MARTIN VIALATTE

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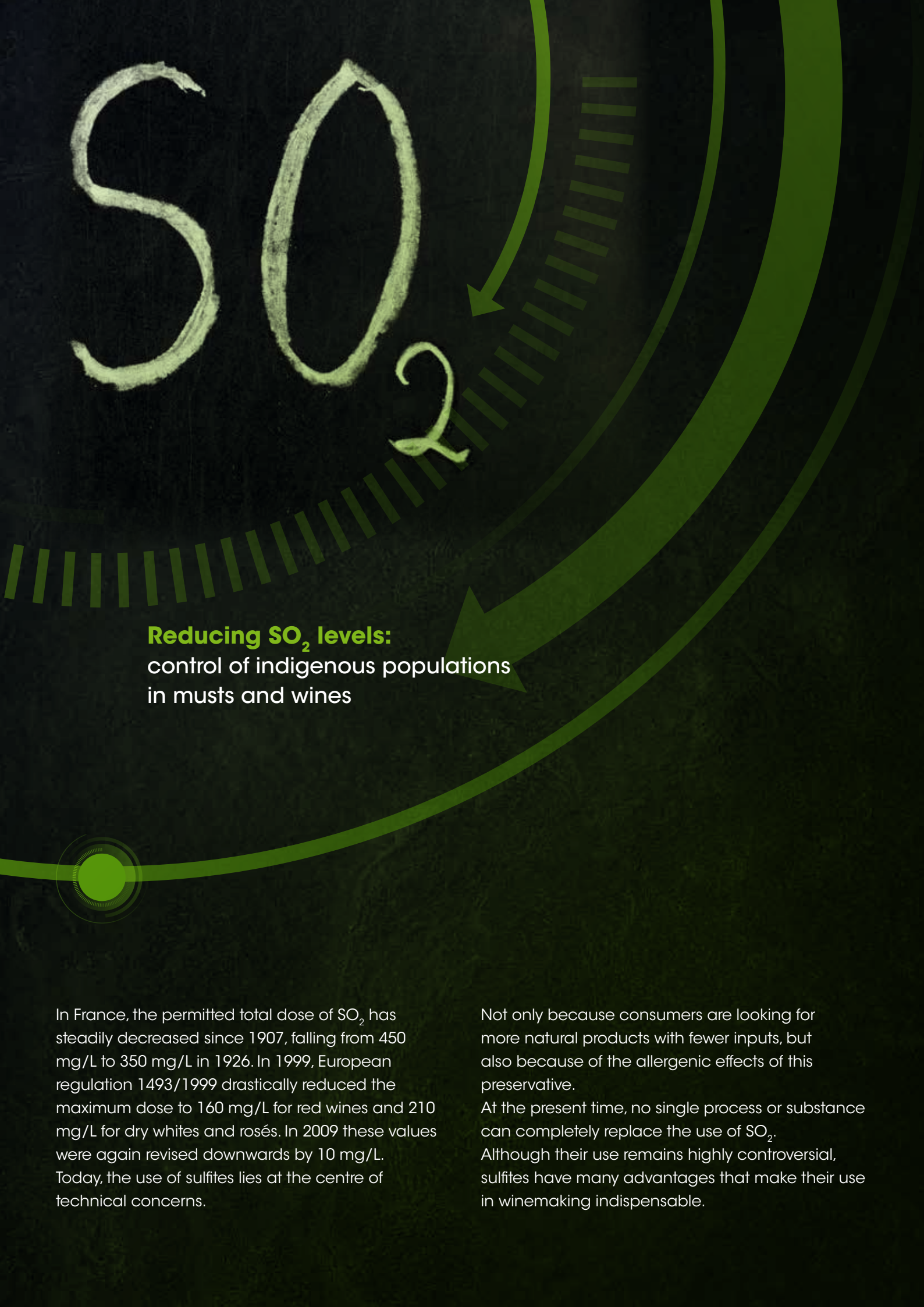
LOW SO_2

TECHNICAL BOOKLET



Reducing SO_2 levels: control of indigenous populations in musts and wines

In France, the permitted total dose of SO_2 has steadily decreased since 1907, falling from 450 mg/L to 350 mg/L in 1926. In 1999, European regulation 1493/1999 drastically reduced the maximum dose to 160 mg/L for red wines and 210 mg/L for dry whites and rosés. In 2009 these values were again revised downwards by 10 mg/L.
Today, the use of sulfites lies at the centre of technical concerns.

