ENOLOGICA VASON S.P.A.

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• • • INACTIVATED YEASTS: PRESENT INNOVATION, FUTURE NECESSITY • • •

Introduction

The research of viticultural and wine making practices that respect both territory and fruit, for the production of healthy wines, represents today's hardest challenge that operators in the oenological sector have to face.

One of the crucial aspects is the progressive reduction of additives and processing aids levels, with the risk of obtaining atypical, oxidized or spoiled wines. Sulphites is certainly one of the most delicate issue, given the importance of this adjuvants in the preservation of chemical and organoleptic characteristics of wine. Reduction of sulphur dioxide rates in wine, if not dialed in correctly, leads the way to oxidative processes, with deterioration in wine quality after just few months post bottling. At the moment, though, there are no valid natural alternative products with the same anti-oxidant action of SO_2.

A possible solution is provided by the analysis of wines refined on lees. Aging *sur lies*, in fact, represents a perfect example of how it is possible to increase the longevity of wines, thanks to the contribution of molecules with reducing action released by yeasts during the autolysis process, without the addition of chemical additives. Fining on "fine lees" in the cellar, however, is a long procedure, applicable only to wines and therefore not to grapes and musts, where the oxidation reactions are among the most active. Furthermore, this activity of the yeast occurs naturally within a limited period of time that goes from the end of the alcoholic fermentation up to 5-6 months, neglecting moments in which wine is very sensitive to oxidative processes, as in the case of bottling phases or even later.

These assumptions led to the diffusion in the market of inactivated yeasts and/or yeast derivatives, with the aim of reproducing the effects of fine lees, without the problems related to their management. However, not all derivatives are equal and effective, and a key role, in addition to the yeast strain, is given by the inactivation and dehydration technique used. Essential remains the correct application of methods of use.

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Inactivation technique: how much does affect the properties of inactive yeasts and/or yeast derivatives

The last few years have seen the appearance on the market of numerous inactive yeasts/yeast derivatives (FIG. 1) capable of making several improvements to wine on both colloidal and on organoleptic aspects, but it is difficult to understand the real differences between products. Even at the scientific level, unfortunately, there are not many studies that have provided exhaustive answers on the mechanisms of action of inactive yeasts and/or yeast derivatives, making it difficult to make an informed choice of the best product, according to the needs of the cellar.

FIGURE 1. Inactive yeasts and yeast derivatives in commerce. Differences in color and aspect (powder or granular) depend on the production process, which consists of an inactivation phase followed by a dehydration of the powder.

The inactivation technique is certainly one of the most important aspects in diversification in terms of compositional characteristics of inactive yeasts. The process' temperature greatly influences the content of the thiol molecules responsible for antioxidant activities, in addition to the polysaccharide and mannoprotein content as reported in FIG. 2.

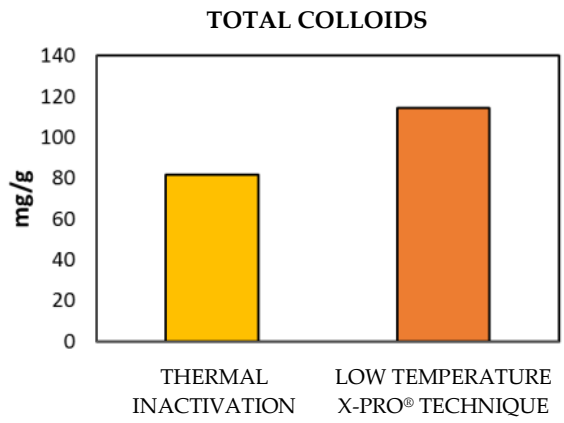

FIGURE 2. Influence of process temperature on concentration of the colloidal content in inactivated yeasts.

As noted by some authors, the inactivation technique also has a strong effect on the sensory impact of inactivated yeasts and/or yeast derivatives in wine, with effects that can be ameliorative (increasing the volatility of typical wine aromas) or detrimental (impoverishment of the aromatic profile of wine) according to their composition and dosage used (Comuzzo et al., 2006; Comuzzo et al., 2011; Del Barrio - Galán et al., 2018). Undesirable effects in wine may also occur due to direct inputs of aromas from commercial preparations. High temperatures, especially in the final phase of the production process (dehydration), can in fact promote Maillard reactions with the consequent formation of undesired odorous compounds that can also be found in wine (smell of broth, cheese).

