

# Conventional Yeast Derived Products VS. X-PRO® Process.

*The Natural Option to Enhance and Protect the Identity of Wine*

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## **New yeast derived products: stabilization capabilities and concerns**

The antioxidant capacity of inactive yeasts and yeast derivatives (YD) is the latest among the many properties that have been ascribed to this kind of products.

In the early 2000s first has been noticed that some of these products, naturally rich in Glutathione (GSH), had the capacity of reducing the loss of aromatic compounds from the wine over the time during storage (Andújar-Ortiz et al., 2010). In the wake of these results, the GSH has been initially assumed to be the main responsible of the antioxidant properties of the YD.

Further studies proved that, along with the tripeptide glutathione, some other molecules can contribute to such a result, and in particular those peptides that contain methionine, tryptophan, and tyrosine (Rodríguez-Bencomo et al., 2014).

As in the case of the lees, the thiolic components play a key role when it comes to the antioxidant properties of the inactivated yeast.

According to Gallardo-Chacón et al. (2010), the thiolic groups found in the yeast's cell wall represent one of the most active antioxidant constituents of the fresh lees; and the same components are part of the YD.

Among the products available on the market, the content of those thiolic compounds can vary consistently: the soluble fraction (like GSH) goes from 0 to 15 mg/g, while the fraction bound to the cell walls goes from 0 to 3 mg/g (Molteni, 2018).

Despite the concentration of these compounds seems to be low, the presence of those molecules makes the YD effective for the protection and against the browning of the phenols. The products that contain a conspicuous thiolic portion function somewhat like sulfites acting as quinone scavengers (Molaro, 2017; Comuzzo e Toniolo, 2018), thereby protecting the wine from browning during storage (Comuzzo et al., 2015a; Rosso, 2017).

The production technology has a great influence on the thiolic molecules concentration in YD.

It's known that the thiols decrease as they come in contact with oxygen. This occurs during the aging on the lees so the antioxidant capacity of the fresh lees gradually diminishes along the "elevation" (Gallardo-Chacón et al., 2010).

The traditional techniques to obtain the lysis of the cell that are based on the use of heat (i.e. thermolysis) dramatically reduce the content in GSH and other thiolic molecules in YD (Ferretti, 2014) therefore decreasing the capacity of the product to reduce the quinones and fight the browning.

Recently the new technologies based not on heat, but on ultrasound (US), pulsed electric fields (PEF), and high pressures (HP) are becoming interesting for the wine sector and, despite not being usable in the winery due to some law restriction, have been subjected to many trials in lab or on a pilot scale to demonstrate their effectiveness as an alternative to the traditional heat treatments.

Some of these technologies have proven to be responsible for inducing the lysis of the yeast (Cacciola et al, 2013; Comuzzo et al., 2015, 2017); therefore, they can be very interesting for the production of non-heat-induced yeast derived products for oenological use.

Recent experiences shown that some of these technologies are potentially able to provide YD products with low residual cell vitality (following the prescription from OIV), a proportion of soluble polysaccharides comparable to the one present in the products obtained by heat processing, and a higher proportion of thiolic compounds if compared to the traditional methods (Ferretti, 2014). This could cause a higher antioxidant capacity of the new kind of YD. Moreover, the reduction of process temperatures seems to have a potentially favorable impact on the final aminoacidic rate and on the reduction of the smells that come from the YD itself (Voce, 2014).

This concern about the off flavors that may come from the YD has been pointed out by various authors and could be attributable to the deterioration of the molecular structure connected to the "Maillard reaction" - but not only- (Tirelli et al., 2009), that has been caused by the traditional thermic processing of the YD.

### **A new method explored for the production of hi-end yeast derivatives**

In response to the indications that came from the latest studies, it's more than five years that the R&D department of **VASONGROUP** is doing research on this topic, together with the top Universities and with GiottoConsulting -which is an inter-disciplinary consulting company about vine growing and winemaking-.