Not only because consumers are looking for more natural products with fewer inputs, but also because of the allergenic effects of this preservative.
At the present time, no single process or substance can completely replace the use of SO_2.
Although their use remains highly controversial, sulfites have many advantages that make their use in winemaking indispensable.

SULFITES

Sulfites have a particularly broad spectrum of action. They combine **antioxidant, anti-oxidasic** and **antiseptic** properties. In addition, they are easy to use, while their low cost is a major advantage.
As a result, in order to reduce doses of SO_2, it is necessary to act on both its protective effects: against **oxidation** on the one hand, and against **undesirable microorganisms** on the other.
To do this, a thorough understanding of its oxidation mechanisms as well as its interaction with microorganisms in musts and wines is required.

The term 'SO_2' actually refers to several forms of sulfur dioxide (Figure 1).

"SO_2": refers to several forms of sulfur dioxide

$$\mathbf{SO_2} + H_2O \leftrightarrow \mathbf{H_2SO_3} \qquad pKa_1 = 1{,}77$$

$$\mathbf{H2_SO_3} + H_2O \leftrightarrow \mathbf{HSO_3}^- + H_3O+ \qquad pKa_2 = 7{,}08$$

$$\mathbf{HSO_3}^- + H_2O \leftrightarrow SO_3^{2-} + H_3O+$$

Molecular
When dissolved in water, it yields **sulfurous acid**
The bisulfite ion

Figure 1: the different forms of SO_2 in equilibrium when dissolved in water

The relative abundance of each of these forms depends on the pH *(Figure 2)*.

As Figure 2 shows, the bisulfite ion is largely dominant at the pH of wine. However, it is the molecular form of SO_2 that exhibits antiseptic activity. Thus, the higher the pH of the wine, the less significant is its antiseptic action.
Global warming and climate change have led to a constant increase in the pH of musts and wines over the past few years. Moreover, since the use of sulfites has been widespread for several years now, some microorganisms appear to have developed resistance, especially in the case of *Brettanomyces*.

Bearing in mind all the above observations and the current tendency to reduce the use of sulfites, it clearly appears necessary to provide novel solutions to supplement the antiseptic action of molecular SO_2.

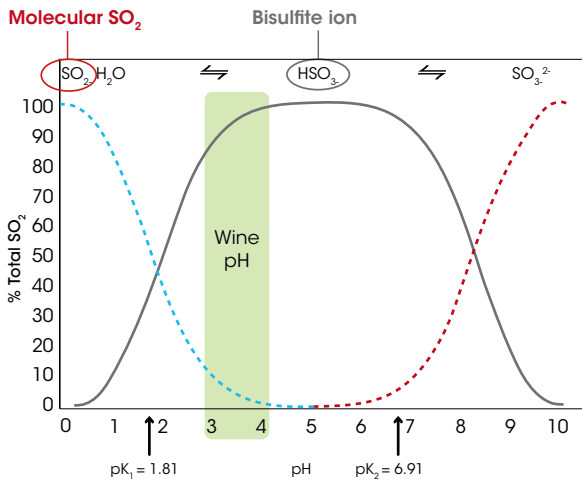


Figure 2 : Percentage of different forms of SO_2 as a function of pH

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CHITOSAN AND MICR

Why use chitosan?

Chitosan is a cationic polysaccharide produced by deacetylation of chitin. Chitin is a polymer naturally present in the cell wall of *Aspergillus niger*. Chitin and chitosan were discovered in the nineteenth century.

In December 2010, the EU approved the use of chitosan and chitin glucan derived from *Aspergillus niger* in winemaking.

Among the applications described by the OIV, chitosan can be used to reduce the microbial population, especially *Brettanomyces*, at a maximum dose of 10 g/hL.

How does it work?

