agrovin

Harvest Catalog

Winemaking proposals

Agrovin Group

Synonymous with QUALITY

Quality and Food Safety are two fundamental pillars of our daily activity. Implementing the international standard FSSC 22000 guarantees our commitment to offering safe, high-quality products.

The support of our laboratory accredited by ENAC in the ISO 17025 standard not only guarantees the reliability of the accredited parameters, the daily training of technicians, and the fine-tuning of equipment and methodologies but also ensures that the controls carried out on raw materials and formulated products are exhaustive and comply with the highest quality standards imposed internally.

Our commitment to research in the oenological sector is still active, and together with national and international universities, we continue to search for solutions that will help the development of this sector.

AGROVIN, RESEARCH AND DEVELOPMENT

Product/Technology	Project / Reference studies / Patents
Actimax VARIETAL	**NUTRIAROMA** "Nitrogen nutrition and its influence on the release of thiolic varietal aromas by yeasts." The European Union financed the project through FEDER and CDTI (Ministry of Industry) funds (85% EU Co-financing). University of Rovira i Virgili. Complutense University of Madrid.
Actimax NATURA	**CENIT-DEMETER** "Viticultural and oenological strategies and methods in the face of climate change. Application of new technologies that improve the efficiency of the resulting processes." This alcoholic fermentation nutrient results from research carried out within specific lines. CSIC. DEMETER. University of Rovira i Virgili.
viniferm ÉLITE	**NUTRIAROMA** "Nitrogen nutrition and its influence on the release of thiolic varietal aromas by yeasts." *Saccharomyces cerevisiae* var. *cerevisiae*. This strain was isolated from vineyards of *Vitis vinifera* cv. Merlot, Bordeaux (France), Complutense University of Madrid.
viniferm 3D	**CENIT-DEMETER** "Viticultural and oenological strategies and methods in the face of climate change. Application of new technologies that improve the efficiency of the resulting processes." It was developed in collaboration with Bodegas Torres SA and the ICVV of the CSIC.
viniferm NS CHANCE	**LOWpHWINE** "Factors (soil, plant and oenological microbiota) that influence the balance of acidity, quality assurance and stability of wines in warm climates." The selection of NS CHANCE results from extensive research activity in collaboration with the Microbiology III department of the Complutense University of Madrid. This activity, which generated prior know-how, has culminated explicitly in the framework of the LOWphWine project with the Polytechnic University of Madrid. This public-private consortium project (IDI-20210393) has financial support from the CDTI Innovation through the CIEN Strategic Program (National Strategic Research Consortiums).
viniferm NS-TD	Selection from the DO Ribera del Duero. Research team: Department of Microbiology III - Complutense University of Madrid.
Proveget PREMIUM	**WINEBALANCE** "Improving the colloidal structure of wine - New bioactive tools of interest." University of Murcia. Ministry of Science, Innovation and Universities CDTI. EU FEDER funds.
Tanicol VINTAGE	The Università di Torino has confirmed its effectiveness in increasing and stabilizing the color of Barbera variety grapes (Vendimia 2018).
Tanicol ONE / L	The Università di Torino has confirmed its effectiveness in its antioxidant effect and color stabilization on Barbera variety grapes (Vendimia 2018).
SuperBouquet EVOLUTION	**VINNOSO$_2$** "Development of an oenological itinerary to produce high-quality wines free of sulfur dioxide." Its high antioxidant capacity helps to preserve the aromatic fraction of wines, guaranteeing the protection of thiols and eliminating reactive quinones, limiting browning. Its action allows reducing SO2 during preparation.
ULTRAWINE PERSEO	**ULTRAWINE** "Eco-innovative maceration system based on lfhp ultrasound technology for winemaking". Innovative ultrasound technique in winemaking processes developed by Agrovin within the framework of the HORIZON 2020 aid of the European Union. **Patent:** WO2015136130 A1.

Content summary

01. White wines

02. Successful rosés

03. Structure without astringency

04. Microbiology

01 — White wines
Production itineraries

The demand for white wines is currently on the rise.

The tools available allow it to extend the consumption time in optimal conditions and increase the quality of second-grade musts, obtaining wines with a low tendency to oxidation and high aromatic potential.

1.1 LONGER THE LIFE OF WHITE WINES

The objective is to maintain the wines in optimal conditions after production to guarantee that they remain in the best conditions during their most significant demand.

Aromatic fraction. Protection of varietal and fermentative aromas

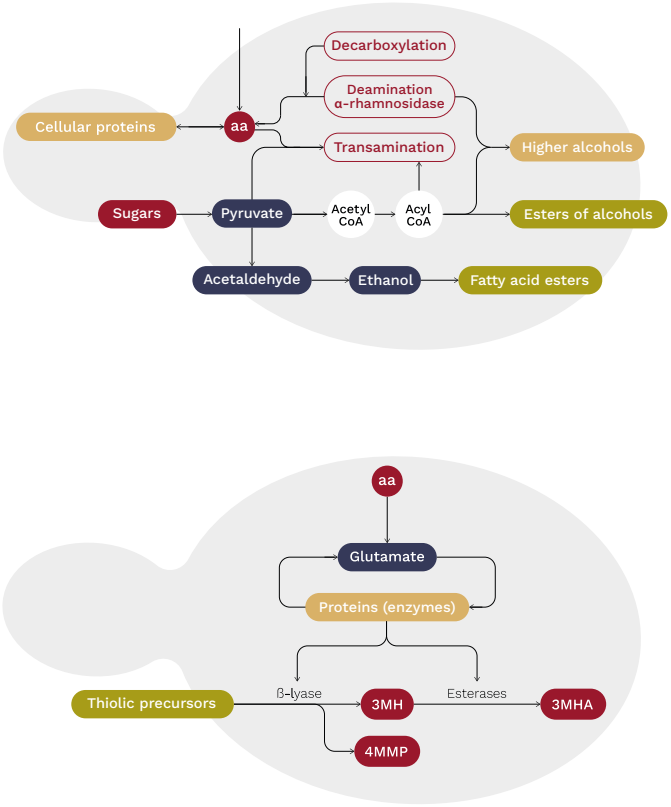
However, before its appearance, the aromatic intensity decreases, with thiols and terpenes being the first to be affected. Therefore, generating a high concentration of aromatic compounds during fermentation is essential to maintain the wine's quality over time.

Organic nutrition based on amino acids (aa) is the basis for forming fermentative aromas and the nitrogenous structure for generating enzymes that reveal varietal aromas.

The nutritional protocol will be based on the contribution of **Actimax Natura** or **Actimax Varietal** in the initial phases of AF. To favor the assimilation of aa, organic nutrition must be applied before reaching 5% vol and in the absence of ammonium salts that activate the NRC (Nitrogen Catabolic Repression) mechanisms, preventing the entry of AA and aromatic precursors (Cooper, 2006).

At this time of the multiplication phase, a contribution of sterols, such as ergosterol, will guarantee more excellent resistance of the yeasts. **Actimax Natura,** in addition to increasing the content of YAN in the form of NOPA, stands out for its high contribution of ergosterol and essential vitamins.

We will achieve greater complexity and aromatic longevity by starting fermentations with our non-Saccharomyces yeast strain **Viniferm NS TD,** in combination with **Viniferm Revelación** to enhance a thiolic profile with a prevalence of passion fruit aromas (Ac3MH) or **Viniferm Elegancia** for a more floral terpene profile.

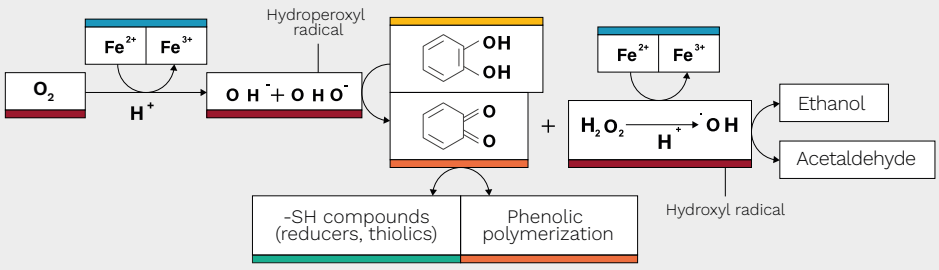


Formation schemes of aromatic compounds by yeast.

Color protection

Hydroxycinnamic acids (HCAs) and flavan-3-ols, such as catechins, are the mainprimary oxidation substrates. Their entry into cascade reactions results in the formation of quinones, with a great oxidative capacity, increasing the color and tone of the wines.

Keep the tones greenish will happen due to the decrease in the content of these substrates, as well as the presence of oxidation catalysts (Fe/ Cu).



Chemical oxidation process. Fenton reaction.

The reduction in the content of these substrates will be obtained with high affinity and reactive protein clarifiers such as **Proveget Premium,** whose novel treatment increases the pure protein fraction, increasing its capacity for action.

The transition metals Fe and Cu are catalysts for the formation reaction of the hydroperoxyl radical. Its elimination from the medium through chelating agents such as PVI/PVP allows for reducing oxygen reactivity and, therefore, oxidation reactions.

Solutions such as **Actimax Varietal** during fermentation help reduce oxidation processes thanks to their contribution of PVI/PVP.

Conservation in optimal conditions

Chemical oxidation of the wine must be avoided during its stay in the winery to prolong its freshness and aromatic profile. Therefore, the strategies will be based on:

- Inertize the circuits before transfers.
- Avoid cold stabilization (low temperatures increase the solubility of O_2).
- Preserve wines in the presence of lees.
- Store wines at temperatures around 10ºC, where oxidation reactions are slower.
- Preserve wines at low oxidation-reduction potentials.

In highly clarified wines and the absence of lees, using **SuperBouquet Evolution,** an inactivated yeast rich in glutathione, will allow the wines to be preserved in more reductive conditions.

Once in the bottle, low dissolved oxygen values will allow us to delay its evolution.

AGROVIN SOLUTIONS

Product	Description	Benefits
Actimax VARIETAL	Complete autolysis yeast with great power antioxidant (glutathione) and metal sequestrant (PVI/PVP).	Organic nutrient. Indicated to express varietal potential, it allows the genesis of enzymes responsible for releasing aromatic precursors. It has a high natural antioxidant capacity due to the double effect provided by its high content of reduced glutathione and metal sequestering capacity.
SuperBouquet EVOLUTION	Inactive yeast *(Saccharomyces cerevisiae)* with excellent antioxidant power (glutathione).	Its high antioxidant capacity helps preserve the aromatic fraction of wines, guaranteeing the protection of thiols and eliminating reactive quinones, limiting browning. Its action allows SO_2 to be reduced during production by reducing its combination.
viniferm NS-TD	Non-*Saccharomyces* yeast strain *Torulaspora delbrueckii.*	Bioprotective character. Intensifies floral records due to the production of ß-phenyl ethanol (rose, white flower) and varietal character due to its potent ß-lyase activity (grapefruit, boxwood). Significant release of mannoproteins.
viniferm REVELACIÓN	*Saccharomyces* yeast with high ß-lyase activity.	Aromatic fullness. Revelation of thiols, capable of converting thiol-type precursors into perceptible aromas, in particular: 4MMP (boxwood, cassis), 3MH (grapefruit, citrus), and 3MHA (passion fruit, mango, pineapple) together with the development of fermentative aromas floral type.
viniferm ELEGANCIA	*Saccharomyces* yeast with ß-glucosidase, α-rhamnosidase, α-arabinosidase and α-apiosidase activity.	Cryophilic yeast is suitable for fermentation at low temperatures. Its activity allows the release of aromatic terpenes. Thanks to its rapid lysis, it accentuates the sensations of volume and smoothness in the mouth, ideal for barrel-fermented whites.
Proveget *PREMIUM*	Fining agent in liquid format of plant origin with high reactivity, obtained from pea protein.	It significantly reduces hydroxycinnamic acids, oxidation substrates, and catechins. The flocs' high compaction also considerably improves performance.

