TECHNICAL BOOKLETS

VINIFICATION OF HARVESTS AFFECTED BY *BOTRYTIS CINEREA*

LAFFORT

l'œnologie par nature

Vinification of affected harvests: Practical application

*Botrytis cinerea i*s a mycelium fungus commonly called grey mould. It develops on the surface of the grapes and forms small grey-white filaments.

This fungus produces extracellular enzymes, notably **laccase**, which is responsible for the oxidation of numerous phenolic compounds in the contaminated grapes and for the degradation of certain aromas. During white wine vinification, laccase degrades white grape phenolic compounds into quinones (an oxidation process), then into brown pigments which are characteristic of rotten grapes. Humidity, lack of aeration and temperature are factors which have a high influence on the dispersal of the fungus.

Botrytis also produces **glucan**, a polysaccharide subsequently responsible for clarification and filtration problems in wine. This glucan can be degraded by using ß-1-3 and 1-6 glucanases derived from *Trichoderma harzianum*.

Œnological consequences of contamination by the fungus *Botrytis cinerea*

- Risk of « mouldy » or « compost » type aromatic deviations.
- Aroma alteration.
- Rapid must oxidation by laccase.
- Fermentation (yeast) problems due to *Botrytis* toxins.
- Alteration of the must colour.

Level of laccase activity

The level of contamination can be evaluated by measuring **laccase activity**. Several manual or automatic tests exist on the market. This evaluation must be carried out before sulphiting. A healthy harvest with no oxidative risk will have a laccase level of < 1 laccase unit /mL, whilst an affected harvest will have a level of over 2 laccase units /mL.

Laccase is an enzyme with a high resistance to SO_2. With an affected harvest, the initial strategy consists of depriving the enzyme of oxygen until it becomes deactivated. Put simply, no oxygen = no oxidation. This is the role of SO_2 which, in its free form, is more reactive towards oxygen than laccase. The oxygen is ideally then rapidly consumed by the initiation of fermentation. Thus, yeasts with a fast fermentation start and a short latency phase must be used. The yeasts both consume the dissolved oxygen and release CO_2, which drives oxygen from the must.

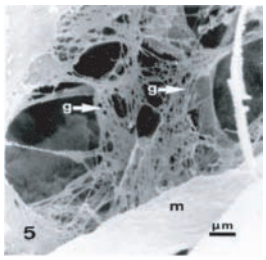
NB. Heating the must (pasteurisation) can be justified for altered harvest vinification, although negative impacts on the organoleptic characteristics of the finished wine may be an undesired side-effect of this process. Laccase, like most enzymes, is destroyed by a 10 second thermal treatment at 80°C. The pectinase activities of the grape are also destroyed in this process, and the use of commercial enzyme preparations after must pasteurisation enables the enzymes destroyed by the heat to be replaced, facilitating juice clarification.

Importance of tannin addition

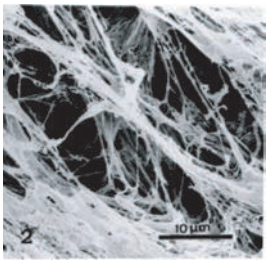
Tannins are extracted to a greater extent by alcohol, hence during pre-fermentation cold soaking minimal native tannin will be present in the must. To correct this situation it is recommended to add œnological tannins during cold maceration to ameliorate several factors, such as a tannin deficiency of the grapes, to permit improved protection of the colouring matter and to reinforce the structure of the finished wine. The early addition of tannins also has a strong, permanent inhibitory effect on laccase. Tannin addition, when used in combination with SO_2 and the exclusion of oxygen from the must, is thus the most effective way to inhibit laccase activity whilst minimising quality loss.

Degradation of *Botrytis cinerea* glucan

The glucan in *Botrytis* acts as a wine-clogging colloid. It increases the juice's viscosity and turbidity, and disrupts both the clarification and filtration of wines, which in turn lowers the quality.



Frozen *Botrytis* glucan



Grapes infected by Botrytis
Source: Dubourdieu, 1978.

Structure du glucane de *Botrytis cinerea*

Alcohol test

It is possible to carry out a simple measurement of glucan presence from *Botrytis* in the wine cellar (detected from 15 mg/L glucan):
Pour 5 mL of wine into a graduated cylinder. Carefully add 2.5 mL of alcohol (96%) down the sides of the cylinder. Shake slowly then leave to stand for 30 minutes at room temperature. If a haze forms and whitish filaments appear on the surface of the wine, this indicates the presence of glucans in the wine.

If this test is positive, the wine contains enough glucan to disrupt clarification and filtration. In the case of a severely infected harvest it is possible to see long, whitish, tangled filaments on the surface of the tank. These are *Botrytis* glucans.

ß-1,3 and 1,6-glucanases are enzymes extracted from the fungus *Trichoderma harzianum*. They selectively degrade the ?-(1,3:1,6)-glucans and polysaccharides produced extensively by the *Botrytis cinerea* fungus. It is recommended to add the ß-glucanase at mid-fermentation, under the cap in order to reduce clarification and filtration problems generated by the *Botrytis* glucan. Moreover, these enzymes (**Extralyse®, Filtrozym®**) will permit some of the yeast-derived polysaccharides to be degraded, which will increase the wine's roundness.