Chitosan is a polysaccharide belonging to the family of glycosaminoglycans. Its chemical structure is a chain of β-D glucosamine monomers linked via 1→4 glycosidic bonds (Figure 3).

Since chitosan is obtained by deacetylation of chitin, it is characterised by its degree of deacetylation (DD), i.e. the ratio of the number of **amine groups** to **acetamide groups**. It is also defined by its number of **hydroxyl groups**, which determines how hydrophilic it is, as well as by the **length of the macromolecular chain** (number of repeat units in the molecule), on which its **molecular mass** depends (Figure 4).

The conditions under which **chitin** is extracted depend on several variable parameters (temperature, time, concentration of basic solutions). The chitin thus obtained varies in the degree of amide groups and in molecular mass.

The conditions under which **chitosan** is obtained from chitin by deacetylation also leads to chitosan having differing degrees of deacetylation and molecular masses.

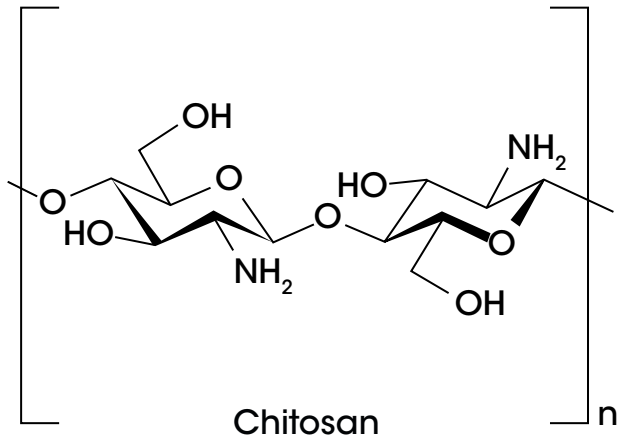

Figure 3 :
Chemical structure of chitosan – Skeletal formula

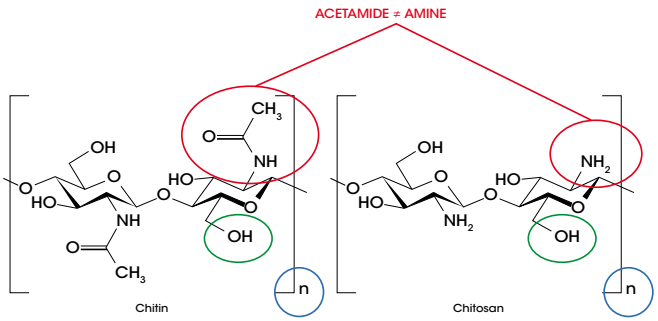

Figure 4:
chemical structures of chitin and chitosan – Skeletal formula – Showing key functional groups

The conditions of extraction and deacetylation of chitin impact the final structure of the chitosan and hence its functional properties.

In reality, the term chitosan does not designate a single molecule but rather the whole family of copolymers whose degree of deacetylation exceeds 50%.

LOW SO_2

OORGANISMS

The properties of chitosan

The properties of chitosan are determined by the ratio of its degree of acetylation to its degree of deacetylation, which in turn determines its solubility in an acid medium and the flexibility of its macromolecular chains, and hence its conformation and viscosity in solution. The lower the degree of deacetylation of chitosan the less soluble it is. Chitosan's molecular mass also affects not only its solubility but also its rheological properties. The higher its molecular mass, the more amine groups it is likely to have, facilitating high degrees of deacetylation and hence the number of possible charges.

It should be noted that the antimicrobial activity of chitosan has been attributed to its positive charges, which are thought to interfere with negatively charged residues on the surface of the cells of microorganisms, eventually leading to the death of the cell (Valencia-Chamorro et al. 2011). The molecular mass and degree of deacetylation of chitosan are therefore parameters that guarantee its effectiveness.

KTS® and biocontrol at Martin Vialatte®:

results of an innovative study carried out by Martin Vialatte®'s Innovation, Research and Development department

KTS® FA

The aim of this innovative study, carried out at a winery on musts from various wine-growing regions in France, was to trial the effect of a specific preparation based on activated chitosan on indigenous populations.

