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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 changes. With the recent approval of products such as chitosan and PVI/ PVP, it is now easier to replace sulfur dioxide.

SO_2 ACTIVITIES

Three reasons to use SO_2 during pre-fermentation:

1. Protect juice from oxidation
2. Minimize laccase activity in the case of *Botrytis* infected grapes
3. Control growth of microorganisms that can harm 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 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 in juice 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.

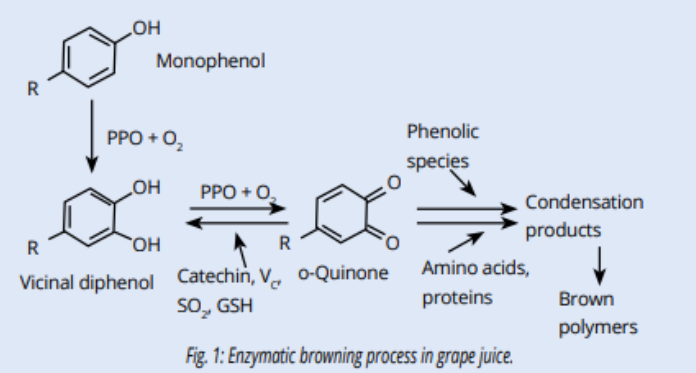

Fig. 1: Enzymatic browning process in grape juice.

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 non-enzymatic oxidation of phenols.
Activated chitosan application during the juice stage can reduce laccase activity. The mechanism is not yet clear - chitosan could reduce laccase activity by either 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.

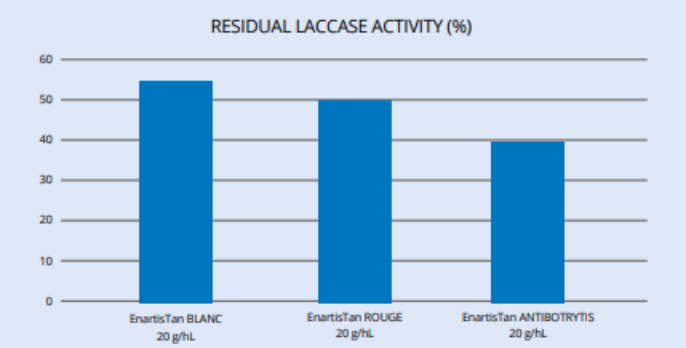

Fig.2: Tannin inhibiting effect on laccase activity

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

ALTERNATIVES TO SO2 ANTIMICROBIAL ACTIVITY

The antimicrobial activity of sulfur dioxide is mainly due to the molecular form whose concentration depends on free sulfur content, pH, temperature and alcohol content.
SO_2 is effective in controlling both yeast and bacteria. It is able to interfere with microbe metabolism in different ways: modification 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.
Each microorganism has a different sensitivity to SO_2; therefore, the quantity that is necessary to block the growth is specie dependent. Grape pH 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 juice. Subsequently, it alters the permeability of the cell membranes and causes microorganisms to die from osmotic shock. Like SO_2, chitosan is a non-specific antimicrobial compound and can control both yeast and bacteria.

VINIFICATION PHASE	TYPE OF WINE	PRODUCT	ACTIVE MOLECULES	APPLICATIONS
Grapes and Must	🟢🟠🔴	**AST**	Ascorbic acid, gallic tannin and potassium metabisulfite	• Reduction of dissolved oxygen • Blocks radicals
	🟢🟠🔴	**EnartisTan BLANC**	Gallic tannin	• Reduction of dissolved oxygen • Blocks radicals • Reduction of laccase and tyrosinase activity
		EnartisTan AROM	Gallic tannin, GSH and cysteine	
	🟠🔴	**EnartisTan BLANC**	Gallic tannin	• Reduction of dissolved oxygen • Blocks radicals • Reduction of laccase and tyrosinase activity
		EnartisTan ROUGE	Condensed, ellagic and gallic tannin	
		EnartisTan COLOR	Grape seed tannin, ellagic tannin, GSH and cysteine	
	🟢🟠	**CLARIL SP**	Bentonite, PVPP, potassium caseinate and silica	• 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**	GSH and cysteine, free mannoproteins and PVI/PVP	• Removal of copper and iron • Removal of catechins • Reduction of laccase and tyrosinase activity
		EnartisPro XP	Free mannoproteins and PVI/PVP	
	🟢🟠🔴	**EnartisPro AROM**	GSH and cysteine, mannoproteins	• Reduction of quinones (reduction of browning)
		EnartisPro BLANCO	GSH and cysteine, free mannoproteins	
Fermentation	🟢🟠	**EnartisFerm Q9**	Yeast for thiolic varieties	• Low SO_2-producing strains
		EnartisFerm ES181	The "never go wrong" yeast for all varieties	
	🔴	**EnartisFerm ES488**	Yeast for blackberry aroma and "Ripen Up!" strategy	• Low SO_2-producing strains
		EnartisFerm WS	Yeast for the fermentation of high-alcohol, big red wines	

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