1.2 OPTIMIZE THE QUALITY OF SECOND GRADE MUSTS

The amount of suspended solids in white musts is directly related to the quality of the wines. Plant particles favor the formation of substances with herbaceous tastes and aromas (alcohols and aldehydes with six carbon atoms). The remains of stems and leaves also favor the formation of C6 compounds with a herbaceous flavor. The high concentration of polyphenols, heavy metals, and oxidizing enzymes favors oxidation reactions, reducing the aromatic fraction and causing the browning of the wines.

Furthermore, un-decanted must have a greater tendency to form sulfur compounds with unpleasant odors. Finally, musts with high turbidity have a more significant microbial load that may give rise to more significant deviations in fermentation (Hidalgo, 2003). All this makes second-class must products with specific needs at each stage of production:

Grape reception

Ellagitannins provide excellent antioxidant protection with three routes of action:

1. They present a high oxygen consumption rate, reducing their availability in oxidation processes.
2. They can change the three-dimensional conformation of polyphenoloxidases, inactivating them.
3. Hydroxyl groups in hydrolyzable tannins can complex metal cations such as Fe^{+3} and Cu^{+2}, oxidation catalysts.

Therefore, adding ellagic tannins such as **Tanicol ONE / ONE L** will protect the musts from reception.

Limiting the microbiological population will be a consideration during reception. To achieve this, the early application of chitosan in the hopper or the must, after pressing, will act on bacteria and non-*Saccharomyces* yeasts.

Type of tannin	O_2 consumption (mg O_2/day.g)	RAC* (%)
Sulfur dioxide	11.90 ± 0.63	100
Seed tannin	0.19 ± 0.05	13
Skin tannin	0.40 ± 0.10	27
Quebracho tannin	0.57 ± 0.15	38
Gallotannin	0.06 ± 0.05	4.2
Ellagic tannin	1.81 ± 0.15	122

*RAC = Relative Antioxidant Capacity referred to sulfur dioxide

The antioxidant capacity of different tannins depends on their origin. (Pascual, 2017)

This will facilitate the implantation and desired sensory characterization of the chosen Viniferm LSA.

Microstab pH is a formulation based on chitosan of fungal origin and L (+) tartaric acid, whose low pH promotes a high load of the chitosan present, increasing its action on the indigenous flora from the earliest stages.

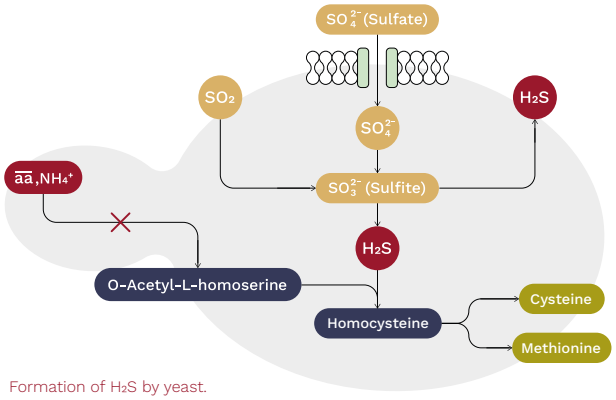
Desludging

When working with second-grade musts, the settling stage can be difficult for two reasons: the high microbiological load we will treat with **Microstab pH** and the high concentration of suspended solids (>10%), which can hinder dynamic settling. Therefore, to reduce the turbidity of these musts, we must carry out static settling.

Adjuvants such as **Proveget BC** will help lessen the musts' polyphenolic load and facilitate the sludge's precipitation and compaction.

Fermentation

During fermentation, we must encourage aromatic production and avoid the formation of sulfur compounds that can create unpleasant aromas.



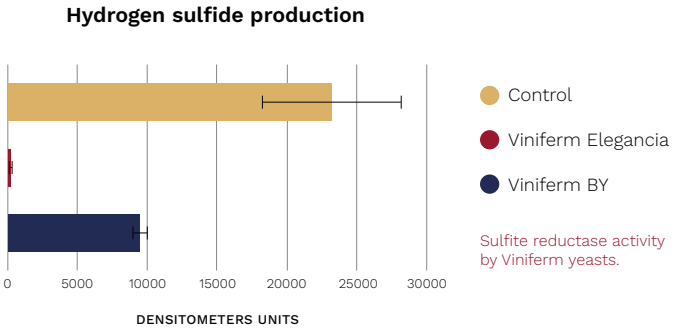
Formation of H_2S by yeast.

The formation of sulfur compounds can occur in two ways:

- **Metabolic pathway:** yeasts need to synthesize sulfur amino acids (cysteine and methionine) for survival. The absence of sufficient nitrogenous bodies (aa) results in the formation and accumulation of H_2S.
- **Spontaneous chemical route:** The sudden drop in redox potential recorded during the exponential phase of yeast multiplication can lead to values lower than -70 mV. This poses a risk because, at these values, the spontaneous chemical transformation of elemental S into H_2S can occur.

The use of organic nutrients with high amino acid content, such as **Actimax Natura,** allows the control of the decrease in the electrochemical potential during the multiplication phase of the yeast, as well as avoiding nitrogen deficiencies that lead to the formation of H_2S.

On the other hand, yeasts with low sulfite reductase activity, such as **Viniferm Elegancia** and **Viniferm BY,** are recommended.



Sulfite reductase activity by Viniferm yeasts.

Alternative: Hyper oxidation

Hyper oxidation pursues the oxidation of the polyphenols in the must for their subsequent elimination in settling. Thus, after fermentation, we obtain wines with a lower content of oxidation substrates and, therefore, with a better evolution, reducing aromatic losses due to oxidation and browning and extending the life of these wines.

	HIP	Control	D %
DO 420	0,078	0,077	N/V
IPT	4,56	5,48	16,79
DO 320	2,72	3,49	22,06
DO 440	0,056	0,057	N/V

Comparison of the different absorbances of a hyperoxidized wine (HIP) versus a conventional production (Control). Harvests 2021, DO Mancha. Airen must.

Method of action: Oxygen is added to the appropriately acidified must at doses between 20 and 60 mg/l—depending on the variety of must and its phenolic concentration—and the oxidation process can last 2 to 5 hours. After hyperoxidation, settling is carried out. The application of **PVPP** during this process favors the elimination of oxidized polyphenols.

For fermentation, the use of **Viniferm BY** fast-acting yeasts and **BCP XII** fermentation complex clarifier is recommended to selectively eliminate catechins and leucoanthocyanidins formed by the oxidation reactions promoted.

AGROVIN SOLUTIONS

Product	Composition	Benefits
Tanicol ONE / L	Chestnut ellagic tannin.	Antioxidants have three effects: oxygen consumption, an antioxidative effect, and precipitation of heavy metals (catalysts of chemical oxidations). Antioxidant protection in harvests of poor sanitary quality due to laccase inhibition.
MICROSTAB pH	Liquid formula based on chitosan of fungal origin in suspension and L (+) tartaric acid.	They are applied during harvest to prevent the development of spoilage populations, yeasts, and bacteria, reducing their load and thus avoiding possible deviations in FA. Reducing the population of bacteria reduces the risk of unwanted malolactic.
Proveget BC	Pure vegetable protein from peas and selected bentonites.	Fresher and more frank aromas, elimination of astringent and bitter notes. Eliminates oxidizable and oxidized compounds, contributes to protein stabilization, and improves balance in the mouth: fast flocculation, more compact lees, and higher yield.
Actimax NATURA	Complete autolysis yeast *(Saccharomyces cerevisiae).* An essential source of primary amino acids, slow assimilation.	There is a high contribution of amino acids (37%), precursors of fermentative aromas, and the basis for correctly synthesizing the enzymes responsible for developing varietal precursors (glycosidases, lyases). Its high YAN contribution limits the production of hydrogen sulfide. Its high content of ergosterol and essential vitamins guarantees the viability of the yeasts.
viniferm BY	*Saccharomyces cerevisiae* var. *bayanus*.	It has fermentative abilities in difficult fermentation conditions: low temperatures, highly clarified musts, and nutritional deficiencies. Low production of volatile acidity.
BCP XXI	Bentonite, PVPP, and cellulose.	Strong deproteinizing action selectively eliminates leucoanthocyanins and catechins, preventing and eliminating browning phenomena in whites and protecting the color of rosés and reds. Improves compatibility of the lees.

1.3 SPECIAL SITUATIONS

A complication during the production of white wines is the start of fermentation in the presence of sludge. These fermentation starts with unsuccessful racking may be due to the entry of grapes at high temperatures, a high microbial load, peaks of grape entry that are difficult to manage, as well as final days of harvest when there is a high load of microorganisms.

In this case, it will be necessary to clean the musts during fermentation to avoid the appearance of sulfur compounds with reductive aromas and reduce their polyphenolic load.

Key intervention points will focus on:

- The application of bentonites to help compact sludge.
- The elimination of polyphenols that reduce the risk of browning in wine.
- Racking of the must in the final stages of fermentation (D~1030) to complete the alcoholic fermentation in the absence of gross lees.

The application during the first stages of fermentation of **Proveget Cristal,** a mixture of PVPP, pea protein, bentonite and cellulose with a high affinity for hydroxycinnamic acids, catechins and leucoanthocyanidins, reduces the risk of browning and the formation of unpleasant aromas during fermentation. In addition, its cellulose fraction favors the development of fermentation by adsorbing compounds harmful to yeasts.

Absorbance	Compounds	Solutions
320 nm	Hydroxycinnamic acids	Vegetable proteins
420 nm	Yellow color	Vegetable proteins and PVPP
440 nm	Oxidized polyphenols	PVPP
280 nm	TPI	Vegetable proteins and PVPP

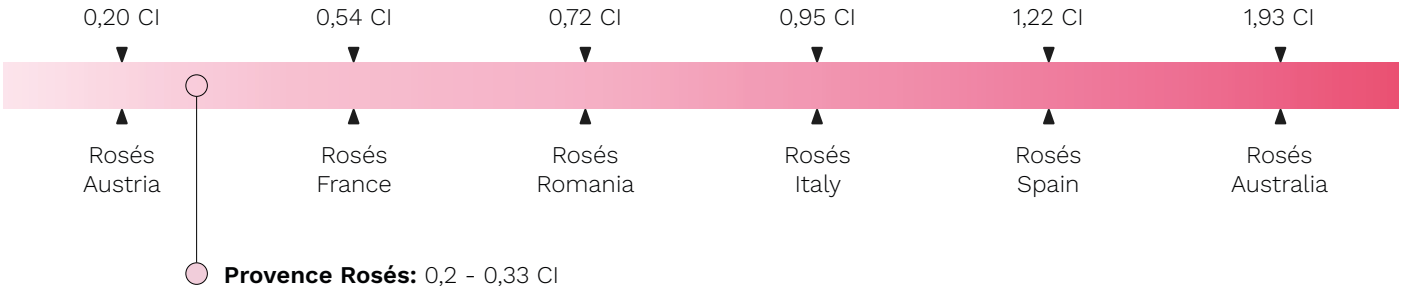
AGROVIN SOLUTIONS

Product	Composition	Benefits
Proveget *CRISTAL*	Pure vegetable protein from pea, PVPP, bentonite powder and cellulose.	The synergistic combination of PVPP and plant protein allows the elimination of a wide range of oxidizable and oxidized phenols. Promotes protein stability. Reduces the concentration of compounds toxic to yeasts.