These considerations have led Enologica Vason's R&D department to focus the attention on the research for a new inactivation technique alternative to traditional thermal processes, with the awareness that the use of low temperatures was the main possible resource to fully exploit the potential of yeasts. From a 5-year research the X-PRO® technique is born: in a completely innovative way, allows to obtain inactive yeast through a process that takes place under vacuum and with temperatures close to ambient. In this way it is possible to preserve all the most interesting components of the yeast without having the denaturation that inevitably occurs in a thermal inactivation process. This innovation has allowed or developed a range of new products, including X-PRO® PROTECTION, an inactive yeast characterized by a remarkable reducing capacity with a specific protective action to counteract and cure wine oxidation.

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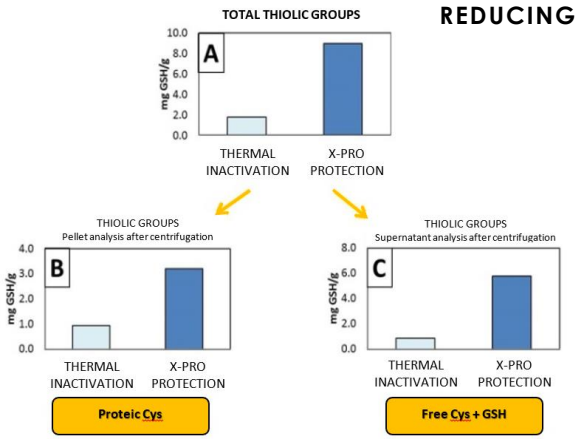
Mechanisms of action of inactivated yeasts in the prevention of oxidative processes

The cascades of oxidative reactions responsible for the deterioration of the organoleptic, chemical and chromatic characteristics of the wines have not yet been fully described by the scientific community, due to the multitude of actors involved (including quinones, polyphenols, metals, antioxidant substances) and the complexity of the interactions between them. In the light of current knowledge, it becomes therefore complicated to establish with precision what are the mechanisms of action at the base of the antioxidant action expressed by the inactive yeasts and/or yeast derivatives added to the wine. The adsorption by the yeast's walls of the flavanes (catechin and epicatechin), considered among the main responsible for the browning of white wines, is one of the hypotheses suggested by some authors (Razmkhab et al., 2002).

Another hypothesis is that the thiol functions of reduced cysteine forms (**cysteinyl thiols**) present in inactivated yeasts can act as nucleophilic centers against quinones; the high reactivity of the cysteinyl thiols would allow to protect and preserve over time the molecules responsible for the aromatic component of wines, such as volatile thiols, characterized instead by a lower reactivity. The different reactivity of quinones towards the molecules present in wine, such as polyphenols, amino acids, volatile thiols and antioxidant substances (sulphur, glutathione, ascorbic acid), would in fact appear to be the focal point at the base of the oxidative evolution of wines (Nikolantonaky et al ., 2012) and would explain why some wines are able to maintain the aromatic profile for years, while others would undergo rapid alterations after minimal exposures to oxygen. The choice of inactive yeast/yeast derivative based on the presence or absence of reduced cysteine forms thus becomes a fundamental aspect.

Contrary to expectations, actually, many of these commercial oenological additives have very low levels of cysteinyl thiols (due to temperature damage) and are able to bind free thiol molecules present in wine, with deleterious consequences in terms of both aroma and protection against oxidative processes (Tirelli et al., 2010).

As reported in FIG. 3, X-PRO® PROTECTION is characterized by much higher cysteinyl thiols levels than inactivated yeasts obtained through heat treatment, testifying the importance that temperatures play in preserving the antioxidant characteristics of the yeast. The contribution of other molecules in determining the antioxidant power of inactivated yeasts such as methionine/tryptophan/ tyrosine-containing peptides cannot be excluded (Bencomo et al., 2014).



REDUCING-CHARACTER COMPOUNDS

FIGURE 3. Influence of process temperature on concentration of thiolic groups in inactivated yeasts. The tests reported compare an inactive yeast obtained by heat treatment and X-PRO® PROTECTION. The results show that the X-PRO® technique, performed at low temperatures, allows the preservation of thiol group levels much higher than traditional inactivation techniques (**A**), both in terms of reduced protein cysteine (Cys) present in parietal structures of yeasts (**B**), and of free reduced cysteine (Cys) and glutathione (GSH) (**C**).