KTS® FA is a preparation based on activated chitosan and yeast hulls that aims to control microbial populations present in musts.

KTS® FA is used as a tool for bioprotection. It helps to lower doses of sulfite and reduces contamination caused by spoilage microorganisms.

IMPACT on INDIGENOUS FLORA

KTS® FA and **non-*Saccharomyces* yeasts**

A study by Kisko *et al.* showed that chitosan inactivated certain spoilage yeasts. It was shown that treatment with chitosan at 5 g/hL totally inhibited the growth of the yeasts *Kloeckera apiculata and Metschnikowia pulcherrima* during alcoholic fermentation. However, the growth of *Saccharomyces cerevisiae* and *Pichia spp* was not impacted by the treatment (Figure 5).

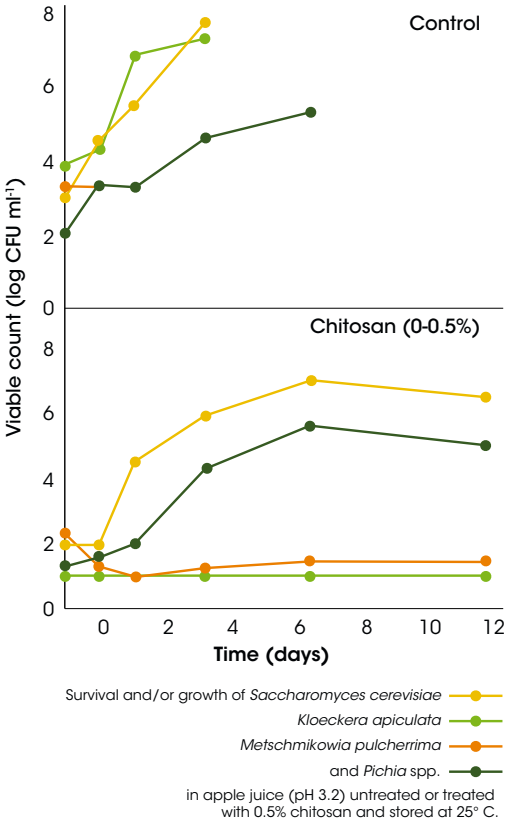

Figure 5:
Growth of microorganism population (log CFU/mL) in function of time for a control fermentation (top graph) and one treated with chitosan at 5 g/hL.

USE ON MUSTS: impact on indigenous flora, application and precautions

The microbiota in must is extremely diverse, and controlling the various species present in the absence of SO_2 is not devoid of risks. Moreover, populations change during the winemaking process, as is shown in the following Figures 6 and 7:

Yeasts: dominant species from grapes to wine

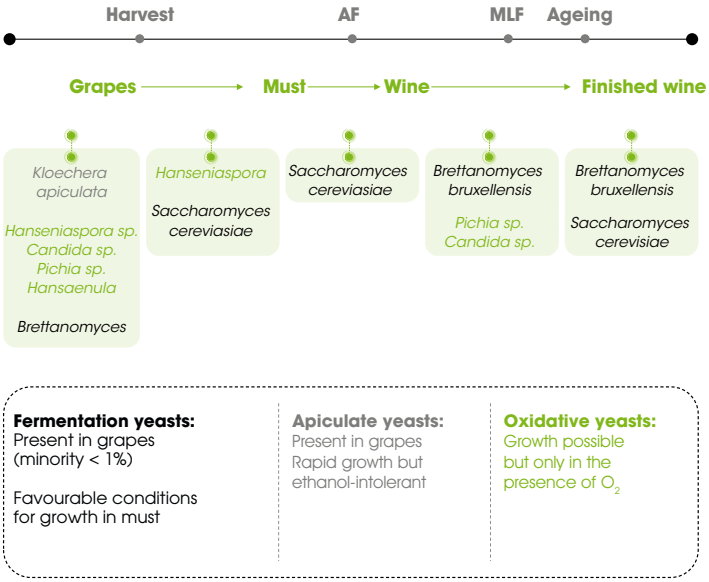

Figure 6:
Changes in species of dominant yeasts during the winemaking process

Bacteria: dominant species from grapes to wine

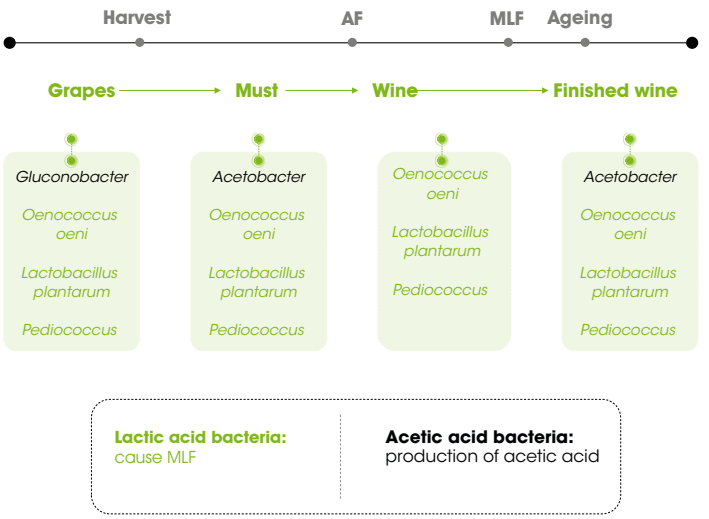

Figure 7:
Changes in species of dominant bacteria during the winemaking process

KTS® FA and ***Saccharomyces cerevisiae***

Since chitosan is an antimicrobial agent, the first question to ask when it is used on must is that of its impact on *Saccharomyces* fermentation yeasts. Several studies in the literature have already shown that these yeasts are not very sensitive to chitosan. As it grows, *Saccharomyces* produces chitinases and chitosanases, which affect the structure of chitosan. For a concentration of pure chitosan between 20 g/hL and 200 g/hL, the extent to which the latency phase of *Saccharomyces* is impacted depends on the treatment dose. However, a concentration exceeding 400 g/hL is required for the growth phase of the yeast to be affected. The *maximum* treatment dose permitted by European regulations is 10 g/hL, well below the doses likely to affect Saccharomyces.

This study thus broadly confirms that the use of KTS® FA at 20 g/hL does not have a negative impact on the latency phase or kinetics of fermentation.

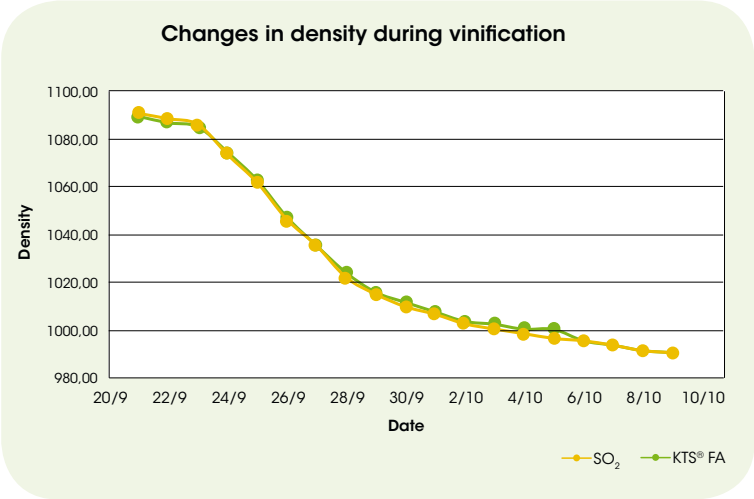

Figure 8:
Change in density during vinification

KTS® FA and **management of *Brettanomyces* after harvesting**

Research carried out by our R&D department confirms the advantage of using KTS® FA preventively at vatting with the aim of reducing SO_2 doses. The product allows improved control of *Brettanomyces* populations during vinification compared to a tank treated with SO_2 (Figure 9).