The work began with the choice of the yeast that could give the highest stability to the wines, through extensive ageing tests until the right strains were selected. Then the work focused on the study of the variables that take part in the production of the YD, giving priority to those processes of lysis that are better suited to keep the original structure of the components of the fresh yeast. From these trials stands out the excellence of the "X-PRO®" process that operates the lysis with a physical method that works at low temperature and in the absence of oxygen.

This proprietary process offers a great advantage to the researchers as all the parameters of extraction can be set and varied to adjust the properties of the final product without changing the core characteristics.

As already mentioned, the choice of the yeast strain offers the opportunity to adjust the final results according to the desired effect on wine or must. This has been the way to obtain some specific action for each type of product, although they all share the capacity of managing the redox equilibrium of the wines and better the tartaric and protein stability; in particular "Identity Red" betters the color stability of the wine, "Identity White" betters the protein stability, and "Finesse" betters the perlage of the sparkling wines.

## Great results right from the first scientific trials.

The most remarkable result is the way X-PRO® interacts with the redox potential of the wine. The aim of the research has been to extend the protective and stabilizing effect of natural lees that remain after the acholic fermentation.

But a great surprise has emerged from the results of the first experiments on musts and wines: with X-PRO® products is not only possible to achieve the goal that was expected, but it is also able to remedy and revive the smell and the color of a wine that is getting old. An easy way to check this refreshing effect is to add some product to a bottle of wine that has been uncorked a couple of days before; it can be noticed how fast the wine that was decaying goes back to its original pleasant bouquet.

But is not easy to explain how is it possible to get those striking results on wine from Specific Inactivated Yeasts (SIY), since the exact mechanism of action of the yeast in the prevention of the oxidative processes have not yet been fully clarified.

Some authors propose that the effect of the inactivated yeast occurs through the adsorption of flavans, as catechins and epicatechins (Razmkhab et al., 2002), but that's not the case of X-PRO® products where the effect seems to be stronger; maybe the thiolic functions "-SH" of the reduced cysteines (FTCR) of the inactivated yeast can be the nucleophilic center for the quinones; this seems to be the most likely mechanism of action, due to the high reactivity of the FTCR against the effect that quinones has on polyphenols, amino acids, volatile thiols, and the various antioxidants like SO<sub>2</sub>, GSH and ascorbic acid (Nikolantonaky et al., 2012). Contrary to the expectations, many among the inactivated yeasts on the market have a very low level of FTCR due to the thermic damages that undergone during the industrial processing.

The traditional products can bind the thiolic molecules that are found into the wine thus negatively affecting the aromatic profile (as already described), and do not protect against oxidation (Tirelli et al., 2010).

As shown in **fig. 1**, X-PRO® Protection has a much higher content of FTCR compared to the inactivated yeast that undergone a thermic processing, as an evidence of the importance of temperature control in order to keep the antioxidative properties of the yeast.

It's possible to notice how the X-PRO® production process, since it's done under anaerobic conditions and at low temperature, can preserve the thiolic groups in comparison to the traditional way of processing (A); and this is true both for the non-soluble thiols (supposedly proteins containing cysteine, inside the cell walls) (B), and the soluble thiols in the supernatant (i.e. cysteine and glutathione) (C).

Those observations suggest that with the SIY it is possible to overcome the general view about the longer waiting times (Del Barrio-Galan et al., 2018) that the yeast needs to take effect in the wine, and get good results even with a low dosage, without changing the natural character of the wine.

All of these molecules, together with the others that have been already discussed -like peptides that contain methionine, tryptophan and tyrosine- could be involved in the antioxidative power of the inactive yeast (Bencomo et al., 2014).