Application of inactivated yeasts for the prevention of oxidative processes

As anticipated, the bio-protection against oxidation through the use of inactivated yeasts implies a change in the approach to winemaking, involving all the phases that go from harvesting/pressing the grapes to bottling. Below we will present some of our research in the application of X-PRO® PROTECTION at different stages of the winemaking process, offering the CIELAB color analysis as an index of the evolution of musts/wines.

GRAPES AND MUSTS

Added to grapes/musts, the yeasts inactivated by the X-PRO® technique are able to guarantee a high level of protection against oxidative processes. Preliminary tests, carried out on Glera and Garganega musts, show an immediate improvement on the chromatic characteristics of the two musts, with a younger color (a * and b * reduced, L * increased) than the control and musts treated with heat inactivated yeasts (**A**). This effect also extends over time, as shown in the data on musts stored for 12 days at 30°C (**B**).

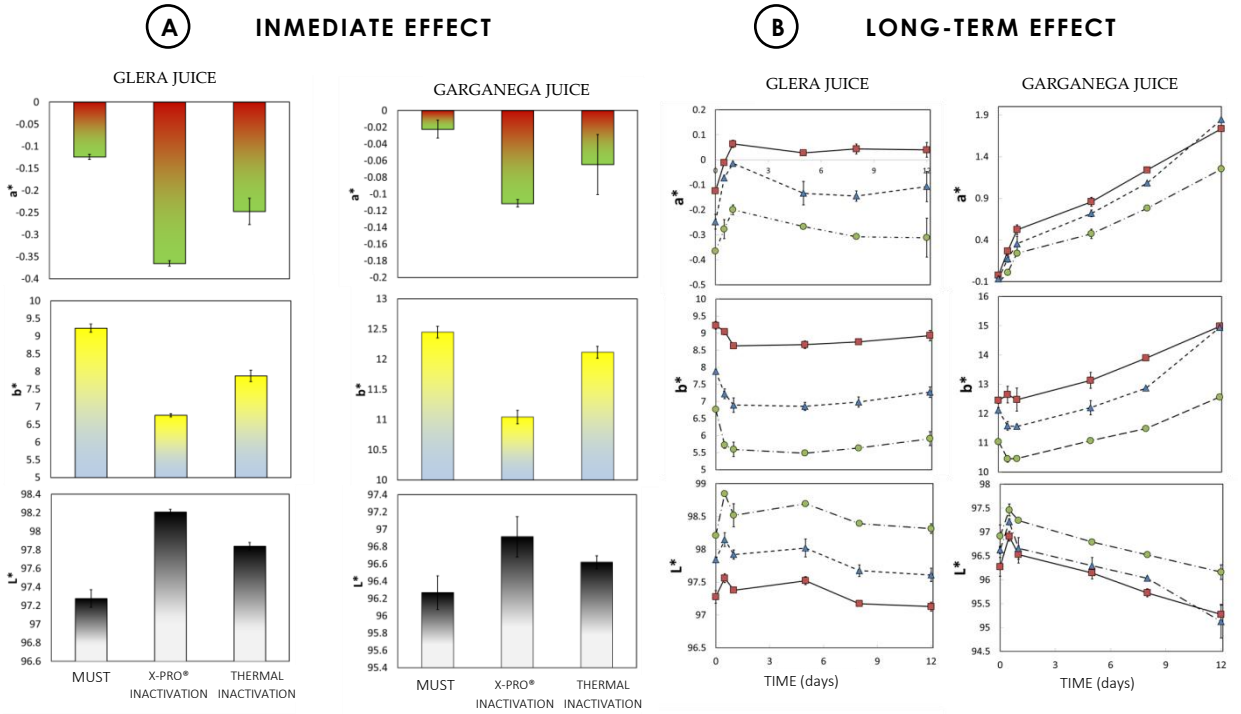


FIGURE 4. Antioxidant action of X-PRO® PROTECTION added to grapes/musts. A. CIELAB analysis of two musts a few hours after the addition of 30 g/hL of heat inactivated yeast and X-PRO® PROTECTION. B. CIELAB color evolution of two musts placed at 30°C for 12 days. IN RED: control musts; IN BLUE: musts added with 30 g/hL of a heat inactivated yeast; IN GREEN: musts added with 30 g/hL of X-PRO® PROTECTION. All tests were done in triplicate.