02 — Successful rosés

In the last twenty years, consumption of rosé wine has expanded significantly, increasing production by 25% between 2001 and 2021.

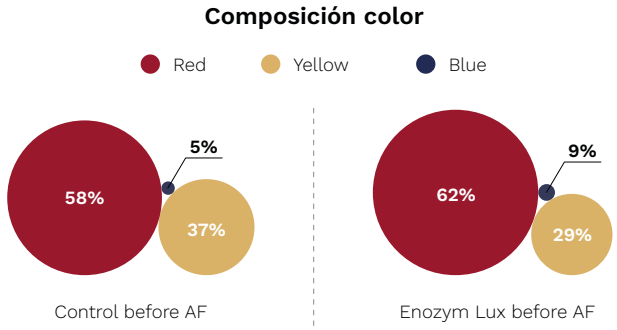
This increase is mainly due to the consumption of rosé wines with low color intensity. The great challenge is to obtain a rosé with low color intensity that lasts over time and in which the aroma and the palate are the protagonists.



2.1 COLOR DEFINITION

The main objective of obtaining low-layer wines is to obtain them by direct and gentle pressing, preferably in closed presses, trying to maintain good protection against the oxygen of the must with the addition of nitrogen gas. To help obtain it with the minor color extraction and maximum aromatic precursors, we recommend the addition of **Enozym Lux** and **Enozym Extra Arome**, highly concentrated and liquid pectinase enzymes. Furthermore, its incorporation from reception, directly on the grapes, can imply a modulation of the tone of the color obtained (reduction of yellows).

Temperatures above 16oC will discourage the use of enzymes to avoid excessive extraction.



Comparison of the color composition of a rosé must with and without the application of Enozym Lux.

Extraction optimization

The optimal tools to modulate color are based on bleaching carbons or formulations with PVPP and vegetable proteins such as **Proveget Cristal** and **Triplex R.** It is essential to work differently on the must fractions obtained:

Yolk must

Light refining. Application of preventive doses with **Proveget Cristal**.

— There are three applications of 10-20 g/hl at the beginning, middle, and 2/3 of alcoholic fermentation.

Press must (<0.8 bars)

Intensive refining. Application of corrective doses with **Triplex R.**

— Dose of 20 g/hl decreases 15% CI
— Dose of 40 g/hl decreases 25% CI
— Dose of 60 g/hl decreases 45% CI

During the life of the wine, it will be essential to minimize corrections with SO_2 discolors the wine; therefore, we will need to have good management of oxidations, avoiding the formation of acetaldehyde that results in the combination of SO_2 and the recurring need to make corrections.

Actimax Varietal helps eliminate metals and oxidation catalysts, and it can be used with inactive yeasts rich in glutathione, such as **Super Bouquet Evolution.** It will keep the wine in a more reductive situation of great interest in wines with a more thiolic profile.

AGROVIN SOLUTIONS

Product	Composition	Benefits
Enozym LUX	Liquid preparation is highly concentrated in pectin lyase (PL). Free of cinnamyl esterase activity.	High PL activity whose action favors the release of aromatic precursors and phenolic compounds in soft pressings, reducing yellow tones.
Enozym EXTRA AROME	Liquid preparation is highly concentrated in pectin-lyasa (PL). Incorporates ß-glucosidase activity.	Optimizes skin maceration with its high PL and ß-glucosidase activity, releasing varietal aromatic precursors, and is suitable for working at low temperatures.
Proveget CRISTAL	Pure vegetable protein from pea, PVPP, bentonite powder, cellulose.	Preventive color refinement in low extraction yolk musts. Reduction of yellow tones and hydroxycinnamic acids.
TRIPLEX R	Complex adjuvant based on PVPP, carbon, and bentonite.	Corrective refining of the color of rosés in musts with a high tone. Reduction of yellow tones, improving its longevity. Improves compaction, optimizing performance.

2.2 DEFINED AROMATIC PROFILE

Despite being very light, the most popular rosé wines are intensely aromatic, so, during fermentation, we will enhance the aromas with our **Actimax Varietal / Actimax Natura** nutrients and Viniferm yeasts.

Organic nutrition with a high amino acid content will provide the nitrogenous bodies necessary for the yeast to define the aromatic profile. **Viniferm Revelación** has high ß-lyase activity to obtain a thiolic profile, and **Viniferm Emoción** forms fermentative esters towards an amyl/fruity profile.

	Amilic profile	Thiolic profile
		
Turbidity	**+/- 50 NTU** A higher proportion of ester formation	**+/- 100 NTU** Higher content of aromatic precursors
LSA strain	**Viniferm Emoción** High formation of fruity aromas	**Viniferm Revelación** High ß-lyase activity
Temperature	**14 ºC** Less aromatic loss	**16/18 ºC** Greater release of thiol compounds
Nutrient	**Actimax Natura** High contribution of amino acid bases	**Actimax Varietal** Reduction of oxidation losses of varietal compounds.
Sensory improvements	**Intensify fruit potential** Tannins from the wood of red fruit trees such as **Tanicol Red Vintage** enhance the fruit characters (strawberry, cherry, bach flower).	**Stabling** Maintain the wort with high turbidity (400-600 NTU) at 4ºC for 1-2 weeks. Later addition of **Enozym LUX** (3-5 ml/hl) to adjust turbidity to desired values for fermentation.

AGROVIN SOLUTIONS

Product	Composition	Benefits
Tanicol **RED VINTAGE**	Condensed tannin from grape seeds and wood from red fruit trees.	Increase in the perception of red fruit due to the contribution of volatile compounds (ethyl benzoate → cherry; acetophenone → strawberry; 2-octanone → red fruits).
Actimax **VARIETAL**	Complete autolysis yeast with great power antioxidant (glutathione) and metal sequestrant (PVI/PVP).	Organic nutrient. Indicated to express varietal potential, it allows the genesis of enzymes responsible for releasing aromatic precursors. It has a high natural antioxidant capacity due to the double effect provided by its high content of reduced glutathione and metal sequestering capacity.
Actimax **NATURA**	Complete autolysis yeast *(Saccharomyces cerevisiae).* An essential source of primary amino acids, slow assimilation.	There is a high contribution of amino acids (37%), precursors of fermentative aromas, and the basis for correctly synthesizing the enzymes responsible for developing varietal precursors (glycosidases, lyases). Its high YAN contribution limits the production of hydrogen sulfide. Its high content of ergosterol and essential vitamins guarantees the viability of the yeasts.
viniferm **REVELACIÓN**	*Saccharomyces* yeast with high ß-lyase activity.	Aromatic fullness. Revelation of thiols, capable of converting thiol type precursors into perceptible aromas, in particular: 4MMP: (boxwood, cassis), 3MH: (grapefruit, citrus) and 3MHA: (passion fruit, mango, pineapple) together Development of floral fermentative aromas.
viniferm **EMOCIÓN**	*Saccharomyces* yeast with specific adaptation to the conditions of rosé wines.	Increase in aromatic intensity due to its high activity in forming fermentative esters, giving rise to wines with a fruity-amylic profile. Very stable aromas over time. Strain adapted to fermentation at low temperatures.
Enozym **LUX**	Liquid preparation is highly concentrated in pectin-lyase (PL). Free of cina-mil esterase activity.	Due to its high concentration of PL activity, it allows reductions in turbidity at low temperatures. Its application after housing requires an increase in usual doses.

2.3 INCREASE IN UNCTUOSITY

Once the FA is finished, the work with lees will allow the release of polysaccharides that increase the oiliness of the wine by filling the edges derived from low pH. Furthermore, lees are avid consumers of oxygen, so preserving wines in their presence will guarantee conservation in better conditions.

Resuspension of the lees 1-2 times a week will favor the release of polysaccharides, with contact times of between 2-4 months being necessary to improve results.

In highly clarified wines or when we want to accelerate this treatment, it is advisable to add exogenous lees such as **SuperBouquet Evolution,** which provides a more excellent sensation of fresh acidic fruit, or **SuperBouquet MN,** which increases smoothness and sweetness, in combination with **Enozym Glucan** ß-1 enzymes, 3-1,6—glucanase, which optimizes the release of mannoproteins.

The refinement of already clarified wines can be adjusted by using alternatives that increase the sensations of fresh fruit, such as the combination of **Spirit Smoothie + Spirit Nuance** and oak toppings that release quickly without adding astringency.

AGROVIN SOLUTIONS

Product	Composition	Benefits
SuperBouquet EVOLUTION	Inactive yeast *(Saccharomyces cerevisiae)* has excellent antioxidant power (glutathione).	Obtaining a reductive state during wine conservation due to the presence of reductive lees (glutathione). Antioxidant protection of wine. Increased sensation of fresh fruit in the wine
SuperBouquet MN	Yeast rind rich in yeast polysaccharides (48-53% by weight). High content of soluble mannoproteins (20-22%).	Increase in creaminess and sweetness due to the contribution of polysaccharides and mannoproteins. Fixation of aromas by interaction with the transferred mannoproteins. Increase in fine lees.
Enozym Glucan	Enzyme ß 1,3-1,6 glucanase.	Accelerates the release of parietal polysaccharides when aging on endogenous or exogenous lees.
Spirit Smoothie	Oak alternative in topping format.	Its combined use enhances the fruit intensity, and the increase in smoothness and center of the mouth, in turn, balances wines with more acidity.
Spirit Nuance	Oak alternative in topping format.	

03 — Structure without astringency

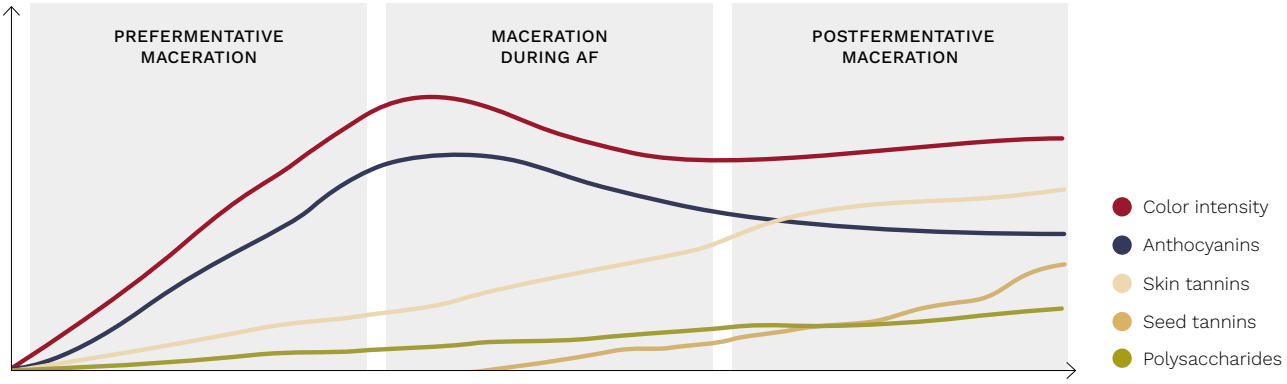
Wines with a high structure must balance the tannic composition with its organoleptic antagonists to obtain structured, unctuous wines with a pleasant palate. The maceration processes will focus on extracting the most noble compounds and the balancing agents is necessary.