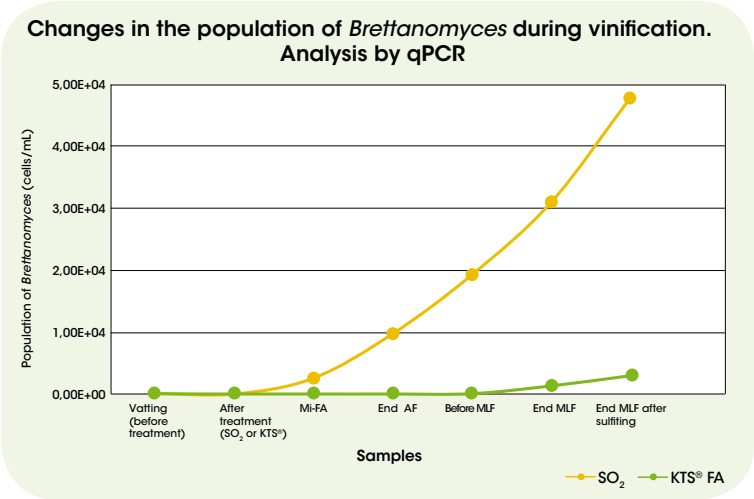

Figure 9:
Changes in the population of Brettanomyces during vinification. Analysis by qPCR

Application

KTS® FA, at a dose of 15-20 g/hL, can be used on all types of must before yeasting, or directly by spraying into the reception bin, as long as the grapes do not subsequently undergo heat treatment at a temperature exceeding 40°C.

Once the grapes are in the tanks, it is advisable to carry out yeasting directly after adding KTS® FA (time less than 2 hours), especially in the event of a warm harvest or high temperature of must on reception.

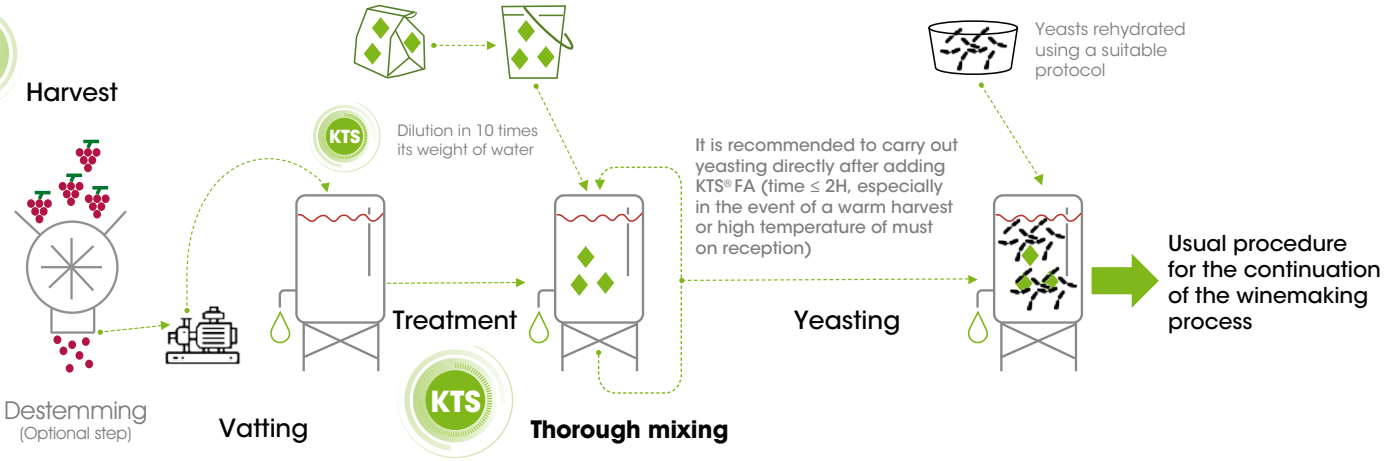

Figure 10:
Example of a protocol for treatment of must with KTS® FA at vatting

KTS® FA is a particularly interesting **antimicrobial alternative to sulfites.**

Caution

KTS® FA and heat treatment

The use of KTS® FA on must that is to undergo heat treatment is highly inadvisable. This is because chitosan partially breaks down at temperatures exceeding 40 °C. However, it is possible to use KTS® FA, after the must has returned to room temperature and just before inoculation, in order to protect such musts, which are especially sensitive to any spoilage microorganisms.