AGEING/STORAGE

X-PRO® PROTECTION is the ideal instrument for the protection of white, rosé and red wines even during storage or aging, phases in which SO_2 levels are very low and therefore wines are more exposed to oxidative processes (e.g. sparkling wine bases, red wines in barrique).

From the comparison tests carried out by treating a white wine with X-PRO® PROTECTION and with two other types of yeasts inactivated by heat processes, the importance of the inactivation temperature on the properties of inactivated yeasts emerges again. After 30 days from the addition, in fact, the white wine treated with X-PRO® PROTECTION preserves fresher chromatic characteristics, typical of young wines, with yellow shades tending to green and a greater luminosity than the control wine and the other tests (FIG. 5).

The evolution of the hue of the wines perfectly reflects the olfactory and gustatory profile of the various tests, with less hints of oxidized and bitter notes in the wine treated with X-PRO® PROTECTION, where predominantly floral and fruity aromas emerged, otherwise missing in the control wine.

It should be highlighted that, in the case of X-PRO® PROTECTION, the tasting panel did not perceive olfactory anomalies due to the use of inactive yeasts/yeast derivatives.

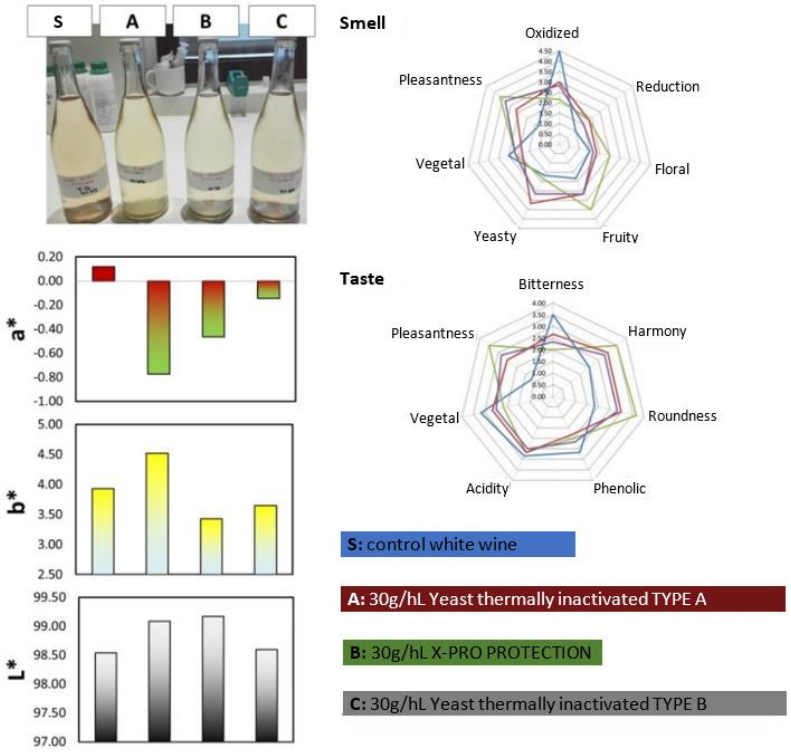


FIGURE 5. Evolution chromatic and organoleptic characteristics of a low sulphur content white wine treated with 30 g/hL of X-PRO® PROTECTION and 30 g/hL of two other heat inactivated yeasts, after 30 days of storage at 20°C.

FINING

X-PRO® PROTECTION, with an almost immediate effect, can be considered a valid alternative or co-adjuvant to some conventional fining agents, with important improvement effects on the oxidative and chromatic characteristics (FIG. 6) as well as sensorial characteristics of the wines.

