# OENOBIO

# Organic wine production principles

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# INTRODUCTION TO ORGANIC

## BASIC DEFINITION (OIV, 2021)

**Wine is the beverage resulting exclusively from:**

- **the partial or complete AF of fresh grapes;**
- **crushed or not, or of grape must;**
- **actual alcohol content shall not be less than 8.5% vol.**

**Nevertheless, taking into account climate, soil, vine variety, special qualitative factors or traditions specific to certain vineyards, the minimum total alcohol content may be able to be reduced to 7% vol. by legislation particular to the region considered.**

**Complementary definitions relating to sugar content (S = Glucose + Fructose):**

- **Dry:** with a maximum of either 4 g/l sugar or 9 g/l when: ***TA > S - 2**
- **Medium dry:** up to 12 g/l or 18 g/l, when: ***TA > S - 10**
- **Semi-sweet**: up to a maximum of 45 g/l.
- **Sweet:** minimum sugar content of 45 g/l.

**TA – total acidity expressed as g/l tartaric acid.*

# INTRODUCTION TO ORGANIC

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## BASIC DEFINITION

- Organic wines are made from organic grapes (certified) complying with the organic production rules.

- The organic grapes are obtained from vines grown without chemical fertilizers and herbicides with pesticides of chemical origin, but not synthetic. Organic vineyards needs to achieve certification from an inspection organism that guarantee that no chemical fertilizers, synthetic pesticides or herbicides have been used on the vines.

- The organic winemaking rules came into effect on 1 August 2012. The requirements are the same for all EU countries. Since 2012, the term ‘organic wine’ can be used if the whole process from grapes to wine is managed under winemaking regulations (EU No. 203/2012 amending Regulation (EC) No 889/2008).

- The organic wine production is more restrictive than conventional wine production.

- The biodynamic wine production is more restrictive than organic wine production. Basically, biodynamic wine is organic with certain restrictions and adaptations. Biodynamic viticulture use plant extracts, quartz, with specific protocols and in relation to the moon cycles.

# INTRODUCTION TO ORGANIC

## BASIC DEFINITION

- The rules for organic wine production have to do with food additives, processing aids, and authorized techniques allowed during the vinification process (EU No. 203/2012 amending Regulation (EC) No 889/2008).

- They are based on four main points:

    1. ingredients must be derived from raw materials of agricultural origin and certified as organic;

    2. restricted list of additives and processing aids used under well-defined conditions;

    3. restrictions or interdictions on use of certain physical procedures;

    4. restrictions on total $SO_2$ level in final wines;

## PRINCIPLES OF ORGANIC PRODUCTION (EC) No 834/2007

### Art. 4. Overall principles

Organic production shall be based on the following principles:

(a) the appropriate design and management of biological processes based on ecological systems using natural resources which are internal to the system by methods that:

- (i) use living organisms and mechanical production methods;
- (ii) practice land-related crop cultivation and livestock production or practice aquaculture which complies with the principle of sustainable exploitation of fisheries;
- (iii) exclude the use of GMOs and products produced from or by GMOs with the exception of veterinary medicinal products;
- (iv) are based on risk assessment, and the use of precautionary and preventive measures, when appropriate;

## PRINCIPLES OF ORGANIC PRODUCTION (EC) No 834/2007

### Art. 4. Overall principles

Organic production shall be based on the following principles:

(b) the restriction of the use of external inputs. Where external inputs are required or the appropriate management practices and methods referred to in paragraph (a) do not exist, these shall be limited to:

(i) inputs from organic production;
(ii) natural or naturally-derived substances;
(iii) low solubility mineral fertilizers;

## PRINCIPLES OF ORGANIC PRODUCTION (EC) No 834/2007

### Art. 4. Overall principles

Organic production shall be based on the following principles:

(c) the strict limitation of the use of chemically synthesized inputs to exceptional cases these being:

- (i) where the appropriate management practices do not exist; and
- (ii) the external inputs (par. b) are not available on the market; or
- (iii) where the use of external inputs (par. b) contributes to unacceptable environmental impacts;

(d) the adaptation, where necessary, and within the framework of this Regulation, of the rules of organic production taking account of sanitary status, regional differences in climate and local conditions, stages of development and specific husbandry practices.

## PRINCIPLES OF ORGANIC PRODUCTION (EC) No 834/2007

### Art. 6. Specific principles applicable to processing of organic food

In addition to the overall principles set out in Art. 4, the production of processed organic food shall be based on the following specific principles:

(a) the production of organic food from organic agricultural ingredients, except where an ingredient is not available on the market in organic form;

(b) the restriction of the use of food additives, of non organic ingredients with mainly technological and sensory functions and of micronutrients and processing aids, so that they are used to a minimum extent and only in case of essential technological need or for particular nutritional purposes;

(c) the exclusion of substances and processing methods that might be misleading regarding the true nature of the product;

(d) the processing of food with care, preferably with the use of biological, mechanical and physical methods.