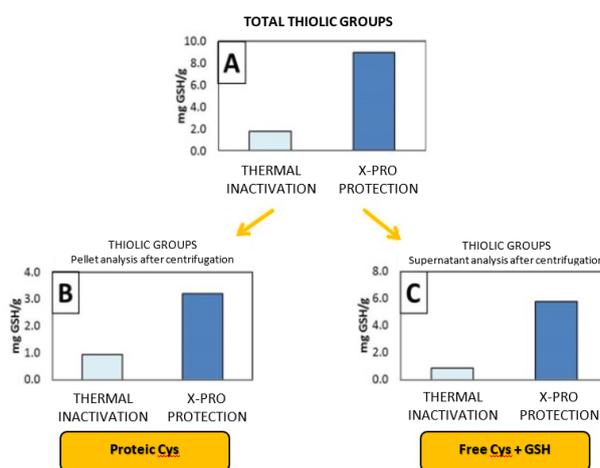


Fig. 1 – Influence of process temperature on the concentration of thiolic groups in the inactivated yeast

Following this logic could be possible to clarify the reason why some wines are able to keep the aromatic profile for years, while others undergo rapid deterioration after the slightest exposure to oxygen.

The use of SIY rather than the traditional chemical antioxidants could be considered a key factor in the natural management of the redox balance of the wine; and among those products X-PRO® seems to be the most satisfactory solution, precisely because the strong presence of natural reduced molecules.

In the market many products are available, but most are inefficient for the redox control since they lack of those molecular structures that can be active against oxidation. Among the available products only two of them have shown somehow a similar capability, so that the following comparisons will consider only those products Vs. X-PRO®. The research proceeded rapidly with the examination of parameters like polyphenols, redox, color (CIE L\*a\*b\*), and those stabilities related to the aging on lees (mostly protein, tartaric, and color).

#### X-PRO® - THE TECHNIQUE

*The innovative yeast inactivation process named XPRO is done under anaerobic conditions, at low temperature and without the use of enzymes. The precious raw material is thus protected from possible alteration. Then adjusting the parameters of the process, it can be diversified according to the required features to enhance. No chemicals added, only the strong commitment of Enologica Vason to provide the native and well-known properties of the natural substances contained in the yeast cell.*

#### Application of X-PRO® Protection on white musts

The first test for the purpose of controlling the redox of the fresh musts have been carried out during 2016 vintage. This practice should lead to a dramatic reduction of the traditional chemical antioxidants (SO<sub>2</sub>, Ascorbic acid...) giving an important contribution when dealing with “low SO<sub>2</sub>” or “no sulfites” procedures.

When added to the must X-PRO® Protection can provide a high degree of protection against the oxidative process; this result clearly emerges from the shift in the CIE L\*a\*b\* color coordinates, following a 10 g/hl addition to the must. (Fig. 2).

Those parameters were measured after 2 hours from the addition of X-PRO® Protection and can be used as markers to follow quickly and easily the progress of the reaction during the test (i.e. the effect of quinines that originated from phenolic oxidation).

These first observations have been done on Glera and Garganega musts: it is noticeable the rapid improvement of chromatic characteristics, namely a “younger” color (decreasing a\* and b\*, rise of L\*), in comparison to the blank test and to those that have been treated with “conventional” inactive yeast.

In Fig. 3 it is visually noticeable the anti-browning effect: on the Glera must the color shifted toward the green, while on Garganega must the brownish oxidation tones have been clearly reduced.

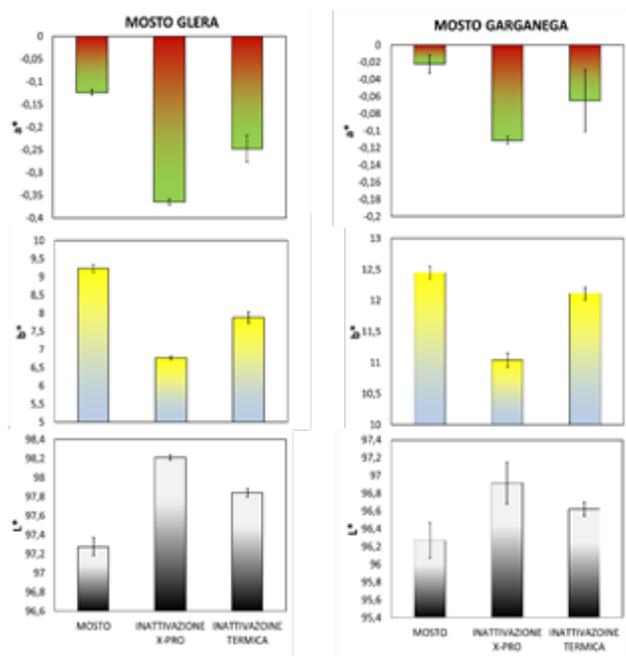


Fig. 2 – Improvements on the redox system on Glera and Garganega musts following the addition of X-PRO® Protection, in comparison to the “traditional” yeast YD.