The perception of astringency is influenced by the concentration of tannins, their type, and the matrix of the wine, where polysaccharides play a fundamental role.

3.1 PREPARATION OF BALANCED RED WINES

Three variables, such as maceration time, temperature, and pumping over frequency, condition the extraction of compounds from grapes.

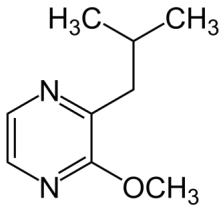
The maturation balance reached at the time of harvest, along with the character of the wine sought, are determining factors in determining and adjusting these variables, taking as reference the extraction process of the different components during the maceration phases.



Extraction kinetics of compounds during maceration

During the first moments of alcoholic fermentation, anthocyanins, and varietal aromatic compounds are extracted. However, there are varietal compounds that, in high concentrations, can be undesirable and cause sensations of lack of maturity or greenness. We are talking about pyrazines, present in quality varieties widely used in world viticulture (Cabernet Franc, Cabernet Sauvignon, Merlot).

Among the methoxypyrazines, iso-butyl-methoxy-pyrazine (IBMP) is of particular importance, and it is responsible for the green pepper aroma characteristic of some wines. Its low perception threshold of up to 1-2 ng/l (Mozzon, 2016) in less structured wines makes it noticeable to many consumers, being considered an olfactory defect at contents more significant than 15 ng/l.



Chemical structure of IBMP

Methoxypyrazines	Aromatic descriptors	Perception threshold (ng/l)
ETMP	Potato, Vegetable, Ground, Red pepper	400 – 425
SBMP	Vegetable, Ivy leaves, Red pepper	1 – 2
IPMP	Ground, Cooked asparagus, Green pepper	2
IBMP	Ground, Vegetable, Red pepper, Humidity	0,5 – 2

Aromatic descriptors and perception threshold in water of the main methoxypyrazines (SALA et al. 2004)

The presence of pyrazines in the grape skin makes it impossible to eliminate them in red vinifications, so they are transferred to the must during maceration.

For this reason, the production strategies to reduce its perception will reside in the masking of these compounds, for which preparations will be carried out in which the increase in tannic structure and fruity profile predominates. This will balance the bouquet of the wine, preventing the herbaceous aromas, specifically the green pepper, from being predominant.

C6 compounds are other compounds responsible for herbaceous aromas. Their presence in the wine provides vegetal sensations associated with grapes with irregular ripening.

C6 Compounds	Aromas
Hexanal	Herbaceous
Acetic acid, Hexyl ester	Resin solvent
1-Hexanol	Herbaceous
3-Hexen-1-ol, (E)-	Green leaves
3-Hexen-1-ol, (Z)-	Green leaves
2-Hexen-1-ol, (E)-	Green leaves

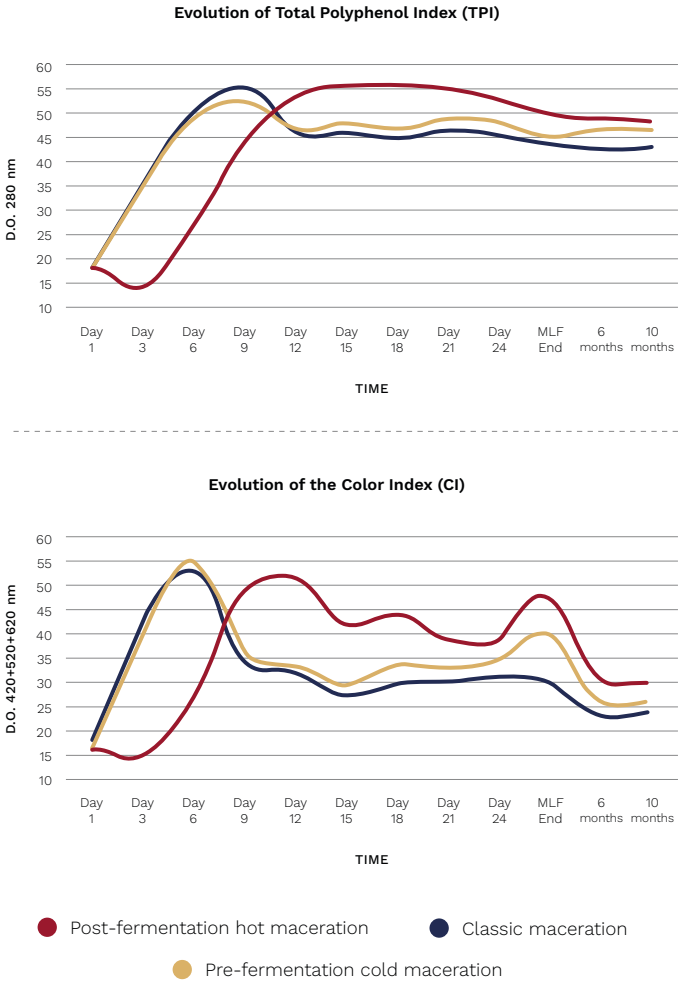
Aromatic descriptors of the main C6 compounds

The appearance of alcohol during fermentation gives rise to a hydroalcoholic solution with greater extraction capacity. Under these conditions, the integuments that cover the seed degrade, resulting in the extraction of seed tannins.

Seed tannins are monomers or polymers of up to 5-6 units. Because they have a low molecular weight, they are very reactive, highly concentrated, and incredibly astringent.

Some of the aromatic compounds described above and included in the well-known C6 compounds are found in the seeds. This is why, at the end of fermentation, the extraction of the most astringent compounds occurs, as well as those responsible for herbaceous aromas.

The strategies to follow in each fermentation will, therefore, have to consider these aspects, assessing how the macerations should be according to the components we want to extract in a majority way.



Comparison of the evolution of TPI and CI depending on the type of maceration carried out during the fermentation of red wines.

During the production of young wines for rapid consumption and especially in grape conditions with irregular ripening, early devatting (D 1030-1010) will reduce the extraction of the most astringent tannins and C6 compounds that provide vegetal sensations, which may be compensated by applying Agrovin's oenological allies.

On the other hand, the use of technology that favors extraction, such as ultrasound, helps to balance the wines by reducing maceration times, thus avoiding the extraction of the compounds present in the seeds in those cases that are not desirable.

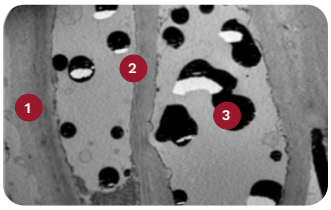
3.2 **ULTRASOUND WINEMAKING.** COLOR AND STRUCTURE WITHOUT ASTRINGENCY

Innovation patented by Agrovin for applying high-power, low-frequency ultrasound in winemaking processes.

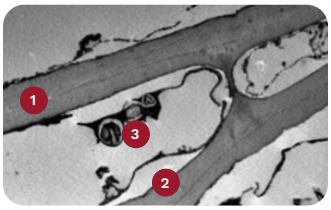
Ultrasounds are applied to crushed grapes after fermentation, causing a process called **cavitation** that results in the collapse of cellular structures.

This breakdown of cellular structures facilitates the passage of compounds in the plastids, such as polyphenols and aromatic compounds, and the release of polysaccharides in the cell wall.

1 Cell wall 2 Membrane 3 Plates



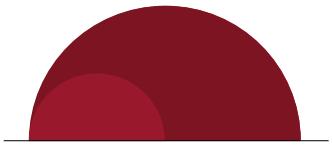
Electron microscope view (970x): Control grape skin cells



Electron microscope view (970x): Sonicated grape skin cells

The oenological benefits of working with Ultrawine Perseo involve a significant increase insignificantly increasing the concentration of components with a sensory effect, giving the wines a 360° global organoleptic improvement, including integration and harmony.

-50%
In times of anthocyanin extraction
More stable over time.
Responsible for color.

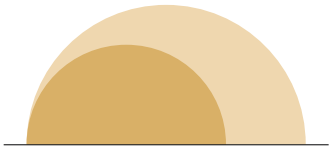


Control Ultrawine Perseo

+30%
Polysaccharide extraction
Including mannoproteins, RG-II, HL, AND PRAG.

Control Ultrawine Perseo

+40%
Tannin extraction of skin
Responsible for the structure and stability of the coloring matter.

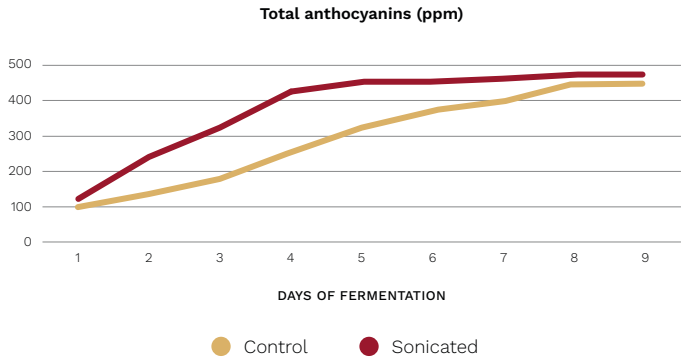


Control Ultrawine Perseo

+30%
Extraction of aromatic compounds
There is no extraction of plant compounds.

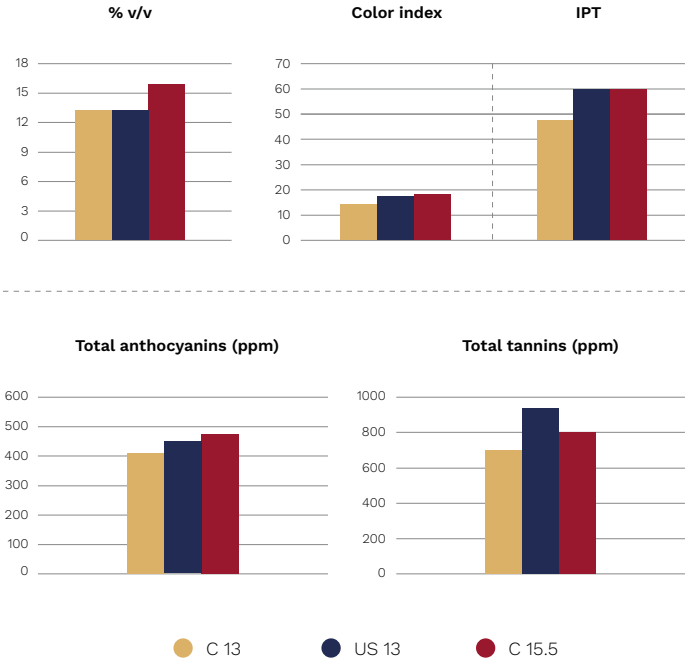
Control Ultrawine Perseo

After the application of ultrasound, the facilitated release of the compounds mentioned above allows for optimizing macerations, reducing the need for time and space in winemaking. **Ultrawine Perseo** enables you to obtain wines with the target anthocyanin content up to 50% faster.



Comparison of total anthocyanin extraction in a traditional winemaking process and in a winemaking process using ultrasound.

Linked to this reduction in maceration times, we can favor the early harvest without the wines being affected in color and tannic structure with the advantage of obtaining wines without vegetal notes. Favoring, yes, less alcoholic wines, with with a more balanced pH.



Results of three elaborations with different technological ripening with and without application of ultrasound (C 13 = traditional grape elaboration with 13° Baume; US 13 = elaboration with application of ultrasound of grapes with 13° Baume; C 15.5 = traditional elaboration with grapes with 15.5° Baume).