## OENOLOGICAL PRACTICES AND RESTRICTIONS (EC) No 203/2012 amending Regulation (EC) No 889/2008

*Article 29d.*

*2.* The use of the following oenological practices, processes and treatments is prohibited:

(a) partial concentration through cooling according to point (c) of Section B.1 of Annex XVa to Regulation (EC) No 1234/2007;

(b) elimination of sulphur dioxide by physical processes according to point 8 of Annex I A to Regulation (EC) No 606/2009;

(c) electrodialysis treatment to ensure the tartaric stabilisation of the wine according to point 36 of Annex I A to Regulation (EC) No 606/2009;

(d) partial dealcoholisation of wine according to point 40 of Annex I A to Regulation (EC) No 606/2009;

(e) treatment with cation exchangers to ensure the tartaric stabilisation of the wine according to point 43 of Annex I A to Regulation (EC) No 606/2009.

## OENOLOGICAL PRACTICES AND RESTRICTIONS (EC) No 203/2012 amending Regulation (EC) No 889/2008

*Article 29d.*

3. The use of the following oenological practices, processes and treatments is permitted under the following conditions:

(a) for heat treatments according to point 2 of Annex I A to Regulation (EC) No 606/2009, the temperature shall not exceed 70 °C;

(b) for centrifuging and filtration with or without an inert filtering agent according to point 3 of Annex I A to Regulation (EC) No 606/2009, the size of the pores shall be not smaller than 0,2 micrometer.

## SPECIFIC RULES FOR THE MAKING OF WINE (EC) No 203/2012 amending Regulation (EC) No 889/2008

### Use of certain products and substances

**Products and substances authorised for use or addition in organic wine**

| Type of treatment | Name of products or substances | Specific conditions, restrictions within the limits and conditions set out in Regulation (EU) No 1308/2013 and Regulation (EU) 2019/934 |
|---|---|---|
| Use for aeration or oxygenation | — Air<br>— Gaseous oxygen | - |
| Centrifuging and filtration | — Perlite<br>— Cellulose<br>— Diatomeceous earth | Use only as an inert filtering agent |
| Create an inert atmosphere | — Nitrogen<br>— Carbon dioxide<br>— Argon | Only for the purpose to handle the product shielded from the air. |

## SPECIFIC RULES FOR THE MAKING OF WINE (EC) No 203/2012 amending Regulation (EC) No 889/2008

### Use of certain products and substances

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| Type of treatment | Name of products or substances | Specific conditions, restrictions within the limits and conditions set out in Regulation (EU) No 1308/2013 and Regulation (EU) 2019/934 |
|---|---|---|
| Antioxidants and antiseptics | — Sulphur dioxide<br>— Potassium bisulphite or potassium metabisulphite<br>— Ascorbic acid | ▪ Red wines, RS<2, ≤100 mg/l $SO_2$<br>▪ White / Rose wines, RS<2, ≤100 mg/l $SO_2$<br>▪ Other wines, a reduction with 30 mg/l $SO_2$ from maximum level in conventional.<br>▪ Ascorbic acid < 250 mg/l. |
| Acidification | — Lactic acid<br>— L-(+)-Tartaric acid<br>— *Calcium sulphate (plastering)** | *Lowering the pH for “vino generoso” or “vino generoso de licor” in accordance with Annex III, point A(2)(b) to Regulation (EC) No 606/2009. |
| Deacidification | — L-(+)-Tartaric acid<br>— Calcium carbonate<br>— Neutral potassium tartrate<br>— Potassium bicarbonate | Homogeneous preparation of tartaric acid and calcium carbonate in equivalent proportions and finely pulverized. |

## SPECIFIC RULES FOR THE MAKING OF WINE (EC) No 203/2012 amending Regulation (EC) No 889/2008

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|---|---|---|
| Alcoholic fermentation | — Yeasts[1]<br>— Fresh lees (recent vinification, undiluted) | Dry or in wine suspension<br>Use in dry wines not exceeding 5% of the volume of product treated |
| Fermentation nutrients / detoxification | — Di-ammonium phosphate<br>— Thiamine hydrochloride<br>— Yeast cell walls<br>— ***Inactivated yeasts, autolysates of yeasts**** | ≤100 g/hl, expressed in salts<br>≤0,6 mg/l<br>≤40 g/hl<br>*They are not on the list, should be included here [Expert Group for Technical Advice on Organic Production, Final Report on Wine, 2015] |
| Malolactic fermentation | — Lactic bacteria | - |

[1]For the individual yeast strains: if available, derived from organic raw material.

