RESOLUTION OIV-OENO 686-2022

REVIEW OF THE CODE OF GOOD VITIVINICULTURAL PRACTICES IN ORDER TO AVOID OR LIMIT CONTAMINATION BY BRETTANOMYCES

IMPORTANT: This draft resolution supersedes the following resolution:
- OIV-OENO 462-2014

THE GENERAL ASSEMBLY,

IN VIEW OF Article 2, paragraph 2 iv of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine,

CONSIDERING the work of the "Microbiology" Expert Group,

CONSIDERING that this code determines the measures to be set up in vineyards and in wineries in order to contribute to reducing the risks linked to the presence of *Brettanomyces*,

DECIDES to replace Resolution OIV-OENO 462-2014, 'Code of Good Vitivinicultural Practices in order to Avoid or Limit Contamination by *Brettanomyces'*, by the following text:

CODE OF GOOD VITIVINICULTURAL PRACTICES IN ORDER TO AVOID OR LIMIT SPOILAGE OF WINES BY *BRETTANOMYCES BRUXELLENSIS*

1. PREAMBLE

- Among processes that deteriorate wine quality, the production of volatile phenols by Brettanomyces bruxellensis is widespread and increasingly problematic. These compounds are characterised in particular by aromas of ink or glue, and horse sweat, leather or stable taints, which can mask wines' fruity characteristics.
- Volatile phenols, mainly 4-ethylphenol and 4-ethylguaiacol, are produced from p-coumaric acid and ferulic acid respectively after enzymatic decarboxylation (cinnamate decarboxylase, PAD) and reduction (vinylphenol reductase, VPR). These precursors are naturally present in grape musts. The decarboxylation step caused by cinnamate decarboxylase activity has been described for many

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bacteria, yeast and fungi species, while the reduction step caused by vinylphenol reductase activity, or VPR, is more specific to the Brettanomyces species.

- Brettanomyces are present on grapes and on winemaking equipment, meaning that these yeasts may proliferate in wines during or after alcoholic and/or malolactic fermentations, during wine maturing or after packaging.

2. INTERVENTIONS IN THE VINEYARD

Not applicable (to our knowledge, no study available). However, *Brettanomyces* yeasts have been detected on grape skins after enrichment steps, from the first stages of berry development. The microbial ecology of grape surfaces has shown great diversity, with a small population for each species.

An initial preventive approach, consisting of rigorous selection of healthy grapes, can play a role in reducing the risk of *Brettanomyces*, which is generally found in greater numbers on spoilt grapes.

3. INTERVENTIONS DURING THE GRAPE HARVEST

Grape management:

Brettanomyces is present on grapes (small population) but is not the major species of yeast. However, the removal of damaged or botrytised grapes could limit *Brettanomyces* spoilage.

The harvest of overripe berries is more and more common, and in this case particular precautions could be considered. These interesting impacts at the organoleptic level may increase the risk of volatile-phenol production because overripe grapes contain more volatile-phenol precursors. Working in these conditions does not necessarily increase the presence of *Brettanomyces*, but increases the risk of activity (a lower total acidity and higher pH directly impacts on molecular SO_2 levels and consequently on the growth of *Brettanomyces*).

4. INTERVENTIONS IN THE CELLAR

Due to multiple factors, including the increase in alcohol content, a reduction in microbial diversity is observed during alcoholic fermentation. However, as *Brettanomyces* has good resistance to ethanol and its presence does not decrease. Therefore, perfect hygiene is essential during winemaking (sound grapes, winemaking

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and storage equipment, etc.).

Pre-fermentative operations and treatments

- Ensuring that suitable hygiene practices are applied in the cellar is recommended.
- The most important factors are sulphiting and temperature:
    - sulphiting is the most effective preventative action at the pre-fermentative stage for limiting the development of Brettanomyces populations, however it is recommended that excessive sulphiting (>8 g/hL), which could delay malolactic fermentation, is avoided,
    - high-temperature, pre-fermentative maceration (above 65 °C) results in the inactivation of Brettanomyces, but also of other microorganisms in winemaking, and cold maceration at a temperature lower than or around 10 °C prevents their proliferation, but does not kill them.
- The use of some enzymes containing cinnamoyl esterase activities may increase the risk of volatile phenol production. In all circumstances, subsequent contamination is possible.