Yeasting management

Fermentation is a guarantee against oxidation. Aside from the chemically reductive environment that active fermentation represents, the production of CO_2, a gas that is quite dense, physically drives oxygen from the juice or must. Fermentation should be initiated as early as possible with an ADY (active dry yeast) inoculation and a nutritive complement to minimise all fermentation risks. The chosen yeast strain must have a short latency phase and strong fermentation kinetics. It is recommended to add yeast with homogenization anaerobically.

Why short macerations?

With infected grapes, maceration duration must be reduced by 20 to 50%. The extraction of phenolic compounds can be accelerated in reds by using extraction enzymes which are added after the start of fermentation (after the first gas releases) in order to ensure that the extracted compounds are protected from oxygen.

Mechanical actions

Mechanical actions (pumping-over, cap punching) must be limited as much as possible to avoid both laccase extraction from the grape skin and the introduction of oxygen during the pre-fermentation phase. All possible measures must be taken to avoid the risks of oxidation during pumping-over. Racking with aeration after alcoholic fermentation (when the laccase activity has disappeared) will prevent the development of off-flavours.

Technical itinerary: red wine vinification of partially infected harvest

1- Grape sorting, if possible.

2- Moderate de-stemming and crushing.

3- Add 8-10 g/hL of SO_2 depending on the laccase activity level (Botrytest or air resistance test) and the pH at the time of tank filling (previously purged with CO_2).

4- Add 30-70 g/hL of **TANIN VR SUPRA** to the must depending on the sanitary state of the harvest. Keep the pre-fermentation phase as short as possible and exclude air.

5- Rehydrate the ADY at 20 g/hL with **SUPERSTART®** at 30 g/hL to ensure a strong fermentation finish. Compensate for nitrogen deficiency if necessary by adding **THIAZOTE** (½ at yeasting and ½ after 1/3 sugar depletion). Recommended yeasts: **ZYMAFLORE F15**, **RX60** or **ACTIFLORE F33**.

6- As soon as fermentation has started, accelerate extraction by using an extraction enzyme: **LAFASE HE GRAND CRU**, **LAFASE FRUIT** or **LAFASE HE** (according to the product objective) at 3 g per 100 kg of harvest.

7- Limit pumping-over and cap punching (mechanical activity). Aerate as much as possible. Tank transfers are not recommended at this stage.

8- At mid-fermentation, add under the cap **EXTRALYSE®** preparation containing the ß-glucanases required for degrading the *Botrytis* glucan. A dosage of 3-5 g/hL is recommended according to the tannin content and infection level.

9- Short vatting time (6-8 days).

10- Carry out running-off anaerobically in a tank purged with CO_2. Maintain anaerobic conditions until all laccase activity has disappeared. Press wines have a higher laccase and blocking colloid content. Enzyme addition with a pectinase of the type **LAFASE 60** at 2 g/hL is recommended for these wines, or a mixed preparation of pectinase/ß-glucanase such as **FILTROZYM®** at 5 g/hL.

For further information, contact LAFFORT (telephone: + 33 05 56 86 53 04, fax: + 33 05 56 86 30 50) or send an email to info@laffort.com info@laffort.com

Technical itinerary: white wine vinification of a partially infected harvest

1- Grape sorting, if possible.

2- Moderate de-stemming and crushing.

3- Add 8-10 g/hL of SO_2 according to the level of laccase activity (Botrytest or air resistance test) and the pH.

4- Add 5-10 g/hL of **TANIN GALALCOOL** at press loading, to reinforce SO_2 efficiency.

5- Press according to the state of the grapes: ideally low pressure pressing to limiting mechanical degradation.

6- Must settling: Load the must anaerobically into the tanks purged with CO_2, and keep the pre-fermentationo phase as short as possible. Add **LAFAZYM CL®** clarification enzyme. It is possible to add 1-3 g/hL of **EXTRALYSE®** ß-glucanase to help clarification. We recommend treating coloured must with **POLYLACT** (a PVPP-casein mixture) at 15-20 g/hL and **MICROCOL ALPHA** bentonite at 30-50 g/hL.

7- Yeasting at 20 g/hL with **SUPERSTART®** at 30 g/hL. Compensate for nitrogen deficiency if necessary by adding **THIAZOTE®** (½ at yeasting and ½ after 1/3 sugar depletion).
Recommended yeasts: **ZYMAFLORE X5, X16, VL1 and ACTIFLORE BO213.**
Turbidity can be adjusted by adding **GRANUCEL®** at 30-60 g/hL

8- Fermentation temperature must be < 20°C.

9- Carry out running-off anaerobically in a tank purged with CO_2. Maintain anaerobic conditions until all laccase activity has disappeared

10- Maturation can be significantly enhanced by using the enzyme preparation **EXTRALYSE®** (3-5 g/hL) which, as soon as fermentation finishes, will allow the *Botrytis* glucan to be degraded, liberating the yeasts' polysaccharides which release sapid molecules and roundness.

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