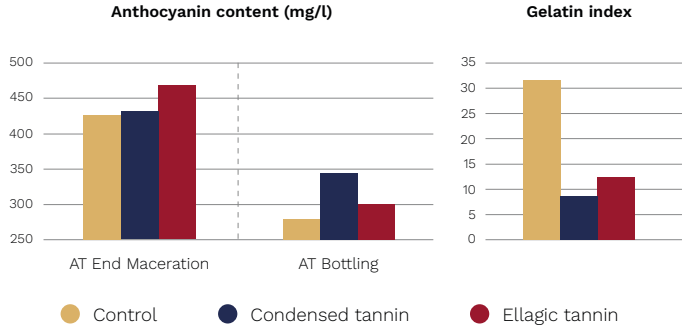
3.3 OENOLOGICAL ALLIES

The oenological proposals available at Agrovin allow modulating maceration to enhance extraction and compensate for components in the wine obtained.

Tannins

Una de las causas de la caída del color tras la fermentación alcohólica es que los antocianos monómericos son fácilmente oxidables, por lo que necesitamos estabilizarlos a través de la condensación con los taninos.

Las maceraciones cortas, ya sean por baja madurez fenólica o por la necesidad de espacio en los vinificadores, puede derivar en la extracción insuficiente de taninos para la estabilización del color. En estos casos, el uso de nuestros taninos de la **gama Tanicol** nos permiten compensar estas deficiencias.



Content of total anthocyanins (TA) and monomeric anthocyanins with the addition of tannins of different natures in bottled wine (Agrovin 2018).

Ellagic tannin	Condensed tannin
Chestnut tannin Oak tannin	Grape tannin Quebracho tannin
Higher AT content at the end of AF	Higher AT content in bottled wine
Prevents incipient oxidation of monomeric anthocyanins	Improves stability over time

The ellagic tannin contained in **Tanicol RED SENSE** provides antioxidant protection of monomeric anthocyanins, preventing their oxidation and precipitation.

Its formulation also collaborates in copigmentation phenomena, forming stable pigments, both to changes in pH and to the addition of sulfur, thus increasing both the coloring intensity in the wine and its stabilization over time.

In the aromatic fraction, **Tanicol RED SENSE** provides the wine with volatile compounds such as ethyl benzoate (cherry), acetophenone (strawberry, cherry, bach flower), and 2-octane (fruity), enhancing the red and black fruit characters and accentuating the varietal typicality of red wines.

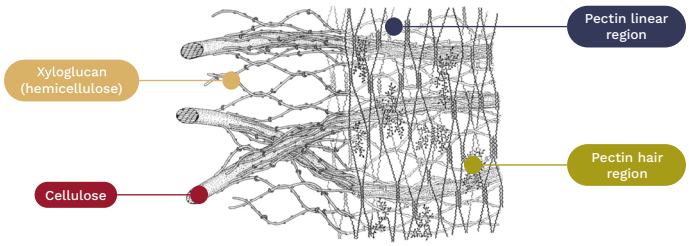
In preparations where maceration times have been limited for different reasons, the application of **Tanicol Vintage** increases the IPT content without providing astringency and helps to polish bitter and green notes and promotes the formation of stable pigments. Its use in vatting helps us achieve an optimal balance in this type of winemaking's anthocyanin/tannin ratio.

Another option for working on the structure without adding astringency is using **Spirit NATURE.** This alternative in rice grain format provides sweetness and smoothness during alcoholic fermentation and increases the content of ellagitannins, preventing the oxidation of the extracted polyphenols.

Extraction enzymes

Enzymes are natural proteins that have catalytic activity in specific biochemical reactions. In the case of the maceration of red wines, the use of particular enzymes with pectolytic activities promotes the degradation of the cell wall, favoring the dispersion of tannins and anthocyanins.

Another option for working on the structure without adding astringency is using Spirit NATURE. This alternative in rice grain format provides sweetness and smoothness during alcoholic fermentation and increases the content of ellagitannins, preventing the oxidation of the extracted polyphenols.



Constitutive structure of the cell wall (Carpita, 1993).

To promote greater extraction of phenolic compounds, greater aromatic intensity, as well as an increase in pressing performance, we will need the action of our liquid enzyme **Enovin CROM.** This presents pectolytic, cellulase, and hemicellulase activities designed specifically for the maceration of red grapes.

If what we intend is not only rapid color extraction but also more excellent stability over time, we will need, in addition to the usual cutting enzymatic activities (pectolytic, cellulase, and hemicellulase), ß-glucanase activity for the extraction of parietal polysaccharides from the cell wall of the yeast, we have our enzyme **Enozym VINTAGE** as an ally.

Polysaccharides

Polysaccharides participate in the color stabilization processes by reaction with anthocyanins; their contribution from the initial phases of maceration allows stabilization from the extraction of anthocyanins in the aqueous phase.

The participation of polysaccharides will benefit wines from grapes with a ripening deficit. To achieve this, **MannoArome** favors stabilizing the coloring matter due to its contribution of polysaccharides, reducing astringency at a gustatory level.

In the same way, the untoasted oak fraction that makes it up reduces the herbaceous sensations at an aromatic level.

Due to its greater capacity to transfer mannoproteins, the selected LSA is also an appropriate strategy for increasing polysaccharides during fermentation. **Viniferm 3D** intensifies the aftertaste and provides presence and volume, clarifying the phenolic fraction, reducing astringency, and enhancing sweet tannins.

In the case of structured wines in which the objective is varietal presence, **Viniferm ÉLITE** encourages the prevalence of varietal aromas with the integration of mature tannins, giving rise to balanced and round wines.

AGROVIN SOLUTIONS

Product	Composition	Benefits
Tanicol RED SENSE	Ellagic tannin *(Castanea sativa)*, condensed tannin from grape seeds *(Vitis vinifera)* and wood from red fruit trees.	Protection against oxidation of monomeric anthocyanins. The CI has more excellent stability due to the tannin-anthocyanin combination. Enhancement of the varietal characters of red wines.
Tanicol VINTAGE	100% condensed tannin from grape seeds *(Vitis vinifera).*	Increase the structure in an integrated and balanced way. Decrease in bitter and green notes. Formation of stable pigments due to balance in the anthocyanin/tannin ratio.
SPIRIT NATURE	Oak alternative in rice grain format.	Increase in sweetness and oiliness during maceration of red wines. Its botanical origin *(Quercus pyrenaica)* provides twice as many ellagitannins as its European and American counterparts.
Enovin CROM	Liquid enzymatic preparation based on pectolytic activities in combination with cellulase and hemicellulase. FCE.	Improvement of the extraction of polyphenolic components. Reduction of maceration times. It improved pressing performance. The ease of application is due to its liquid format.
Enozym VINTAGE	Enzyme complex with pectin lyase, poly-garacturonase, and pectinesterase activities, combined with cellulase, hemicellulase, and ß-1,3-1,6 glucanase. FCE.	Balanced extraction of anthocyanins and tannins, ensuring their stabilization by combination with polysaccharides during maceration. Improvement of the extraction of aromatic compounds. Ease of clarification of musts and wines. Reduction of maceration times by 25%.
MannoArome	Yeast bark (*Saccharomyces cerevisiae*) is specific for its high yield of polysaccharides, medium plus toasted oak tannin, and untoasted oak tannin.	Increase in sweetness and greasiness. Reduction of vegetal notes at low doses (<30 g/hl) and increase in complexity at high doses (>30 g/hl). Reduction of sensations of astringency and greenness in the mouth.
viniferm 3D	Yeast *Saccharomyces cerevisiae var. cerevisiae.*	Significant release of mannoproteins during fermentation and post-fermentation phase that give volume, roundness, and length to the wines.
viniferm ÉLITE	Yeast *Saccharomyces cerevisiae var. cerevisiae.*	Yeast with a varietal profile to obtain structured, unctuous red wines in which the fruit stands out.

04 — Microbiology in wine

Exercising control of the microbiology present in the wine at each of its stages is critical to the quality of the wine:

- Control of the effects of grapes with poor health quality.
- Reactivation of fermentations slowed down or stopped.
- Control of deviations in situations of slowed kinetics.
- Management of the MLF.
- Microbiological safety in wine conservation /aging.

4.1 HIGH MICROBIOLOGICAL LOAD

Microorganisms play a fundamental role in wine production. Thanks to their rich concentration of enzymatic activities, both yeasts and lactic bacteria can metabolize and generate different compounds that can improve the quality of the wine.

However, the microbiological richness present in the fruit of grapes does not concentrate families exclusively with positive impact. For this reason, microbiological control will aim to guarantee the exclusive prevalence of those species whose activity is positive for the quality of the wine, avoiding or alleviating the effects of those that may cause deviations.

Grapes affected by *Botrytis cinerea*

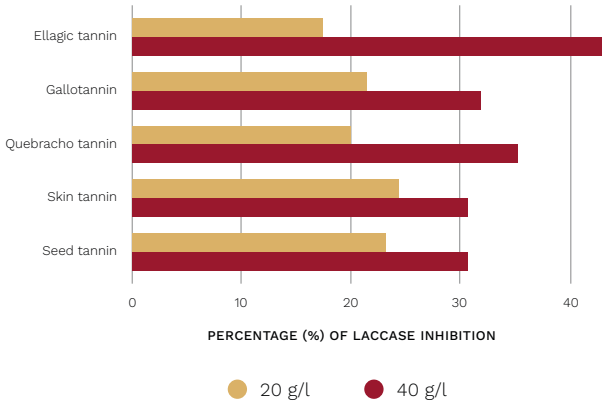
Botrytis cinerea is the fungus responsible for crop loss and a decrease in total acidity, sugars, nitrogen, and an increase in volatile acidity. These affected grapes have greater microbiological complexity and can reach values of up to 10^8 cell/g compared to 10^2 cell/g present regularly.

Furthermore, this microbiological contamination causes the presence of laccase, a fast-acting polyphenoloxidase enzyme, the formation of ß-D-glucans, which in the presence of ethanol forms filamentous clots with great clogging power, and ultimately the production of polysaccharides with an inhibitory effect. For yeasts, phytoalexins (Hidalgo Togores, 2003).

How should we proceed?

Grapes affected by *Botrytis* break easily, releasing must even while still on the vine. Therefore, the phenomena of chemical and enzymatic oxidation as microbiological activity must be controlled during transport with formulations such as **Redoxtanin B** and **T** based on metabisulfite, ascorbic acid, and tannin (gallic or ellagic, respectively).

During reception, the objectives will focus on the inactivation of laccase, which we will achieve by denaturing it thanks to adding tannins such as **Tanicol ONE** 100% ellagic. We will reduce the microbial population of the must thanks to the addition of **Microstab pH,** a liquid chitosan suspension explicitly formulated for application during the harvest.



Inhibitory effect of different oenological tannins on laccase (Vignault et al. 2019).

This biopolymer of fungal origin and derived from chitin has exceptional effectiveness on non-*Saccharomyces* yeasts. and lactic acid bacteria that cause the formation of acetaldehyde and acetic acid or consume nitrogen and thiamine.