Fermentation operations

Alcoholic fermentation (AF):

- During AF, microbial diversity decreases and *Saccharomyces cerevisiae* becomes the main species. However, due to its ethanol resistance and lower nutrient demand, *Brettanomyces* can grow as AF slows down or stops. The oenological practices commonly recommended for management of alcoholic fermentation should be implemented.
- Inoculation of musts with selected yeasts helps to achieve a more reliable AF.
- The environment becomes more favourable to the multiplication of *Brettanomyces* if AF slows down or stops. In the case of the latter, using a process to restart AF as soon as possible is recommended.
- Residual sugars (mainly glucose and fructose) are substrates for *Brettanomyces* growth. Wines are generally considered as dry when the glucose + fructose level is below 4 g/L. A concentration of 0.3 g/L of glucose + fructose is sufficient for the development of *Brettanomyces* biomass capable of producing over 1000 µg/L of

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volatile phenols.

- Yeast nutrients (that may also benefit *Brettanomyces*) should only be added if really necessary to avoid stuck fermentations.

Lag period before malolactic fermentation (MLF):

- Once AF is completed, conditions favour not only lactic bacteria but also Brettanomyces, although proliferation remains slow.
- It is important to monitor the Brettanomyces population since the environment is relatively low in microorganisms.
- Factors favourable to the growth of Brettanomyces during this phase are as follows: final high-temperature macerations (40-45 °C), micro-oxygenation, the release of sugars in the case of uncrushed harvests, and partially raisined harvests.
- Co-inoculation of selected yeasts and lactic bacteria may help to reduce the lag phase between AF and MLF, and consequently the development of Brettanomyces.

Malolactic fermentation (MLF):

- Physicochemical parameters (pH, temperature, total SO_2) affect the progress of MLF. If MLF is delayed, the risk of production of volatile phenols increases because *Brettanomyces* can take advantage of this time to multiply.
- The use of malolactic starters is a good way to limit *Brettanomyces* development.
- After malolactic fermentation, it is recommended to eliminate all microorganisms,
- particularly by adding SO_2. These quantities must be adjusted according to the pH of the wine. The use of physical techniques (HHP or UHPH) is also possible.

Maturation and clarification operations

The first necessary precaution is to carry out regular tasting and a complete microbiological analysis, which includes specific counting of *Brettanomyces* and/or analysis of volatile phenols. This analysis must be repeated throughout the maturation period.

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- SO2 management is crucial to limit the development of Brettanomyces. The recommended concentration is about 0.5 to 0.8 mg/L of molecular SO2[1]. In case of Brettanomyces strains tolerant/resistant to ethanol or SO2, alternative methods are recommended (chitosan, filtration or heat treatment).
- Ageing on lees is an additional risk factor because Brettanomyces are quite able to survive and to proliferate in lees (which release nutrients into wine).
- Clarification by racking, fining and filtration are essential to reduce the viable and viable but non-culturable Brettanomyces populations that can multiply by metabolising residual sugars.
- Some fining agents are more efficient than others. Treatments with fining proteins can reduce populations by a factor of 40-2000. Fining using casein or potassium caseinate may reduce the ethylphenol levels if these are not too high.
- The addition of chitosan is one alternative for controlling the growth of undesirable microorganisms, particularly Brettanomyces and when dealing tolerant/resistant strains to ethanol or SO2.
- Some winemaking operations (racking, topping up, filtration, bottling, etc.) may result in oxygen dissolution in wine, favouring the multiplication of Brettanomyces.
- If micro-oxygenation is practised, the absence of Brettanomyces should be checked using appropriate analyses.
- During wine aging, the cellar temperature should be carefully controlled especially during the summer period, and long periods at over 14 °C should be avoided to prevent Brettanomyces growth.

NB:

1. Upon addition of SO2, the Brettanomyces population can switch (fully or partially) from a viable state to a viable but non-culturable (VBNC) state. These changes lead to a reduction in the size of the yeasts, so it is necessary to adapt filtration.
2. It is also important to note here that enumeration of VBNC microorganisms cannot be performed by routine analysis, for example enumeration on a Petri dish, but rather by qPCR or flow cytometry, which enumerates VBNC and viable forms of Brettanomyces.