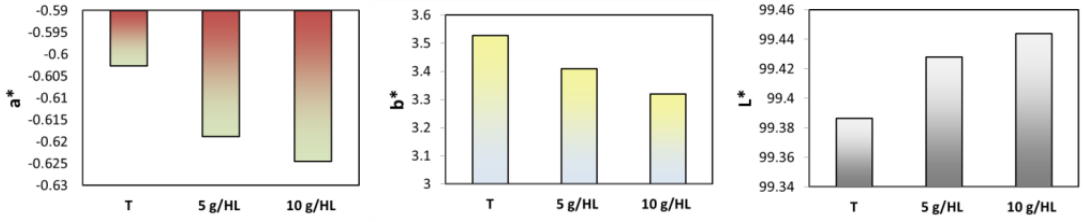


FIGURE 6. CIELAB analysis of a white wine clarified with 5 g/hL and 10 g/hL di X-PRO® PROTECTION.

PRE-BOTTLING

Bottling represents one of the most delicate phases of the production process. Consistent oxygen enrichments in this phase, very often caused by poor performances of the inerting devices installed along the bottling line, can in fact compromise the work of a vintage by promoting oxidative processes in the bottle on which there is no chance to intervene.

As reported in FIG. 7 A, in fact, already two months after bottling, the oxidability of a wine evaluated by TDO (Dynamic Oxygen Test) changes drastically, with kinetics of evolution of the yellow chromatic component (b*) much more marked than the same analysis carried out a few days after bottling. The evolutionary kinetics of the CIELAB color of the wine added with 10 g/hL of X-PRO® PROTECTION in the pre-bottling phase (7 B), on the other hand, are perfectly superimposable (b*) or even lower (a*) compared to those performed at time zero, confirming the high protective power of X-PRO® PROTECTION against oxidative processes that can occur in the bottle.

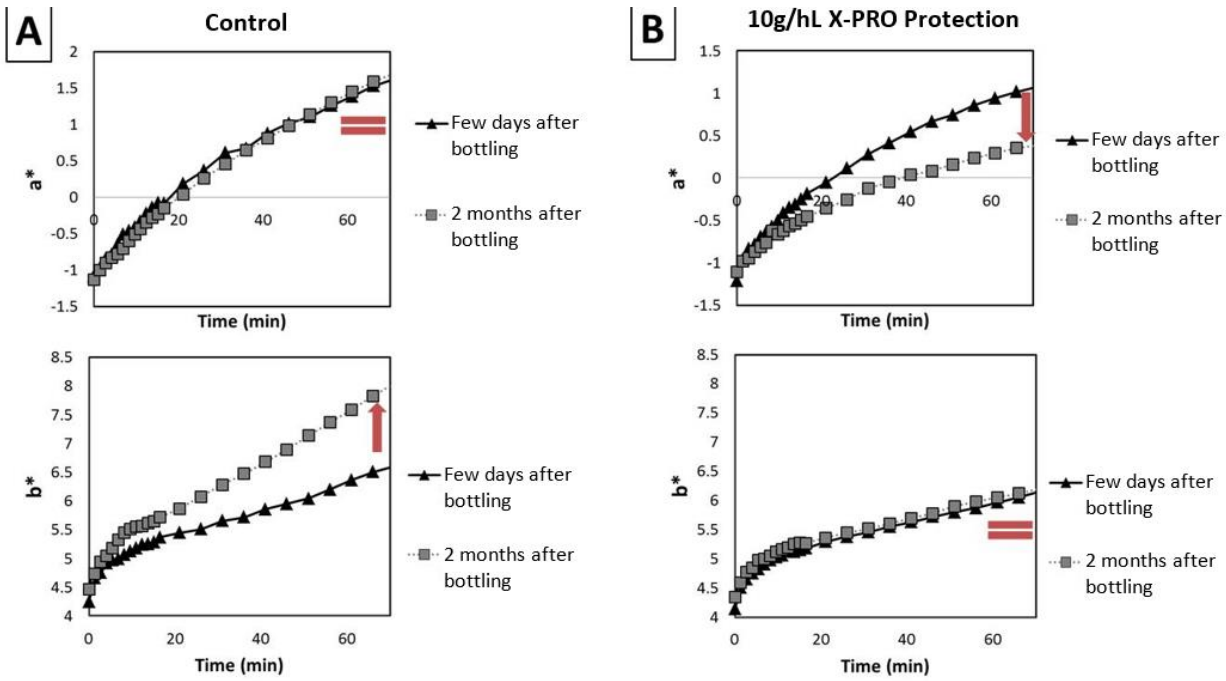


FIGURE 7. Use of X-PRO® PROTECTION during pre-bottling phases. Represented CIELAB colour evolution kinetics of two wines bottled in the absence (A) and in the presence (B) of 10 g/hL of X-PRO® PROTECTION after a few days post-bottling and after 2 months. Colour evolution tests were performed in oxidative conditions (high temperatures and presence of hydrogen peroxide).