## SPECIFIC RULES FOR THE MAKING OF WINE (EC) No 203/2012 amending Regulation (EC) No 889/2008

### Use of certain products and substances

**Products and substances authorised for use or addition in organic wine**

| Type of treatment | Name of products or substances | Specific conditions, restrictions within the limits and conditions set out in Regulation (EU) No 1308/2013 and Regulation (EU) 2019/934 |
|---|---|---|
| Sequestrants and clarifying agents | — Charcoal for oenological use<br>— Edible gelatine[2]<br>— Plant proteins: wheat, peas[2]<br>— Isinglass[2]<br>— Egg white albumin[2]<br>— Tannins[2]<br>— Casein<br>— Potassium caseinate<br>— Silicon dioxide<br>— Bentonite<br>— Pectolytic enzymes<br>— *Potassium alginate** | ≤100 g/hl charcoal (sequestrant)<br><br>**Potato protein** and **yeast protein extracts** should be included here [Expert Group for Technical Advice on Organic Production, Final Report on Wine, 2015]<br><br>*Only for the manufacture of all categories of sparkling and semi-sparkling wines obtained by fermentation in bottle and with the lees separated by disgorging |

[2]Derived from organic raw material if available.

## SPECIFIC RULES FOR THE MAKING OF WINE (EC) No 203/2012 amending Regulation (EC) No 889/2008

### Use of certain products and substances

**Products and substances authorised for use or addition in organic wine**

| Type of treatment | Name of products or substances | Specific conditions, restrictions within the limits and conditions set out in Regulation (EU) No 1308/2013 and Regulation (EU) 2019/934 |
|---|---|---|
| Stabilizing agents | — Meta-tartaric acid<br>— Potassium bitartrate (crystals)<br>— Tannin[2]<br>— Citric acid<br>— Acacia gum (Arabic gum) [2] | Prevent KHT and CaT salts precipitation, < 100 mg/l<br>Assist the precipitation of KHT during cold treatment.<br>Color stabilization.<br>Bind ferric ions in a soluble complex anion < 1g/l<br>Protective colloid with effect on copper and iron haze or pigments in colloidal state <0.3 g/l |
| Other | — Aleppo pine resin (addition)<br>— Carbon dioxide (addition)<br>— Cupric citrate<br>— Oak chips<br><br>— Nitrogen, for bubbling | For 'retsina' wine in Greece, **<** 10 g/l.<br>Still wine: < 3 g/l, less than 1 bar. at 20ºC<br>Correction of taste and odour defects <1g/hl<br>To introduce the characteristics of certain oak wood constituents into wine |

[2]Derived from organic raw material if available.