Finally, the high presence of glucans makes cleaning or filtering musts and wines difficult. For its hydrolysis, we must apply enzymes with ß-1,3-1,6 glucanase activity, such as **Enozym Glucan.**

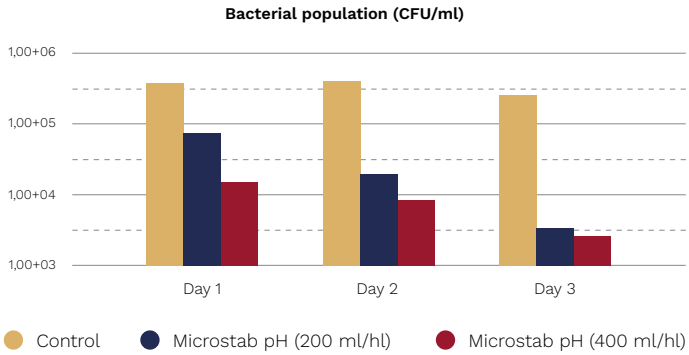
Start of spontaneous fermentations

In recent years, musts arriving at wineries with high pH have increased the risk of microbiological contamination, which can generate spontaneous fermentation, fermentation stops, microbiological deviations, lactic acid spikes, etc..

How should we proceed?

The application of **Microstab pH** allows reducing the microbial load from the reception of the grapes. Its action on microorganisms occurs at two levels. In the first phase, chitosan binds to microorganisms, forming large flocs that, due to gravity, end up precipitating. In the second phase, it causes a breakdown of the membranes, producing cell death.

Microstab pH facilitates the acidification of the wort, correcting the pH and improving the antimicrobial capacity of SO_2. The chitosan, highly activated by the pH at which the product is found, reduces the microbial load of the musts, thus reducing the risk of spontaneous starts and the proliferation of bacteria that can cause lactic acid breakdown.



Antimicrobial effect of Microstab pH on white must with a population of 10^6 CFU/ml of *Oenoccocus oeni.*

Another strategy for controlling microorganisms will focus on the use of LSA adapted to the necessary conditions:

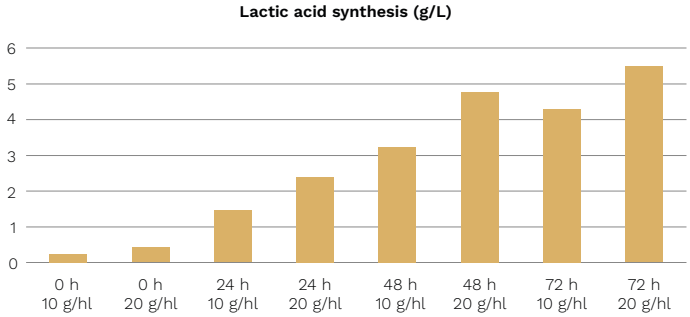
— Early sowing of LSA.

— Use of LSA with Killer factor.

— Sequential sowing of yeasts of different species, starting with yeasts with a greater capacity to adapt to the environment:

- Viniferm NS TD: *Torulaspora delbrueckii* yeast with high ß-lyase activity to enhance the varietal profile of those varieties with cysteine thiols, complementing it with an increase in the oiliness of the wine thanks to its high polysaccharide transfer capacity.

- Viniferm NS Chance: *Lachancea thermotolerans* yeast selected for its lactic acid-generating metabolism, which will allow the management of the total acidity of the wine from perfect integration into the must phase.



According to the dose applied, Viniferm Ns Chance's lactic acid synthesis lasted until the inoculation of *Saccharomyces cerevisiae*.

AGROVIN SOLUTIONS

Product	Composition	Benefits
REDOXTANIN B	Potassium metabisulfite, ascorbic acid, and gallic tannin.	It protects against oxidation, selectively displaces oxygen, and has antioxidative activity. Prevents browning and aromatic loss from the first moments of production.
REDOXTANIN T	Potassium metabisulfite, ascorbic acid, and condensed and hydrolyzable tannin.	Protection of the polyphenol fraction in the harvest of red varieties. It effectively displaces oxygen, reducing its concentration drastically in the first moments.
Tanicol ONE / L	Chestnut tannin.	The antioxidant effect, is expressed in three ways: oxygen consumption, antioxidaticantioxidative effect, and precipitation of heavy metals. Positive effectimpact on the color intensity of red wines, due to the co-pigmentation phenomenon. Protection of the natural phenols of the grape. High efficiency in laccase inactivation.
MICROSTAB pH	The liquid formula is based on chitosan of fungal origin and L (+) tartaric acid.	Highly reactive chitosan helps reduce the microbial load of musts, thus reducing the risk of spontaneous fermentation starts and the proliferation of bacteria that can cause lactic acid breakdown. Control of contaminating microorganisms with a significant presence in grapes attacked by *Botrytis cinerea* as *Brettanomyces*.
Enozym Glucan	Enzymatic preparation with ß-1,3-1,6 glucanase activity.	It enables the degradation of ß-glucans produced by *Botrytis cinerea* in grapes, which prevents the settling of musts and the clarification and filtration of wines.
viniferm NS CHANCE	Non-*Saccharomyces* yeast of the species *Lachancea thermotolerans*.	It was selected for its high capacity to synthesize lactic acid in sequential fermentations with *Saccharomyces cerevisiae*. It gives rise to wines with a balanced and integrated acidity from the alcoholic fermentation.
viniferm NS TD	Non-*Saccharomyces* yeast of the species *Torulaspora delbrueckii*.	Yeast was selected for its bioprotective character and ß-lyase activity. It also improves the tactile sensations of wines - through the formation of glycerol and the release of mannoproteins -reduces the alcoholic level of wines and produces significantly lower quantities of acetic acid.

4.2 ALCOHOLIC FERMENTATION STOPS

Fermentation stops consist of the anticipated decrease in the yeast population, leaving a biological niche free in a favorable environment. The presence of residual sugars in high concentration favors the development of latent microorganisms, causing problems of increased volatile acidity, lactic acid spikes, etc.

Slowed fermentations entail the same risks as a stoppage and potentially leading to it. The sooner the corrective treatments are carried out, the lower the organoleptic and analytical repercussions on the wine.

Knowing the causes that cause fermentation stops can prevent them.

Origin of the stop	Preventive work	AGROVIN tools
Temperatures higher than 35 °C	Management and control of AF temperature	Tank Control
Nutrient depletion	Management of nutritional protocols	Actimax Nutrients
Competition between yeasts	Good yeast implantation	Viniferm Yeasts
	Guarantees obtaining biomass	Actimax Regrowth
High probable alcohol content	Use of yeasts with high resistance to ethanol	Viniferm Yeasts
	Increased ethanol resistance of yeasts	Actimax VIT
Oxidation-reduction potentials < -250 mV	Control of cell multiplication	Actimax Nutrients + Electrowine
Presence of AF inhibitors	Apply detoxifying agents	Actimax Corcell

Causes and solutions for different reasons that trigger a fermentation stop.

Deviation control

Solving a fermentation stop begins by controlling the deviations during the fermentation stop and the early reactivation of the activity.

What should we do?

- Carry out as complete an analysis as possible of the wine (pH, ethanol, residual sugars, volatile acidity, free SO_2/total). Keep track of these parameters every day that the wine remains stopped or slowed down.
- Rack to separate the thick lees/skins and press into the reds (they promote lactic acidity and contain microbial inhibitors).
- In the case of nitrogen deficiencies, correct with ammonium salts in doses of 5 to 20 mg/L (according to YAN values).
- Sulfit appropriately, adjusting the free SO_2 concentration above 20 mg/l.
- Control the pH and correct the acidity if necessary. High pH inhibits the antimicrobial effect of sulfur.
- Keep protected from air in the case of white and rosé wines.
- Keep the wine at a moderate temperature, between 18 and 22°C.

Detoxification

In many cases, the slowdown and even fermentation stops are caused by the accumulation of fermentation-inhibiting compounds, such as short and medium-chain fatty acids (C6, C8, C10, C12), fungicides, and phytosanitary residues.

Yeast hulls, such as **Actimax Corcell,** are rich in mannans and glucans that have a specific adsorption capacity, which is why their use reduces the hostility of the medium and favors the reactivation of fermentation.

Fermentation reactivation

In many cases, eliminating lees and the movement of the wine, together with adsorbent and nutritional treatments, solve the problem. If this is not the case, yeast will be sown and acclimated to the environment.

To do this, you must:

— Know the characteristics of still wine (temperature, free sulfur) and solve its deficiencies (YAN, vitamins), as seen in the previous section.

— If it is a stop due to high fermentation temperatures (>35ºC), wait for the wine to cool before sowing.

— Choosing a suitable yeast strain:

- Do not use the strain initially used.
- Do not use strains sensitive to alcoholic environments, low doses of nitrogen, or Killer factors.

— The most suitable strains for resuming fermentation stops belong to the physiological *bayanus* variety .They are genetically more resistant to ethanol and they work well at temperatures below 20ºC.

- VINIFERM START: strain selected exclusively to resolve fermentation stops, very effective due to its rapid acclimatization to the alcoholic environment. Little production of volatile acidity. It does not affect the aromatic profile of the wine.

— Introduce a sufficient number of yeasts (>10^6 cel/ml); This implies high inoculation doses (30-50 g/HL).

— Acclimatize the yeasts to the alcoholic medium.

Refermentation protocols

From Agrovin Group we carry out refermentation protocols adapted to your particular needs, contact your trusted sales representative.

Fermentation reactivation protocol model
100 hl of must at density 1010

Stage	Operation	Density	Tª / Time
Rehydration	**1.** Add 3 Kg of Viniferm Start yeast **2.** Wait 15 minutes **3.** Stir the mixture **4.** Wait 15 minutes	-	35ºC / 25 min
Acclimatization in the middle alcoholic	30 l of acclimatized yeast + 3'75 Kg of sugar + 330 g of tartaric acid (approximate AT to still wine) + 12 l of wine + 16 l of water + 600 g of Actimax Plus = 65 litros de suspensión	1045 1030	20ºC / 2-6 h (depends on yeast strain)
Preparation of inoculum	65 l of acclimatized yeast + 13,38 kg of sugar + 356,3 g of tartaric acid (approximate AT to still wine) + 297 l of wine + 59 l of water + 1400 g of Actimax Plus = 450 liters of inoculum	1030 1024	20-27ºC / 8-24 h (depends on yeast strain)
Addition of yeast	450 liters of inoculum		20-25ºC / Time needed until the end of the AF

AGROVIN SOLUTIONS

Product	Composition	Benefits
Actimax Regrowth	Complete autolysis yeast, biammonium phosphate, thiamine and chitosan of fungal origin.	It provides the specific needs required by the yeast during cell multiplication, ensuring rapid growth of the yeast population with high fermentative capacities. The fungal chitosan limits the development of contaminating populations.
Actimax Corcell	Yeast cell walls coming from a strain selected from the species *Saccharomyces cerevisiae*.	Adsorbs the main inhibitors of alcoholic and malolactic fermentation: short and medium-chain fatty acids (C6, C8, C10, C12), fungicide, and phytosanitary residues.
Actimax VIT	Inactive yeasts *(Saccharomyces cerevisiae).* Specific strain selected, grown in nutrient-rich medium and inactivated by heat.	Source of primary amino acids - slow assimilation - corrects nitrogen deficiencies in the must. Balanced supply of vitamins and minerals, yeast metabolic cofactors.
Actimax Plus	Inactive yeasts, biammonium phosphate, and thiamine.	The source of primary amino acids—slow assimilation—corrects nitrogen deficiencies in the must. The balanced contribution of vitamins and minerals and metabolic cofactors of yeast.
viniferm Start	*Saccharomyces cerevisiae* var. *bayanus*.	Treatment of all types of wine with stoppage of alcoholic fermentation. Strain very resistant to ethanol. Tolerance > 17% vol.