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Oxidative browning of wines: how to intervene?

As previously stated, the correct management of the transfers, fining, aging/storage and bottling phases all represent crucial aspects in preventing problems associated with oxidative phenomena. An incorrect management of the antioxidant protection in one of these phases can in fact lead to the onset of oxidative processes that determine, depending on the chemical characteristics of the wine, more or less marked chromatic alterations that bring the decrease in brightness and the appearance of orange notes , including the phenomenon known as "browning".

Correcting this type of alteration, may clearly also compromise the aromatic profile of wines, as generally involves the use of fining agents such as PVPP, casein, etc., with the risk of improving the chromatic characteristics of the wine at the expenses of sensory properties and overall quality of the same.

The use of inactive yeasts and yeast derivatives is an important tool to prevent and correct this type of problems. As reported in the literature, indeed, yeasts are able to specifically absorb yellow-brown compounds that originate from the oxidation and condensation reactions of the flavan-3-oils (catechins and epicatechins), among the main responsible for the browning of wines (Razmkhab et al., 2002; Schneider, 1995).

These data were confirmed by Enologica Vason's R&D laboratory which highlighted the high curative properties of X-PRO® PROTECTION in "rejuvenating", both from a chromatic and gustatory/olfactory point of view, wines that had also very marked oxidised characters. In the laboratory tests in FIG. 8, an example of treatment of an oxidised wine (4 months at 30°C) is reported with a dosage of 30 g/hL of X-PRO® PROTECTION which determines a decrease in the yellow value (parameter b*) and an increase in the brightness (L*) at values close to those of the wine at the beginning of the tests.

CURATIVE ACTION

LABORATORY TESTS

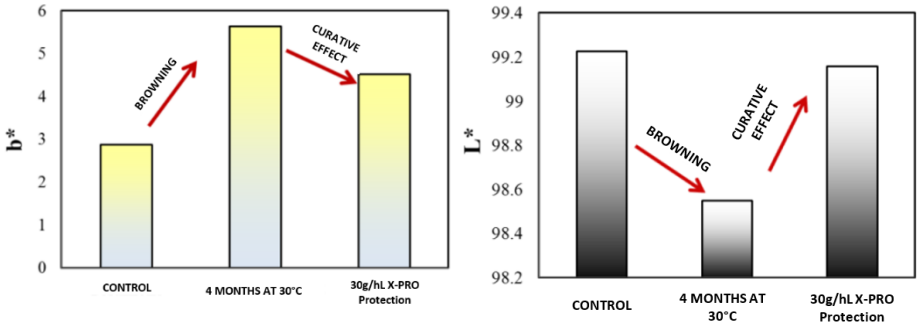

FIGURE 8. CURATIVE ACTION OF X-PRO® PROTECTION. Laboratory tests were performed by measuring the CIELAB analysis of color of a white wine before and after 4 months of storage at 30°C. The healing effect of X-PRO® PROTECTION is evidenced by the decrease in yellow (b*) and the increase in brightness (L*) following a dosage of 30 g/hL.

REAL LIFE EXAMPLES

The healing effects of X-PRO® PROTECTION are visible on two oxidized wines, one of which is characterized by a very marked browning (photo on the right).

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Conclusions

Thanks to the innovative production technique, X-PRO® PROTECTION can be considered an extremely powerful and versatile natural instrument, not only in the protection of musts and wines from oxidative processes, but also a valid aid as a clarifying and stabilizing agent. Its effect is completely comparable to that of yeast naturally present in wine after alcoholic fermentation and during lysis processes, but with the obvious advantage that it is much more flexible in its use and therefore also more precise.

Thanks to its innovative production technique, its use does not lead to gustatory and olfactory alterations, but it is probably the most valid natural tool to enhance and protect the identity of the wine.

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