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3. A PCR test is available to predict Brettanomyces sulphite tolerance/resistance.

Barrel maturation

Barrel maturation is considered to be the period most sensitive to spoilage by *Brettanomyces*, particularly with new barrels.

During sampling, cross contaminations have to be avoided.

As for any microbial spoilage, the wine used for topping up must not be contaminated.

Wood is favourable to growth of *Brettanomyces*, which is capable of growing pseudohyphally, of colonising wood micropores, and of using cellobiose as a carbon source. Barrels are difficult to clean and disinfect.

Old, poorly-cleaned barrels are known sources of contamination by *Brettanomyces.* However, new barrels also favour yeast multiplication and the production of volatile phenols, since they release more nutrients. Moreover, new barrels are more permeable to O_2, which favours a relatively high redox potential and decreases the (active or molecular) SO_2 concentration – two parameters favourable to *Brettanomyces* growth.

Different approaches have been investigated for the sanitisation of barrels, but none of them have enabled the complete removal of *Brettanomyces* on the internal stave surface or bunghole. Indeed, the natural microporosity of the wood makes its complete disinfection difficult because microorganisms stay alive in the cavities of the deep layers of the wood. A deeply-acting treatment is essential for long-lasting efficiency and results over time.

Nevertheless, some techniques for the disinfection of barrels significantly reduce *Brettanomyces* populations and may be used where permitted by the applicable regulations in question, for example:

- steam treatment: deep disinfection requires a sufficiently long treatment time (cold water rinse, hot water rinse at 70 °C and low pressure steam for 10 min). A treatment by immersion in hot water at 60°C for an exposure time of 19 min allowed to eliminate the yeast populations up to a log count reduction of 8,
- ozone sanitisation: performed either with gaseous ozone combined with a hot water treatment at 82 °C for 20 min or with ozonated water, by reacting with materials with a high organic load the ozone does not deeply penetrate into the wood,

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- SO2 sanitisation: a minimum of 5 g per barrel of gaseous SO2 should be used to disinfect empty and dry barrels, with SO2 being very efficient both on the surface and also in deeply penetrating the first millimetres of the wood,
- barrel shaving and re-heating: this treatment does not disinfect the wood but enables the most contaminated part to be removed, enabling an 80% reduction of volatile phenols compared to a non-treated barrel,
- ultrasound: this technique removes more than 90% of viable Brettanomyces (up to 2-4 mm below the internal stave surface).

Pre-packaging operations

The risk of volatile-phenol production should be evaluated before the packaging operations using analytical checks (both chemical and microbiological). When the risk has been evaluated, suitable operations should be planned in order to prevent post-packaging development of *Brettanomyces*:

- sterilisation by membrane filtration (0.45 to 0.65 µm) or cross-flow filtration, for efficient removal of Brettanomyces yeasts, followed by sterile packaging,
- use of DMDC for non-lasting protection,
- use of antimicrobials with lasting protection (sorbic acid, only if lactic bacteria have been removed completely; management of SO2, taking the pH, acquired alcoholic strength by volume, and temperature into account). An online calculator taking into account different parameters (ph, alcohol and temperature) can be used,
- heat treatment,
- use of physical techniques (HHP or UHPH) is also possible.

Storage conditions

In order to prevent *Brettanomyces* proliferation in bottles during storage (and the production of volatile phenols), keeping bottles below 12 °C is recommended, especially for lightly-filtered wines or wines containing low levels of SO_2.

5. CONCLUSIONS

- Frequent analyses are highly recommended in order to detect any contamination

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by Brettanomyces at an early stage. During sampling, particular attention should be paid to avoid cross-contaminations.

- The maintenance of the best possible hygiene conditions in the cellar is highly recommended.
- Sulphiting and molecular SO2 management are recommended.
- Temperature management is recommended.
- Preventive actions are preferable to curative processes.

The present recommendations are based on current knowledge and are liable to be updated according to ongoing research.
Brettanomyces management should be a global preventative approach throughout the winemaking process.

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[1] The final product should comply with the regulations in force regarding total SO_2 limits.

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