The use of KTS® FA on musts inoculated with non-*Saccharomyces* yeasts is not recommended. Although chitosan has very little effect on *Saccharomyces* yeasts at permitted winemaking doses, several studies have shown that chitosan totally inhibits the growth of some yeasts and bacteria at doses of 0.2 g/L or more. Using KTS® FA would therefore risk inactivating such yeasts.

KTS® FA and lysozyme

The use of products in the KTS® range on musts or wines containing lysozyme is not recommended. This is because chitosan and chitin are readily hydrolysed by lysozyme.

Use on musts

Brettanomyces and contamination of wine

For several years now, chitosan-based products have been increasingly used on wine for their action against certain spoilage microorganisms. Its effectiveness in reducing *Brettanomyces* populations has been clearly demonstrated, both in the literature and in the winery.

Treatment at vatting may not be sufficient, depending on the microbiological conditions in the winery. At the end of alcoholic and/or malolactic fermentation, monitoring *Brettanomyces* populations and addition of KTS® CONTROL (a product in the Martin Vialatte® range designed for wine treatment) could be considered in order to control the growth of microorganisms. As with the use of sulfites, inputs of chitosan may be contemplated at various stages and at the most suitable moments during vinification and ageing.

KTS® CONTROL was specifically formulated from chitosan derived from *Aspergillus niger* to **control** the growth of **spoilage microorganisms in wines**.

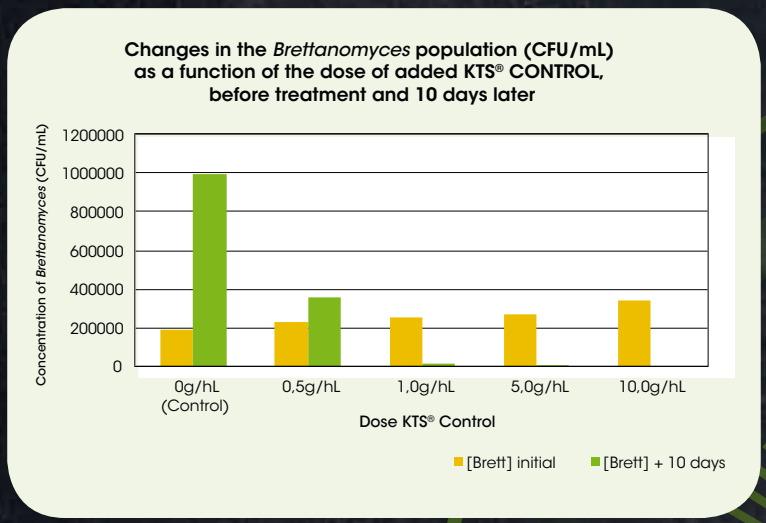

Figure 11:
Changes in the Brettanomyces population (CFU/mL) as a function of the dose of added KTS® CONTROL, before treatment and 10 days later

KTS® FA *versus* BIO-PROTECTION

	KTS® FA (BIOCONTROL)	BIO-PROTECTION
Principle	Addition to must of a chitosan-based product to control indigenous populations with the aim of reducing doses of sulfites	Early, massive addition of non-*Saccharomyces* yeasts to must or harvest, with the aim of reducing doses of sulfites
Effects on must	No modification of nutrient composition of must	Modification of nutrient composition
Effects on fermentation	No impact on *Saccharomyces* at permitted oenological doses. No impact either on latency phase or on growth	What nutrients will remain for *Saccharomyces*? Impossible to predict
Effects on organoleptic characteristics	Helps to produce an enhanced, clearer aromatic profile	What aromatic potential will remain? Some amino acids are precursors of volatile compounds. If they are metabolised by non-*Saccharomyces* to other compounds, there will be a loss of aromatic potential.
Other effects	No competition with *Saccharomyces*, on the contrary, gives them some 'space'	Formation of metabolites by non-*Saccharomyces* which can hinder the growth of *Saccharomyces* (amensalism) Competition with *Saccharomyces*; massive inoculation can hinder their growth

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www.martinvialatte.com

79, av. A.A. Thévenet - CS 11031 - 51530 MAGENTA - France - Tél. : + 33 3 26 51 29 30 - Fax: + 33 3 26 51 87 60