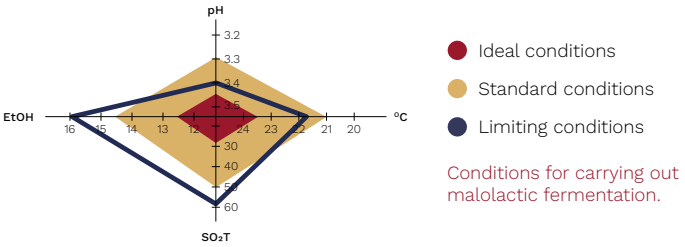
4.3 CONTROL OF MALOLACTIC FERMENTATION

Spontaneous malolactic fermentation, driven by unselected bacteria, can increase volatile acidity and biogenic amine content, and produce excessive amounts of diacetyl. Some biogenic amines (putrescine and cadaverine), at high levels, can provide a retronasal sensation reminiscent of decomposing meat; however, even moderate values of these compounds will have an effect on and produce excessive amounts of diacetyl. Some biogenic amines (putrescine and cadaverine), at high levels, can provide a retronasal sensation reminiscent of decomposing meat; however, even moderate values of these compounds will affect the fruity perception of the wines, reducing their intensity.

Furthermore, some consumers may be susceptible to their presence and relate them to unfavorable hygienic conditions, so it is essential to limit their content.

Completion of the FML with guarantees

Many factors, such as temperature, pH, free sulfur, and alcoholic strength, condition the correct development of malolactic fermentation.



Conditions for carrying out malolactic fermentation.

Other parameters to take into account are the redox potential, low levels decrease the viability of the bacteria due to the low availability of oxygen, and the availability of nutrients: the primary substrate of lactic acid bacteria is malic acid. However, they also need a series of compounds such as amino acids and trace elements to be able to develop fermentation optimally. To do this, nutrients like **Actimax Oeni** provide the amino acids and minerals essential for its development.

Working with directed malolactic fermentations allows us to use strains that do not produce biogenic amines, without increased volatile acidity and minimal diacetyl production.

Viniferm OE is a range of lactic acid bacteria selected with the aim of producing stable, healthy wines (with zero histamine) with a bioprotective character, thus avoiding possible microbiological deviations.

Viniferm OE bacteria are sold in liquid format and adapted to wine conditions, so they are inoculated directly. Thanks to this, the bacteria will present rapid implantation, giving rise to rapid and safe malolactic fermentations. In them, the malate dehydrogenase enzyme is active from the first moment, allowing its application in co-inoculation, avoiding consuming sugars, and minimizing the production of volatile acidity.

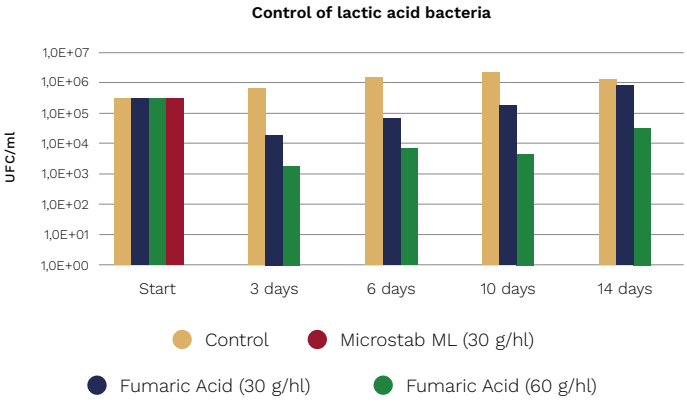
Despite adapting to limiting conditions of alcoholic strength (up to 16%), pH (up to 3.3), IPT, and total SO_2 (up to 60 ppm), the sum of these limitations will represent a difficulty in carrying out the FML, and it is recommended, in those cases, a specialized protocol.

Inhibition of MLF

In many cases, malolactic fermentation is not desired, in the vast majority of white wines, in sparkling wines, in some young red wines, when there is a fermentation stoppage, etc.

Traditionally, MLF has been inhibited with the application of sulfur. However, the developing demand for wines with a lower concentration of sulfur, added to the fact that inhibition with sulfur is not always practical, has led to the development of other alternatives, such as fumaric acid. and chitosan.

Microstab Protect and **Microstab ML** are chitosan-based tools that acts by electrostatic interaction with the cell surface of bacteria, reducing its permeability and blocking the exchange of compounds with the medium.



Comparison of the population of lactic acid bacteria with the application of pure Fumaric Acid and the Microstab ML formulation.

Fumaric acid is an acidic compound recently authorized by the OIV as an oenological product and approved by the European Union in Regulation (EU) 2022/68. Its oenological interest is based on the fact that it is a potent inhibitor of malolactic fermentation, thanks to its bacteriostatic and bactericidal capacity.

Without being an allergenic compound, it is an alternative tool to using lysozyme for those preparations in which it is necessary to work with low doses of sulfur and control the development of lactic acid bacteria. The maximum permitted working dose is 60 g/hl, sufficient to control MLF. Furthermore, the synergistic formulation of fumaric acid and chitosan, such as **Microstab ML,** allows greater effectiveness in controlling lactic bacteria populations, achieving a prolonged reduction of them.

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Product	Composition	Benefits
viniferm OE AG 20	Liquid cultures of lactic acid bacteria *(Oenococcus oeni)* ready for use.	High prevalence -bioprotective character- high tolerance to ethanol, enhances the varietal aromatic characteristics, maintains the fruity expression, absence of lactic aromas, does not produce biogenic amines, accentuates the sensations of body and volume in the mouth.
viniferm OE 104	Liquid cultures of lactic acid bacteria *(Oenococcus oeni)* ready for use.	It is especially indicated for the production of red wines with long macerations and/or high total polyphenol content. It produces excellent results in malolactic fermentation in barrels.
Actimax OENI	Inactive yeast *(Saccharomyces cerevisiae)* and hydrochloride thiamine.	The contribution of primary amino acids is the only source of nitrogen assimilated by lactic acid bacteria. Contribution of minerals such as bioavailable Mg and Mn and group B vitamins. Adsorption of inhibitory compounds and contribution of polysaccharides favoring fermentation kinetics.
MICRSTAB PROTECT	Chitosan of fungal origin, inactive yeast, and gallic tannin.	It effectively reduces lactic acid bacteria populations, helping to control malolactic fermentation. Natural antioxidant effect protects the aromatic fraction and limits the browning of wines.
MICRSTAB ML	Fumaric acid and chitosan.	It combines the bacteriostatic and bactericidal properties of fumaric acid, which acts by permeabilizing the membrane of lactic acid bacteria, with the antimicrobial effect of chitosan, which alters the cellular structure.

Experience and innovation
Lactic acid bacteria producers

After 14 years, Grupo Agrovin has consolidated itself in the market as a producer and marketer of liquid lactic bacteria *Oenococcus Oeni* for direct inoculation, marketed under the brand **Viniferm OE,** the result of close collaboration in consecutive research projects with the Department of Microbiology of the University From Valencia.

The biomass production of the **Viniferm OE** range follows rigorous quality control throughout the process. Genetic control and identification are carried out on each of the production batches using the RAPD-PCR molecular technique, and viable cells are counted, which ensures the effectiveness and homogeneity of each of the containers.

Advantages of liquid cultures

— Fast and safe fermentations.

— Maximum cell viability.

— Quick implementation.

— Crops pre-adapted to complex wines

— Malate dehydrogenase enzyme active from the beginning:

- Limit the consumption of sugars.
- Low production of volatile acidity.

4.4 SPECIAL SITUATIONS: *Brettanomyces*

The presence of *Brettanomyces bruxellensis* in wine gives rise to the formation of volatile phenols described as animal, leather, or horse aromas and to the increase in volatile acidity in addition to synthesizing tetrahydropyridines, esterases, and fatty acids, which give aromas of rancidity, solvent or smoked, among others. All this means the degradation of the fruity aromas, considerably reducing the quality of the wine.

Control of *Brettanomyces bruxellensis* is complicated since it is a yeast that resists ethanol and low doses of sulfur. Although it grows slowly, the long breeding periods allow it to develop until reaching sufficient populations. Grapes with poor sanitary conditions have a high risk of presenting a contaminating load of these yeasts. However, its presence is usually closely associated with quality red wines.

This is because the synthesis of volatile phenols occurs from hydroxycinnamic acids, which are more abundant in these wines. Furthermore, the wooden barrels and vats where these wines are usually made and aged are natural reservoirs for *Brettanomyces* because their porosity prevents effective cleaning and disinfection.

Guidelines are provided to detect yeast *Brettanomyces bruxellensis* early

Preventing this microbiological contamination and ensuring the quality of the wine involves the early detection of the yeast *Brettanomyces bruxellensis*.

For its quantification, the **Agrovin Laboratory** works with the specific DBDM medium that allows colonies to be visualized after 5 days. Its specificity prevents the growth of other yeasts and bacteria, having other characterizations on *Brettanomyces bruxellensis* populations, such as color change and the formation of phenolic aromas.

The development of *Brettanomyces bruxellensis,* as we have seen, is simple in the conditions of the wines, so it is essential to work preventively, preventing populations from exceeding 10^3 CFU/ml, at this level of contamination, the production of phenols is minimal, and acting quickly to reduce them is essential. At these levels, a simple pumping over with a medium filtration and subsequent sulfite treatment will eliminate them.

In addition, it is necessary to carry out controls in the winery at four levels:

1. The health of the grapes, which, if deficient, can represent an increase in the Brett population.

2. The presence of free sulfur at antimicrobial levels.

3. The cleaning and disinfection of the barrels is a critical point of microbiological contamination.

4. The reduction of the deprotection windows between alcoholic and malolactic fermentation with yeast-bacteria co-inoculation strategies.

In this way, the greatest possible guarantees are to avoid contamination by *Brettanomyces bruxellensis*.

Consult our laboratory catalog

Scan the QR code to access the list of Agrovin Laboratory's oenological services and analyses.

ENAC
ENSAYO
ISO 17025
Nº1077 / LE2092

Official Control Laboratory
NºCO/CR/004

How to eliminate *Brettanomyces bruxellensis* yeast?

When risk populations are reached, and the production of phenols is already evident, exceeding perception thresholds (440 ÿg/l of 4-ethyl phenol and 620 for the sum of 4-ethyl phenol and 4 ethyl guaiacol), specific deodorization.

Working from when the grapes enter the winery will help us keep populations at low levels. The health quality of the grapes will be a starting point in the control of Brettanomyces.

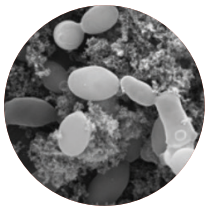


Image of *Brettanomyces* cells trapped in chains of chitosan molecules: Bijlana Petrova/WSU

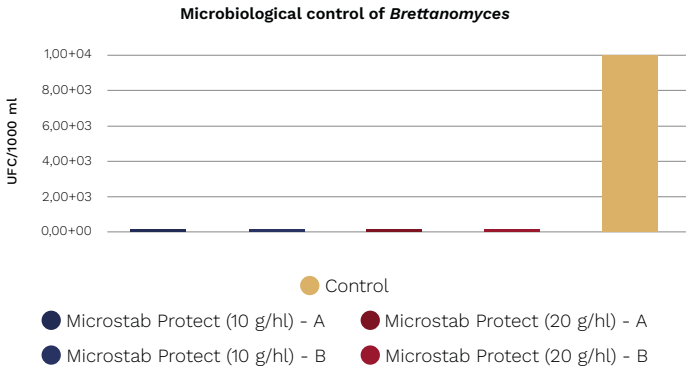
The application of **Microstab pH** at the time of vatting, as well as the use of rapidly implanting yeasts, will reduce the risk of its development in the initial moments of the alcoholic fermentation.

