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Abstract

Microbial contamination and its impact on the sensory profile and chemical parameters of wines have always been one of the major concerns for winemakers. Recent innovation and development offer winemakers an alternative approach to eliminate spoilage microorganisms without compromising wine quality. This process involves the application of EnartisStab Micro M, a pre-activated chitosan product as a fining agent. Chitosan is a natural polysaccharide extracted from *Aspergillus Niger* and can be added at different stages of the winemaking process. It inhibits the cell growth of a wide spectrum of spoilage microorganisms such as *Brettanomyces*, lactic acid bacteria, acetic acid bacteria and non-*Saccharomyces* yeast strains, by altering their membrane wall permeability. This poster summarises the effect of EnartisStab Micro M on inhibition of various bacteria and yeast strains, as well its additional benefits of eliminating undesirable aromas under certain circumstances, through the demonstration of multiple trials.

Effect of EnartisStab Micro M on various microbes

In this trial, sterilized grape juice was inoculated with each microbe from an active culture at standardized dosage. EnartisStab Micro M was applied at two rates, 10 g/hL and 20 g/hL 2-4 hours after inoculation. All juices were kept at 20°C during fermentation. Plating/PCR (population in cells/mL) was performed 4 days after inoculation. Microbes analysed include: *Pediococcus damnosus*, *Lactobacillus*, *Acetobacter aceti*, *Zygosaccharomyces bailii* and *Brettanomyces dekkera*.

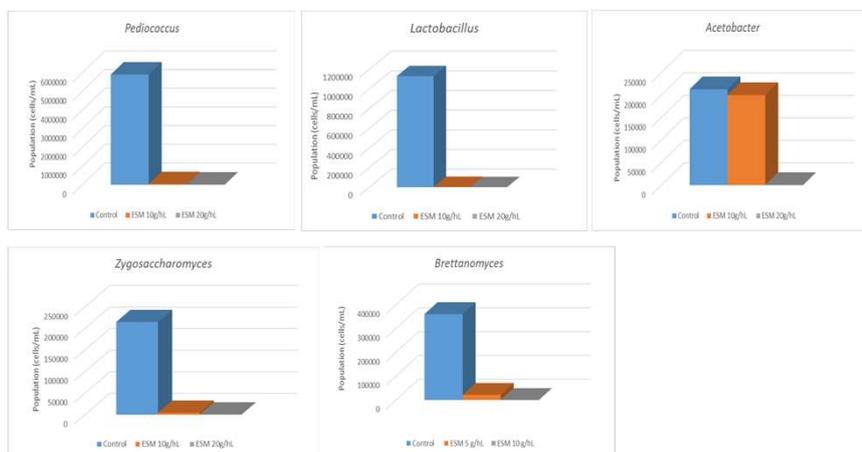


Figure 3. EnartisStab Micro M's impact on microbe population in controlled fermentations – results attained 4 days after inoculation.

Mechanism behind Chitosan and EnartisStab Micro M

Chitosan is a deacetylated derivative of chitin obtained from a fungal source *Aspergillus Niger*. The antimicrobial property of chitosan is credited to its primary amino group (R-NH₂) in the molecule, which is protonated to NH₃⁺ from in wine, thus contributing positive charges which interact with the negatively charged residues on the cell membrane surface of spoilage microorganisms, as well as altering the membrane permeability of the organisms.

Enartis StabMicro M is a pre-activated chitosan formulation which is selective and allergen-free with high efficiency. The pre-activation is carried out with use of organic acids hence increases the molecule's positive charge sites. The formula also contains purified yeast cell walls rich in chitin-glucan which assists the antimicrobial functionality in turbid wine environments. The primary application of EnartisStab Micro M is to control and inhibit the development of undesirable microorganisms in juice, must and wine, such as non-*Saccharomyces* yeast, *Brettanomyces*, lactic acid bacteria and acetic acid bacteria.

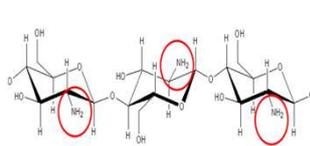


Figure 1. Schematic representation of the chemical structure of chitosan.

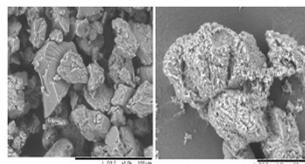


Figure 2. Surface of chitosan vs surface of activated chitosan, 1000x.

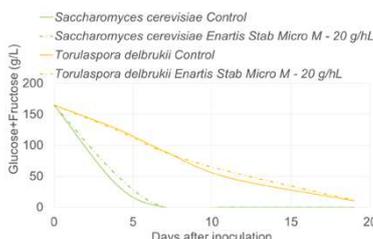


Figure 4. Impact of EnartisStab Micro M on alcoholic fermentation with two yeast strains.