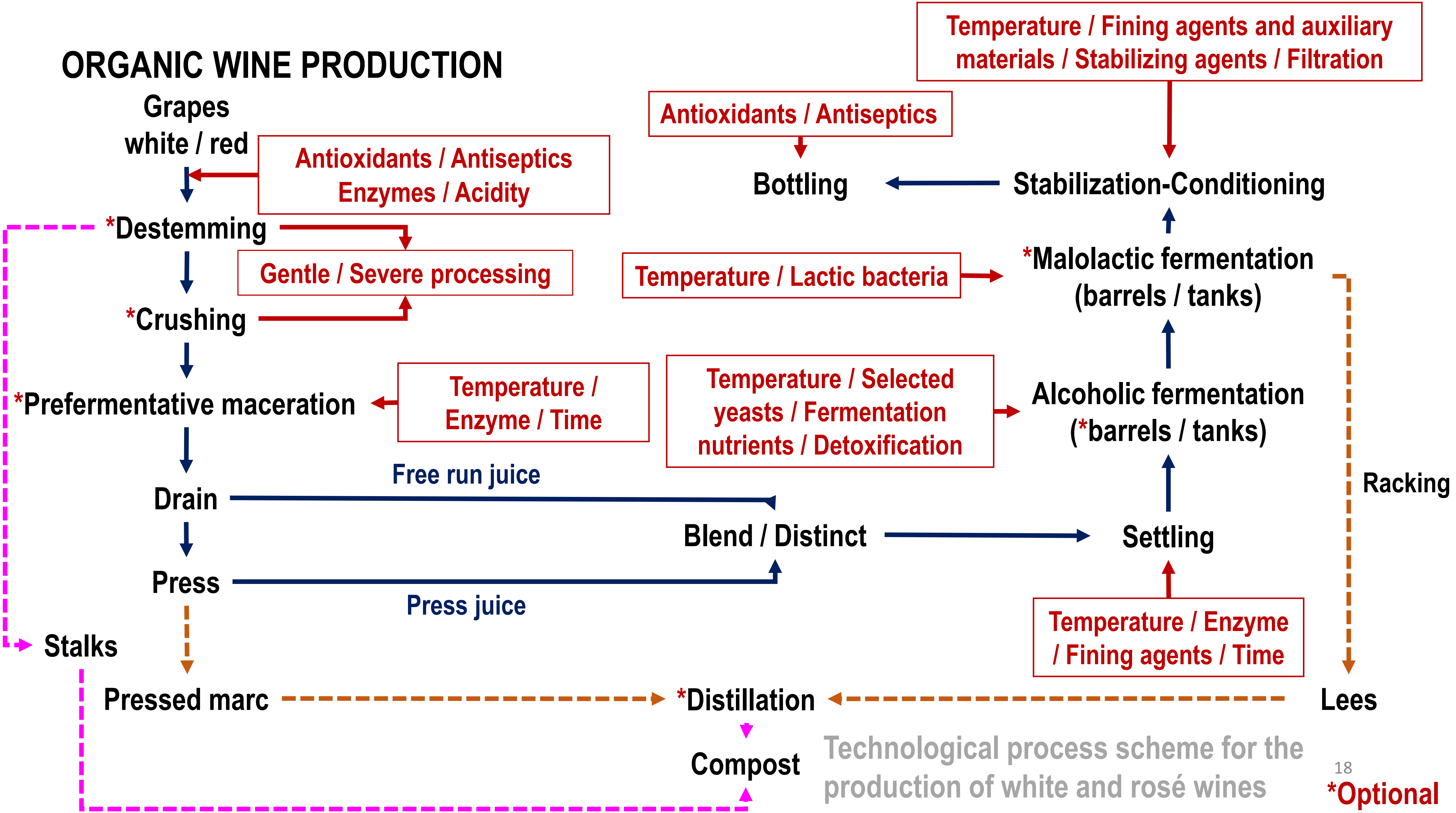
# ORGANIC WINE PRODUCTION

**Maximum allowed sulfite concentrations (mg/l) in various organic and conventional wines produced in EU and USA**

| Type | Sugar | [a]Biodyvin | [b]Demeter | [c]FNIVAB | [c]EU Conventional | [d]EU Organic | [e]USDA Made with Organic Grapes | [f]USDA Organic | [e]US Conventional |
|---|---|---|---|---|---|---|---|---|---|
| Red | ≤5 g/l | 80 | 110 | 100 | 150 | 100-120 | 100 | <10 | 350 |
| | >5 g/l | 105 | 140 | 150 | 210 | 170 | 100 | | 350 |
| White, Rosé | ≤5 g/l | 105 | 140 | 120 | 200 | 150-170 | 100 | <10 | 350 |
| | >5 g/l | 130 | 180 | 210 | 260 | 220 | 100 | <10 | 350 |
| Red, White, Rosé | ≥30 g/l | 175 | 250 | 250 | 300-400 | 270-370 | 100 | <10 | 350 |
| Red, White, Rosé, affected by Rot | ≥30 g/l | 200 | 360 | 360 | 400 | 370 | 100 | <10 | 350 |
| Liqueur Wines, Fortified wines | | 100 | - | 100 | 150-200 | 120-170 | 100 | <10 | 350 |
| Sparkling | ≤15 g/l | 96 | 140 | 100 | 150-235 | 155 | 100 | <10 | 350 |
| | >15 g/l | 104 | 180 | 150 | 185-235 | 205 | 100 | <10 | 350 |