The organoleptic qualities, not reported on the graphics, did improve accordingly to the bettering in color.

### Use of X-PRO® Protection on aging wines

Even with the extremely quick reaction that they show in wine, the most classic application for X-PRO® products is naturally supporting the common techniques of ageing on lees, for white, rosé, and red wines as well.

The protective effect of the lees is well known since a long time, and plays a major role on the prolongation of wine's life.

The use of X-PRO® Protection aims to support and extend the anti-browning effect of the natural ageing on the lees by counteracting the oxidative reactions.

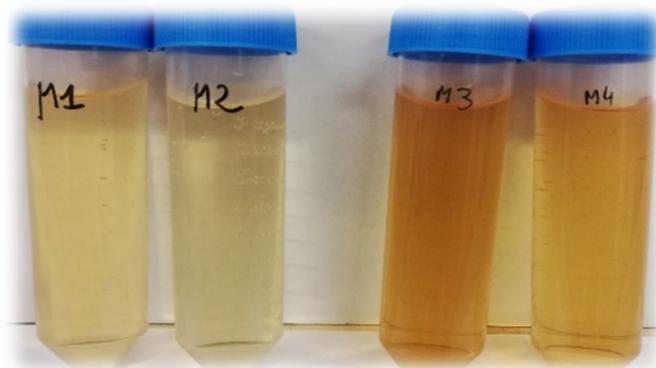


Fig. 3 – Color change achieved on Glera must (M1 and M2), and Garganega must (M3 and M4) following the treatment with X-PRO® Protection

After 30 and 60 days from the addition, white wine treated with X-PRO® Protection keeps better color characteristics, such as in younger wines; the tones are more greenish and brighter in comparison with both the blank test, and the wine that has been treated with some conventional kind of inactivated yeast (Fig. 4).

The protective effect on the color that has been pointed out, can be experienced in the olfactory characteristics as well, since it's possible to feel the reduction of the oxidative notes and the improvement of the fresh, floral and fruity nuances, which are not present in the untreated wine. By no means the use of X-PRO® lead to the comparison of undesired scents of inactivated yeast or yeast derivatives.

		L*	a*	b*
M1	A TEST	99,69	-0,01	1,03
M2	A + 10g/hL X-PRO® Protection	99,82	-0,03	0,66
M3	B TEST	99,34	0,09	1,75
M4	B + 10g/hL X-PRO® Protection	99,42	0,06	1,56

Tab. 1 – Amelioration of the CIE L\*a\*b\* chromatic coordinates in response to an addition of 10 g/hl X-PRO® Protection

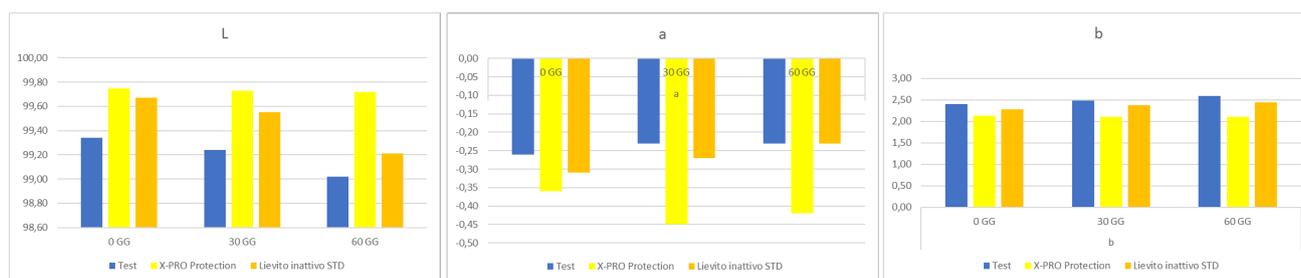


Fig. 4 – Protective effect of the addition of 10 g/hl of hl X-PRO® Protection at point 0, after 30 days and after 60 days, in comparison to the addition of the same amount of conventional yeast, evaluated by means of the CIE L\*a\*b\* coordinates.