Minimal impact on yeast strains

Sterilized juice was inoculated with *Saccharomyces cerevisiae* and *Torulaspora delbruckii* (2 inoculations each) from active culture at a standardized dosage. EnartisStab Micro M was added to half of the treatments 2-4 hours post inoculation. Fermentation kinetics were monitored by measuring glucose and fructose concentration in the juice daily. EnartisStab Micro M does not have an impact on the metabolism kinetics and fermentation rates for both yeast strains.

Reduction of volatile phenol concentration

EnartisStab Micro M is able to absorb undesirable volatile phenol (4-ethylphenol and 4-ethylguaiacol) in wines, which are common traits for wines tainted with *Brettanomyces*. The figure on the right demonstrates level of reduction 7 days after fining, at a dosage of 10 g/hL.

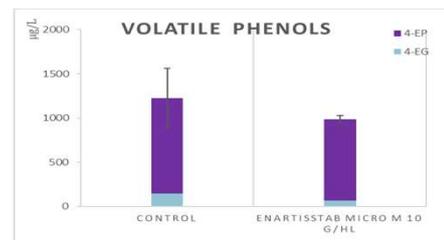


Figure 5. Impact of EnartisStab Micro M on concentration of volatile phenols.

Applications in winemaking

- Postpone, control or circumvent malolactic fermentation.
- Inhibit growth of spoilage microorganisms throughout the winemaking process.
- Assist with re-inoculation of sluggish fermentation.
- Alternative to SO₂ for antimicrobial control.
- Alternative to lysozyme, vegan friendly and allergen-free.
- Reduce volatile phenol concentration and off-odors such as hydrogen sulfide, mercaptan and disulfide.
- Avert formation of volatile acidity during maceration/cold soak.

	Micro-organisms	Enartis StabMicro M
Bacteria	<i>Acetobacter aceti</i>	XX
	<i>Pediococcus damnosus</i>	XXX
	<i>Oenococcus Oeni</i>	xxx
	<i>Lactobacillus</i>	XX
Mold	<i>Botrytis cinerea</i>	xxx
Yeast	<i>Brettanomyces dekkera</i>	xxx
	<i>Zygosaccharomyces bailii</i>	XX
	<i>Schizosaccharomyces pombe</i>	XX
	<i>Torulaspora delbruckii</i>	X
	<i>Saccharomyces cerevisiae</i>	X

Table 1: Reactivity of EnartisStab Micro M with main spoilage microbes. XXX: high reactivity; XX: medium reactivity; X: no significant reactivity

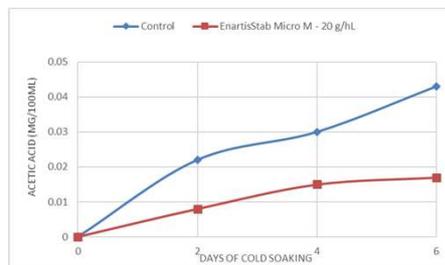


Figure 6. Impact of EnartisStab Micro M on formation of volatile acidity during cold soak.

Controlling volatile acidity

As EnartisStab Micro M inhibits population growth of wild yeast and bacteria in juice, it limits microbial activities consequently result in lower production of volatile acidity in the final wine.

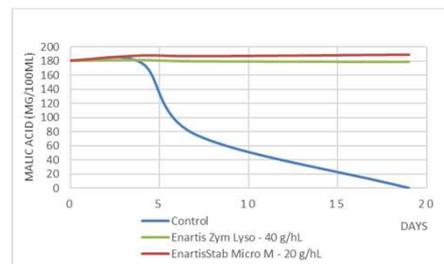


Figure 7. Impact of EnartisStab Micro M and lysozyme (Enartis Zym Lyso) on Malolactic fermentation inoculated with *Oenococcus oeni*.

Alternative to lysozyme

EnartisStab Micro M is able to inhibit growth of lactic acid bacteria such as *Oenococcus oeni*, the most commercially available malolactic fermentation strain, hence it can be used as an alternative to lysozyme when malolactic fermentation is undesired.

Conclusion

EnartisStab Micro M has a wide spectrum of applications in winemaking. As a fining agent, it inhibits growth of spoilage microorganisms, reduce volatile phenol concentration and production of volatile acidity. It can also be utilised as an alternative to lysozyme and SO₂ for antimicrobial control during multiple stages of winemaking.

Recent research has proven chitosan's ability to chelate metals, remove catechins and ochratoxins. Further study and trials will be conducted to testify EnartisStab Micro M's impact regarding these several aspects.