**Sources:** [a]Biodyvin, 2009; [b]Demeter, 2011; [c]FNIVAB, 2011; [d]IFOAM, 2013; [e]USDA NOP, 2011b; [f]ATTTB, 2014.

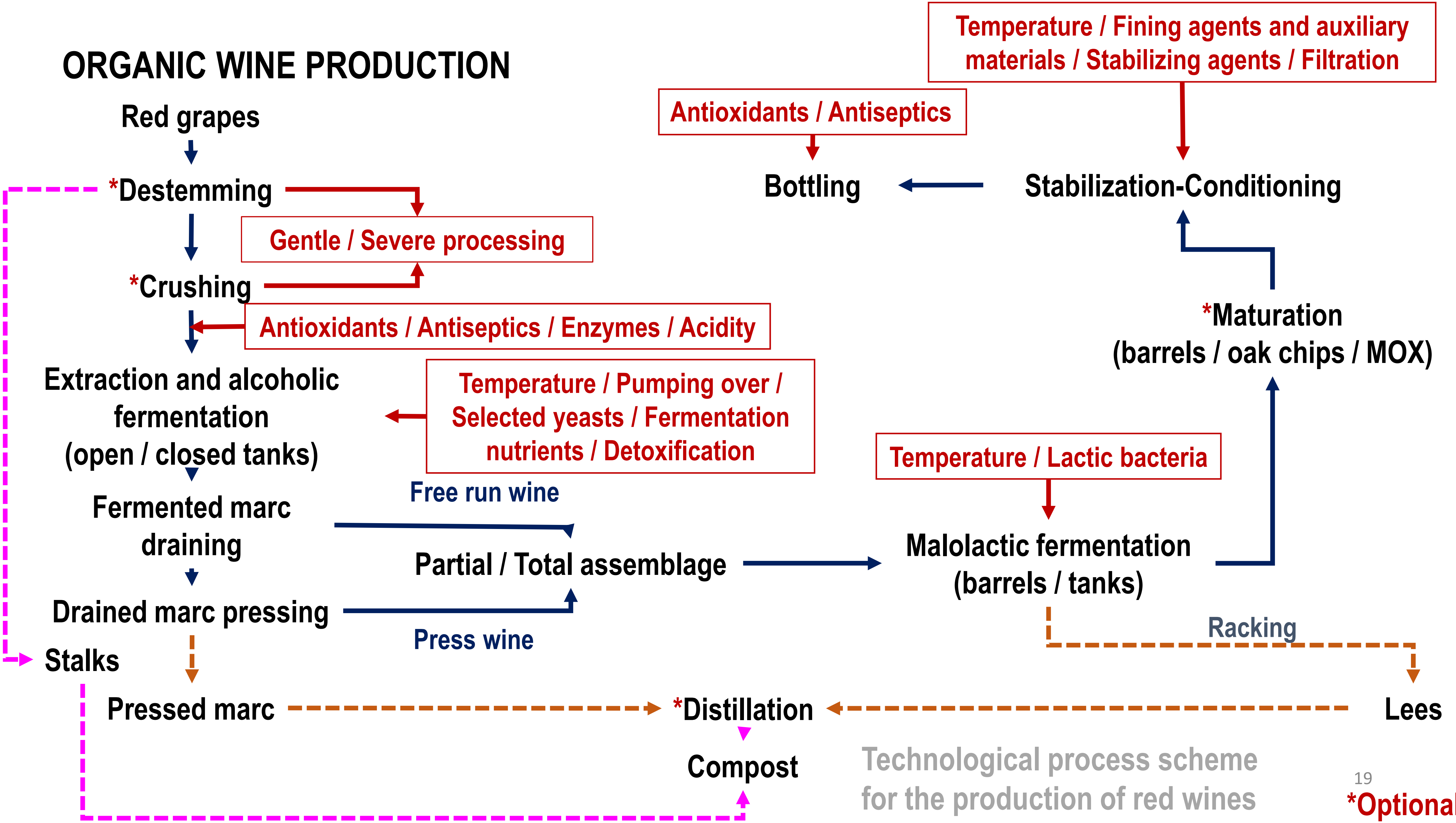
# ORGANIC WINE PRODUCTION




Technological process scheme for the production of white and rosé wines

*Optional

# ORGANIC WINE PRODUCTION




Technological process scheme for the production of red wines

*Optional

# ORGANIC WINE PRODUCTION

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## SUITABLE PRODUCTS FOR ORGANIC PRODUCTION

**List of Products Reviewed (1) or Attested (2) by Ecocert**

Please find below the list of products suitable for International organic farming, in compliance with European, American, Japanese, Canadian or Brazilian regulations:

www.inputs.bio

**ECOCERT is the world leader for certification of eco-friendly products.**

**European Input List - Search**

All products shown in the European Input List comply with the EU regulations No. 834/2007 and No. 889/2008. Products linked to a country code (column 4 of search results) comply with the national requirements for organic production of the respective country and may be legally placed on the market in that country.

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Suitable products
for Organic Farming

## OENOLOGICAL PRODUCTS UNDER ORGANIC PRODUCTION

### Tannins

Tannins are antioxidant substances generally used to facilitate the clarification of new wines by precipitating the excess of proteinaceous matter and to facilitate fining.

They combine with the protein component of oxidase enzymes helping to reduce oxidase activity. In red wines help to stabilize the anthocyanins.

The usual doses of tannin are:

- between 1-5 g/hl for white and rosé wines (pre-fermentative, during grape processing);
- of 2-40 g/hl for red wines (during maceration).
- of 20-80 g/hl for binding and partial inhibition of laccase (grapes affected by *Botritys*).