It must be considered that in real-life tanks it has been possible to keep the right level of SO<sub>2</sub> with a few limited additions of the product, which is a clear sign of redox rebalancing (Fig. 5)

It will be very interesting to go further with the research in order to provide a scientific explanation of mechanism involved in these properties; in the meanwhile, it's highly satisfactory to appreciate the effects on the preservability and on the fragrance of the wines.

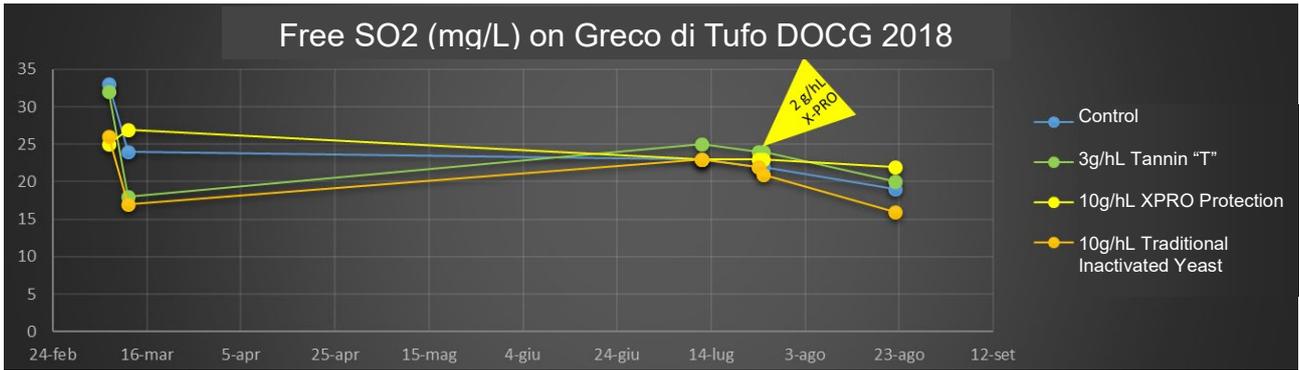


Fig. 5a – The effect of X-PRO® Protection in keeping a higher level of SO2 over time, in comparison to a tannin and to a “conventional” kind of inactivated yeast. 2 more g/hl product have been added on the 24<sup>th</sup> of July. This kept the SO2 level at the right point (analyzed by CEE method)

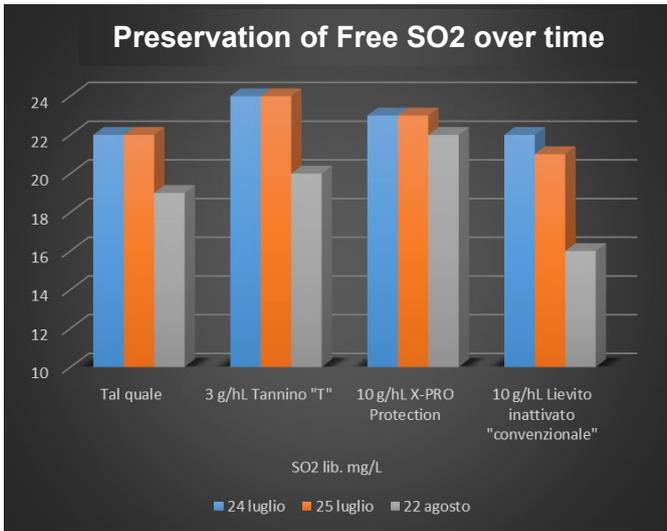


Fig. 5b – More on the data that have been previously examined.

**Use of X-PRO® Finesse on carbonated and sparkling wines.**

In the production of sparkling wines there is plenty of yeast in the tank that will give its contribution both to the wine stability and to the perlage and the texture of the foam. The use of X-PRO® has been repeatedly tested in order not only to extend the shelf life, but also to better the properties of the foam by giving superior volume, longer persistence and more finesse.