On the other hand, the effectiveness of molecular sulfur against *Brettanomyces* will require working properlycorrectly with the pH and aiming to maintain the adequate content of free sulfur, reducing the compounds that produce their combination.

Curatively with high populations, it will be important to limit the batch and opt for curative treatments with **Microstab Protect,** a solution with proven effectiveness on this microorganism, in addition to performing a , with high populations, it will be important to limit the batch and opt for curative treatments with **Microstab Protect,** a solution with proven effectiveness against this microorganism, in addition to performing filtration below one micron.

Microstab Protect in a microbiologically contaminated wine will allow the existing population to be reduced, adjusting the dose according to the starting population.

In the same way, **Microstab Protect** is a prevention tool. After filling the barrels, processing the wines at preventive doses will reduce risks and prevent their contamination. Furthermore, the presence of inactive yeasts rich in glutathione will perform a synergistic protective action with SO_2, maintaining free.



Microbiological control of the Brettanomyces population in a barrel-aged wine with a starting population of 10^4 CFU *Brettanomyces*/ 20 ml. Analysis after 4 months post-treatment with Microstab Protect remaining in barrel (Group A) and racking after 10 days (Group B). All samples had free SO_2 values < 10 ppm and were evaluated in

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Product	Composition	Benefits
MICROSTAB PROTECT	Quitosano de origen fúngico, levadura inactiva y tanino gálico.	It substantially reduces *Brettanomyces* populations, reducing the risk of alterations due to the presence of this yeast. Natural antioxidant effect protects the aromatic fraction and limits the browning of wines.

ANNEX I

Vegan flotation

Dynamic settling allows for managing significant volumes of must and obtaining a turbidity that can be adjusted to the fermentation's needs. Adapting the preparations to vegan markets introduces the use of flocculants of a different nature to gelatins of animal origin, which requires a change in the work protocols to obtain good results in the process.

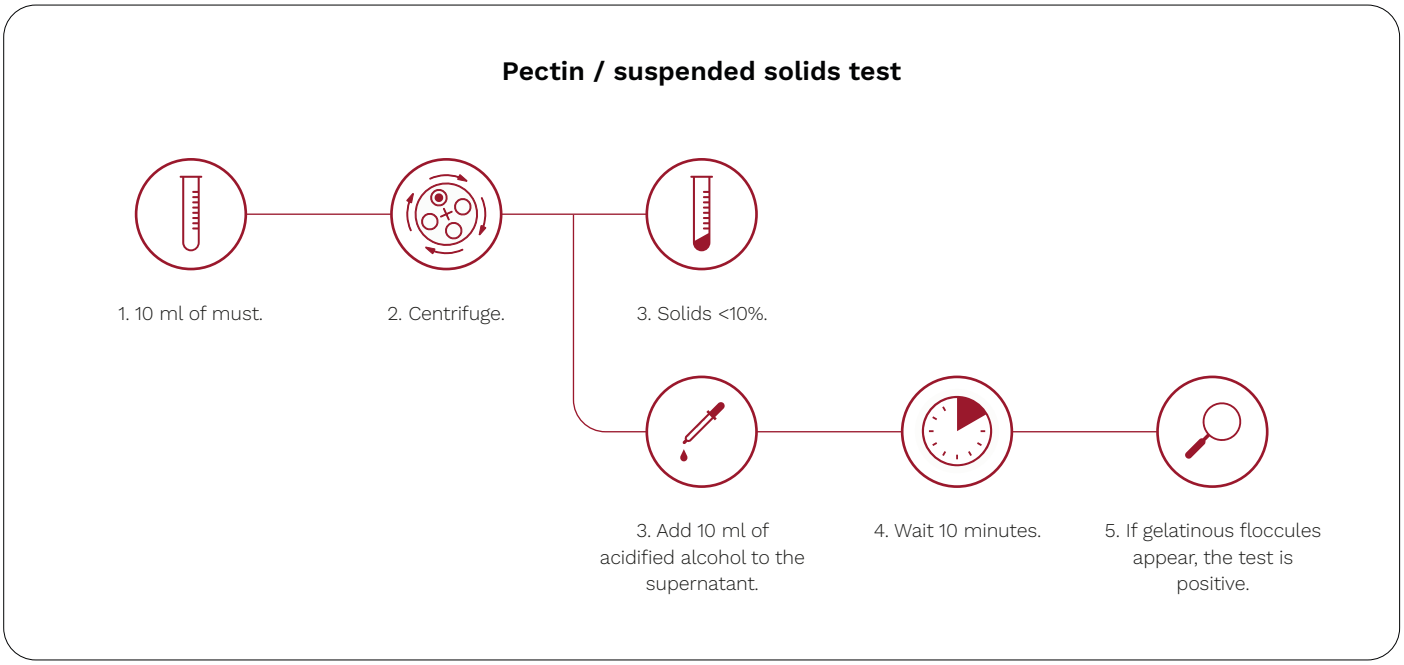
Initial conditions for the flotation process

— It is important to avoid the onset of AF and the consequent release of CO_2, which will stir the must and prevent the formation of the cap.

- Application of SO_2 at a dose of 3-5 g/hl.
Advantages: antimicrobial, antioxidant, and antioxidative action.
- Apply chitosan with **Microstab pH** at doses of 75-150 ml/hl.
Advantages: synergy with the adjuvant in floc formation and acidification of the medium.

— Correct depectinization, the application of specific pectolytic enzymes for dynamic settling systems, such as **Enovin FL,** accelerate the depectinization process

— Moderate suspended solids. Values above 10% make sludge settling by flotation impossible.



Conditions for flotation with vegetable proteins

— Temperature control 15-17 °C. Lower temperatures will increase the viscosity of the must and higher temperatures may favor the onset of fermentation.

— Working pressure (6-7 bar).

— High gas flow rate setting (>20 l/min).

— The recirculation time depends on the float flow rate; it should correspond to the passage of 1.5 times the tank volume.

— The formation and compaction of the flotation cap with vegetable proteins is slightly superior to treatments with animal gelatin.

Work options

Proveget *PREMIUM*

Highly activated pea protein.

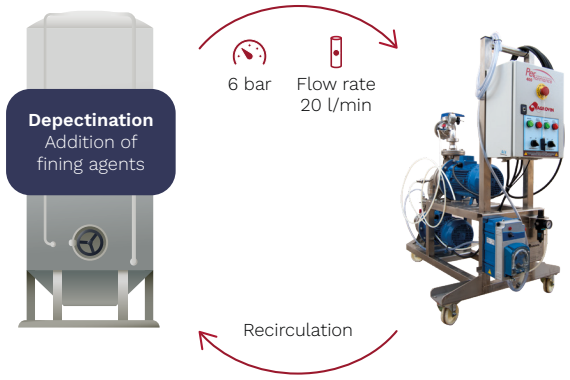
Vegan product Ecological product

Proveget *FLOT*

Pea protein combined with chitin-glucan.

Vegan product

Flotation process with Performance



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Producto	Descripción	Beneficios
MICROSTAB pH	The liquid formula is based on chitosan of fungal origin and L-tartaric acid.	Highly reactive chitosan reduces the risk of spontaneous fermentation starts and helps flocculate prior to the must flotation process.
Enovin FL	Liquid enzyme preparation with pectinase activity, polygalacturo-nase, and pectinate.	A higher proportion of pectinlyasepectin lyase (PL) activity compared tothan polygalacturonase activity allows viscosity to be rapidly reducedreduces viscosity rapidly.
Proveget *FLOT*	Liquid formulated with vegetable protein and chitin-glucan of fungal origin.	The electrostatic characteristics of the plant protein, along with those of chitin-glucan, a highly charged polysaccharide, favor the formation of flocs.
Proveget *PREMIUM*	Pea protein in liquid solution.	Protein with high reactivity accelerates the removal of particles, including oxidized and potentially oxidizable elements. In addition, more excellent compaction of the flocs occurs, significantly improving flotation performance.
Maxibent *FL*	Sodium-activated bentonite powder.	It increases the speed of separation of the bubbles from the wort, thus improving performance during the flotation process.
Silisol	Colloidal solution of 30% silica particles.	It allows for improving the compaction of the lees and flotation performance.

Notes

Calculations and conversion charts

Temperature conversions

°F	0	32	40	50	60	70	80	90
°C	-18	0	4	10	16	21	27	32
	°F = (°C x 9/5) + 32							

Dosage

g/hl	5	10	15	20	100
lbs/10³ gal	0.4	0.8	1.2	1.6	8
ppm	50	100	150	200	1000

Equivalent Units

1 gal = 3,785 L	**1 L = 0,264 gal**	1 lbs = 453,6 g
1 ml = 0,035 fl oz	1 fl oz = 30 ml	1 ppm = 1 mg/l
1 gal = 0,379 hl	1 hL = 26,4 gal	1° Brix = 1% sugar (wt/vol)
1 metric ton =2205 lb	1 metric ton = 1000kg	1 Vol % = 1 ml/ 100 ml
1 US ton = 2000 lb	1 US ton = 907 kg	1 barrel = 225 L = 59,4 gal = 25 cases

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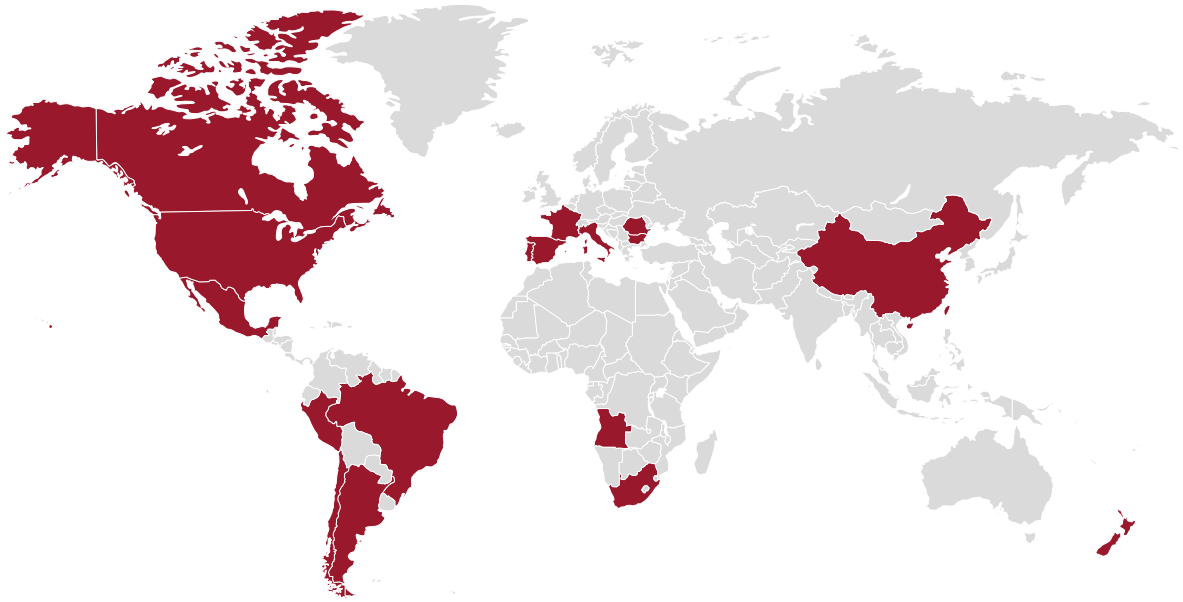

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