# ORGANIC WINE PRODUCTION

## OENOLOGICAL PRODUCTS UNDER ORGANIC PRODUCTION

### Tannins

**The supplementation with all enological tannins caused a significant decrease in the residual laccase activity compared to the control.**

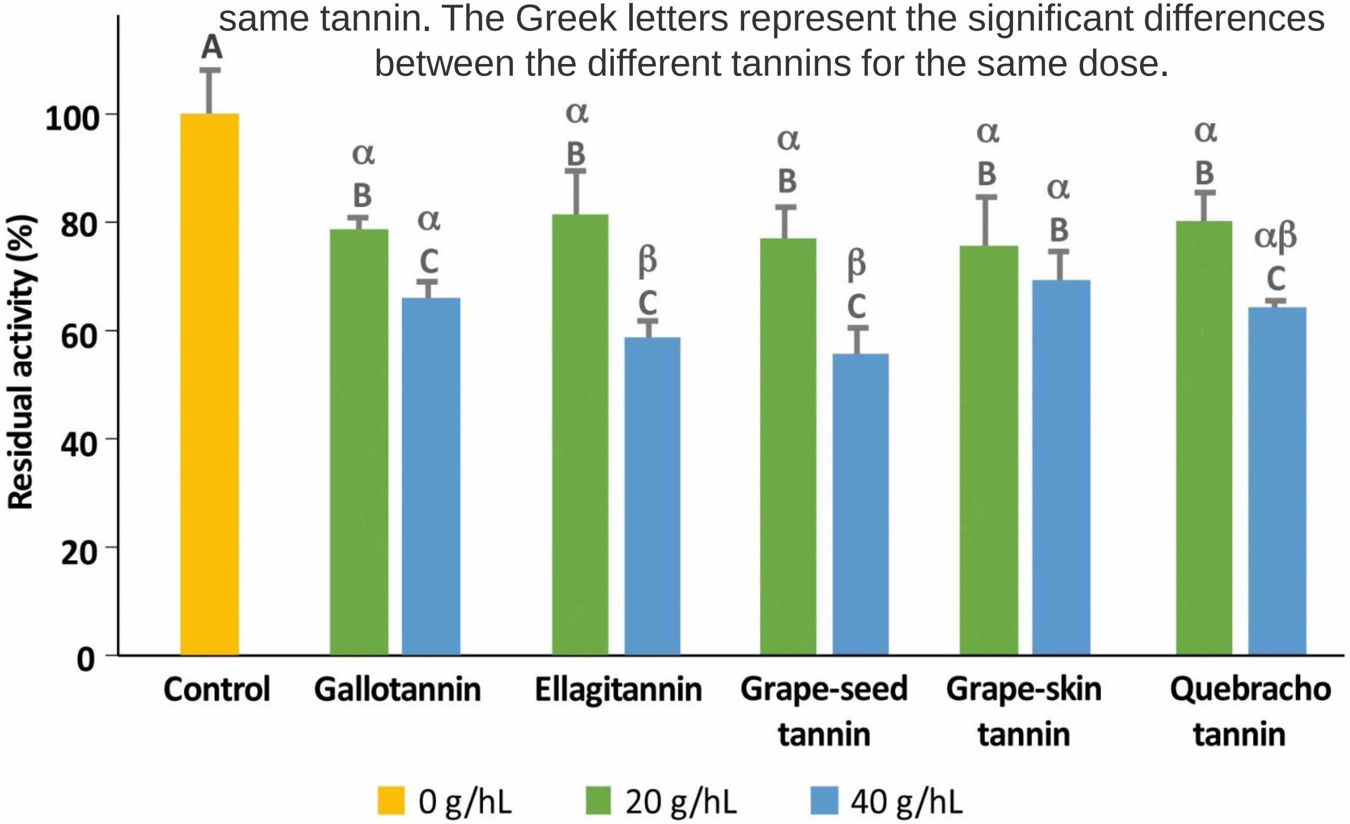
**The residual activities following supplementation with the different enological tannins ranged between 55.3 ± 4.5% (in the case of the grape-seed tannin at 40 g/hL) and 81.5 ± 7.5% (for ellagitannin at 20 g/hL).**

**Regarding the doses of enological tannins used, the higher the dose, the lower the residual activity.**

**Grape-seed tannins and ellagitannins were the most effective, reaching at the highest dose 55.3 ± 4.5% and 58.5 ± 3.1% of residual activities, respectively.**

(Vignault et al., 2019)

All the data are the mean ± SD of three replicates. The capital letters represent the significant differences between the different doses for the same tannin. The Greek letters represent the significant differences between the different tannins for the same dose.