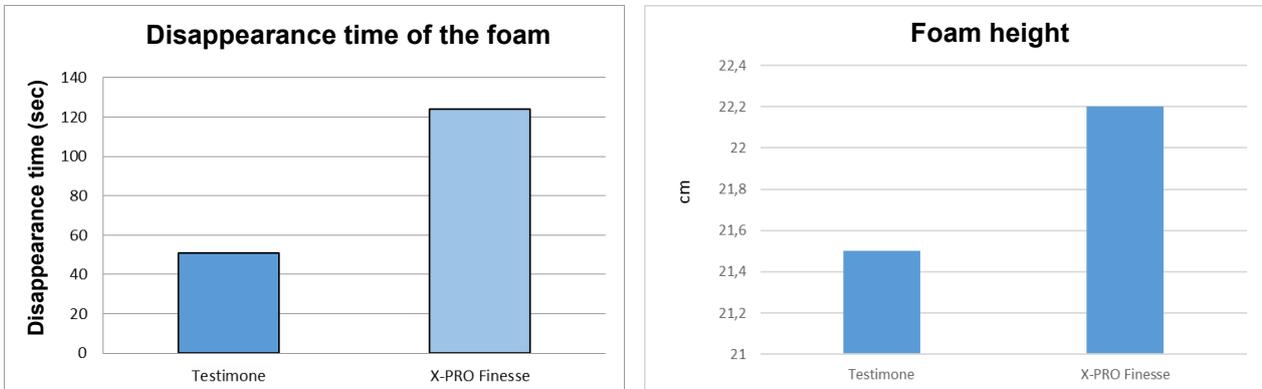


Fig. 6 – Bettering of the foam (thickness and lifespan) obtained by adding 10 g/hl X-PRO® Finesse at the tirage, to a wine processed by Martinotti method.

In order to achieve a better comparability among the tests, the effects of the treatment have been measured by the “Mosalux” system, which can objectively evaluate the capacity of the wine to produce a thick and long-lasting foam.

#### X-PRO® - PRODUCT RANGE

The parameters that have been measured clearly improve after the treatment, and moreover the use of X-PRO® Finesse on premium wines gives them elegance and refinement. The macromolecules that X-PRO® released inside of the wine are able to compensate the dryness and the harsh acid feelings, giving its contribution to the harmony and the length of the mouthfeel.

#### Conclusions

There is good chance that will be necessary to indicate the origin of all the ingredients used in wine in the near future: in this regard the demand of the market is to avoid chemicals in winemaking; this is what drives the research on pursuing the path of physical and biotechnological treatments.

Similarly, this is the path that X-PRO® research project did follow, producing such an amount of scientific evidence and obvious data that make it clear that we are facing a real innovation in technology.

X-PRO® products demonstrate to be of utmost interest in keeping the identity, the fragrance and the elegance of the wines through the time, thanks to their unique ability to manage the redox system -that is not achievable with “conventional” products-. It is the natural option to provide a winemaking procedure that allows the reduction of sulphites.

X-PRO® innovation in yeast lysis processing: no chemicals, but the strong will of Enologica Vason to benefit from the innate and well-known stabilizing capacity belonging to the substances contained in the micro-organisms of the wine.

#### Acknowledgments:

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*The products obtained by the unique process “X-PRO®” give a balanced mouthfeel and preserve the aromatic freshness and the color while respecting the original identity of the wine.*

*X-PRO® PROTECTION has the ability to keep an optimal redox equilibrium and bring back the oxidation of wine. X-PRO® IDENTITY WHITE and X-PRO® IDENTITY RED create a balanced and stable environment during ageing and moreover demonstrate interesting stabilizing side effects.*

*X-PRO® FINESSE instead is ideal to refine the elegance of sparkling wines that reflects in a higher finesse and a durable perlage. X*

*According to the EU Implementing Regulations X-PRO products are allowed to use for the production of organic wines.*

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