarly in wine, in combination with iron, it can catalyze the non-enzymatic oxidation of phenols.
Activated chitosan application during the juice stage is able to reduce laccase activity. The mechanism is not yet clear, chitosan could reduce laccase activity either by its capability in removing copper or by the direct reaction of positively charged chitosan with negatively charged laccase.
Finally, SO_2 can reduce quinones produced by the enzymatic oxidation process back to a re-oxidizable form. This explains the SO_2 bleaching effect when added to brown juice. **Glutathione and cysteine** have a similar ability, when added during the juice stage, they react with quinones formed from the oxidation of caftaric acid (the most abundant hydroxycinnamic acid in grape juice) by tyrosinase activity forming the "Grape Reaction Product" (GRP). GRP is colorless and is no longer a potential substrate for further oxidation by tyrosinase.

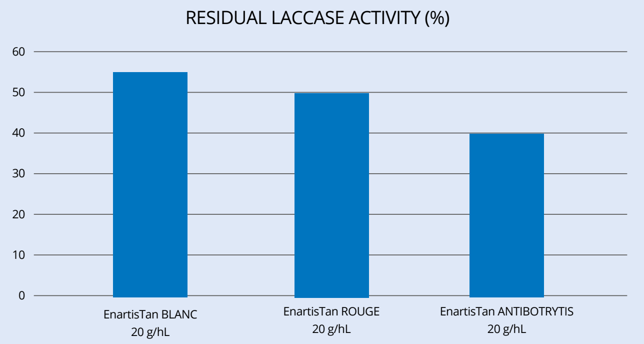

Fig.2: Tannin inhibiting effect on laccase activity

Removing phenolic compounds
Phenols are the main substrate of oxidation. Removing phenolic compounds with **fining agents** such as PVPP, animal or plant proteins and carbon is another way of minimizing the oxidation process.

ALTERNATIVES TO SO_2 ANTIMICROBIAL ACTIVITY

The antimicrobial activity of sulfur dioxide is mainly due to the molecular form whose concentration depends on the free sulfur content, pH, temperature and alcohol content.
SO_2 is effective in controlling both yeast and bacteria. It is able to interfere with the microbes metabolism in different ways: alteration of cell membrane permeability; alteration of enzymatic activity; inhibition of glycolysis; lipid modification; interaction with coenzymes and vitamins; destruction of thiamine; DNA and RNA transformation; and damage to structural proteins.
Every microorganism has a different sensitivity to SO_2 therefore the quantity that is necessary to block the growth is specie dependent. Grape pH's tendency to increase makes microbiological control with SO_2 increasingly difficult.
Activated chitosan antimicrobial activity is much less dependent on juice pH and makes it a reliable alternative to SO_2. It works by contact: the positive charges present on its surface attract negatively charged microorganisms in the juice. Subsequently, it alters the permeability of the cell membrane and causes the microorganism to die from osmotic shock. Like SO_2, chitosan is a non-specific antimicrobial compound and can control both yeast and bacteria.

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VINIFICATION PHASE	TYPE OF WINE	PRODUCT	ACTIVE MOLECULES	REASONS TO USE IT
Grapes and must		**AST**	Ascorbic acid, gallic tannin and potassium metabisulfite	• Reduction of dissolved oxygen • Blocks radicals
		EnartisTan BLANC or **EnartisTan AROM**	**EnartisTan BLANC:** Gallic tannin **EnartisTan AROM:** Gallic tannin, GSH and cysteine	• Reduction of dissolved oxygen • Blocks radicals • Reduction of laccase and tyrosinase activity
		EnartisTan BLANC or **EnartisTan ROUGE** or **EnartisTan COLOR**	**EnartisTan BLANC:** Gallic tannin **EnartisTan ROUGE:** Condensed, ellagic and gallic tannin **EnartisTan COLOR:** Grape seed tannin, ellagic tannin, GSH and cysteine	• Reduction of dissolved oxygen • Blocks radicals • Reduction of laccase and tyrosinase activity
		CLARIL AF	Bentonite, PVPP, pea protein	• Removal of catechins • Removal of iron
		EnartisStab MICRO M	Activated chitosan	• Removal of spoilage microbes • Removal of catechins • Removal of iron and copper • Reduction of laccase and tyrosinase activity
		EnartisPro FT or **EnartisPro XP**	**EnartisPro FT:** GSH and cysteine, free mannoproteins, PVI/PVP **EnartisPro XP:** Free mannoproteins, PVI/PVP	• Removal of copper and iron • Removal of catechins • Reduction of laccase and tyrosinase activity
		EnartisPro AROM or **EnartisPro BLANCO**	**EnartisPro AROM:** GSH and cysteine, mannoproteins **EnartisPro BLANCO:** GSH and cysteine, free mannoproteins	• Reduction of quinones (reduction of browing)
Fermentation		**EnartisFerm Q9** or **EnartisFerm ES181**	**EnartisFerm Q9:** Yeast for thiolic varieties **EnartisFerm ES181:** The "never go wrong" yeast for all varieties	• Low SO_2-producing strains
		EnartisFerm ES488 or **EnartisFerm WS**	**EnartisFerm ES488:** Yeast for blackberry aroma and "Ripen UP!" strategy **EnartisFerm WS:** Yeast for the fermentation of high-alcohol, big red wines	• Low SO_2-producing strains

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