**Inhibition effect of enological tannins on laccase activity (Vignault *et al.*, 2019).**

# ORGANIC WINE PRODUCTION

## OENOLOGICAL PRODUCTS UNDER ORGANIC PRODUCTION

### Tannins

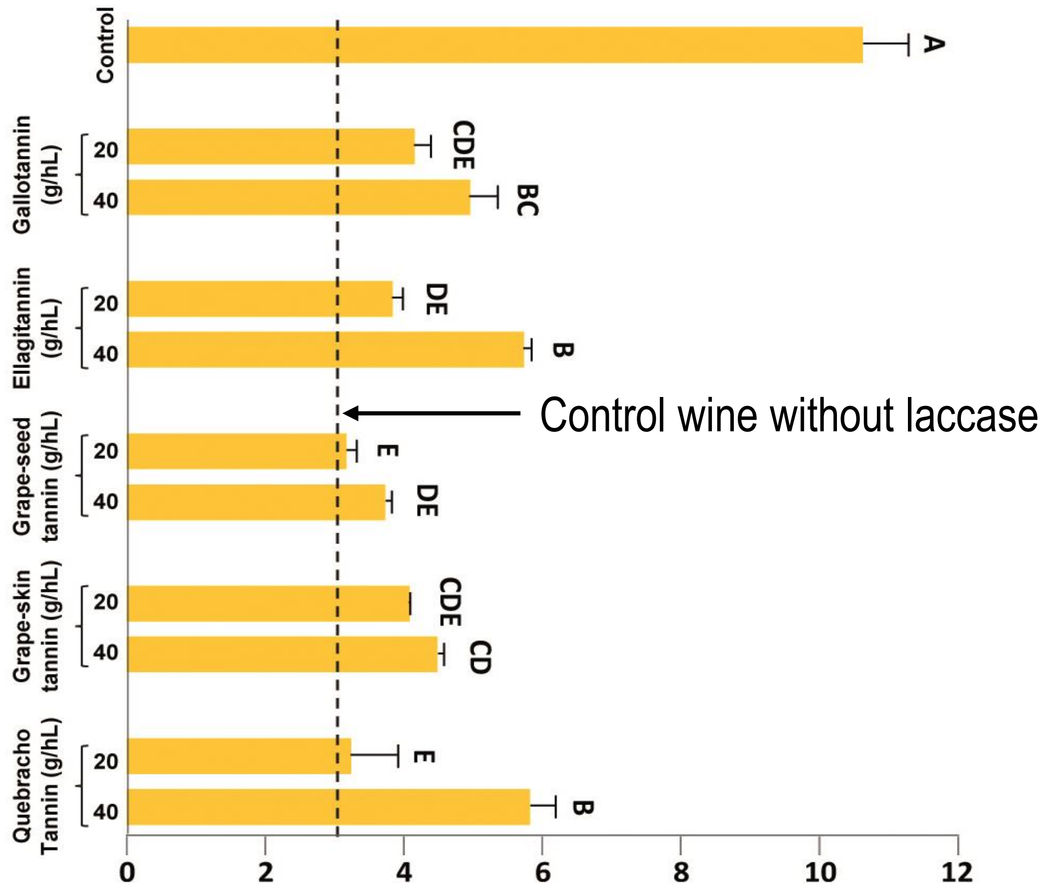
- **Specifically, the figure shows the total color differences obtained with white wines.**

- **The comparison between the two control white wines (with and without laccase) showed a ΔEab* reaching around 10 units.**

- **Since this value is much higher than 3 units, this result indicated that the presence of laccase drastically affected the quality of the wine color.**

(Vignault *et al.*, 2019)

All the data are the mean ± SD of three replicates. The capital letters indicate the existence of significant differences between the different samples.




**Impact of enological tannins added in botrytized wines on color visible to the human eye (Vignault *et al.*, 2019).**

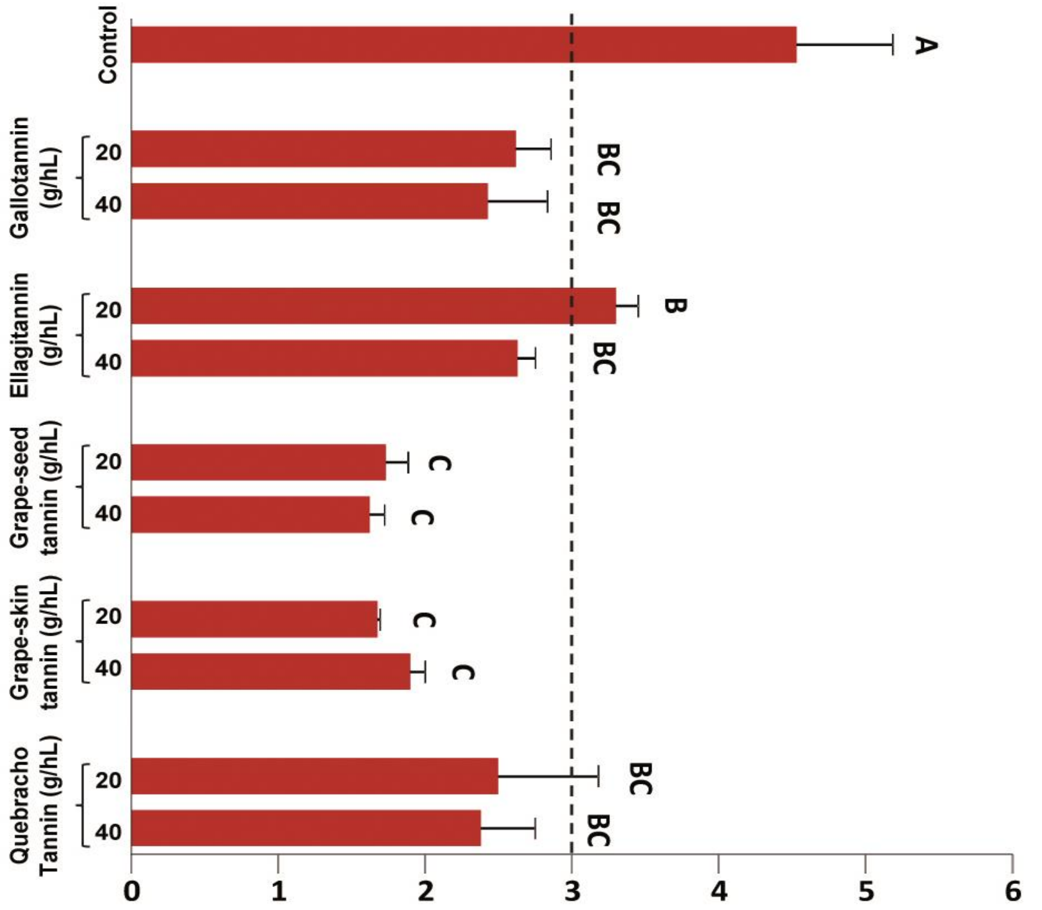
# ORGANIC WINE PRODUCTION

## OENOLOGICAL PRODUCTS UNDER ORGANIC PRODUCTION

### Tannins

- This figure show the results for the total color differences in the white wines supplemented with malvidin-3-O-glucoside.
- Trend was very similar to that observed for the white wines.
- The comparison between the two control red wines (with and without laccase) showed a ΔEab* reaching around 5 units.
- This value being greater than 3 units, the presence of laccase deteriorated sufficiently the color of the red wine to be distinguished by the human eye.
- Moreover, the supplementation with tannins significantly decreased the ΔEab* compared to the control wine without laccase.
- The corresponding ΔEab* values in most of the cases were lower than 3 units.

All the data are the mean ± SD of three replicates. The capital letters indicate the existence of significant differences between the different samples.




Impact of enological tannins added in botrytized wines on color visible to the human eye (Vignault *et al.*, 2019).

## OENOLOGICAL PRODUCTS UNDER ORGANIC PRODUCTION

### Tannins

Important concluding remarks (Vignault *et al.*, 2019):

- enological tannins inhibit laccase activity.
- small differences in the effectiveness of the different types of tannins were observed.
- the duration of contact needed to reach the maximal inhibition of laccase can be considered as very short (around 4 minutes).
- the supplementation with all enological tannins really mitigates the negative effect due to the presence of laccase by affecting the color of white and red wines.
- this effect seems to be more effective in the case of the protection of red color in red wines.

# OENOLOGICAL PRODUCTS UNDER ORGANIC PRODUCTION

## Pectolytic enzymes

Grape enzymes are less active under vinification conditions (low pH, $SO_2$, low temperatures).

Commercial preparations of pectolytic enzymes are usually extracted from *Aspergilus niger* and *Aspergilus aculeatus*.

The usual doses of the enzyme preparation vary depending on the enzymatic activity of the preparation (concentration) and the working conditions.

Usually the usual doses are:

- 1-3 g / hl for clarification
- 2-5 g / q for macerations.

Generally there are no technical risks associated with overdose of enzyme treatment if are used only quality commercial preparations.
In the case of poor quality enzymatic preparations, not properly purified from some secondary activities (eg. cinnamoyl esterase activity involved in the formation of phenolcarboxylic acids from esters of aromatic hydroxycarboxylic acids), some sensory defects may occur.

# OENOLOGICAL PRODUCTS UNDER ORGANIC PRODUCTION

## Pectolytic enzymes

| Variables | How factors influence the reactions catalyzed by enzymes |
|---|---|
| Grapes quality | Optimal ripening of grapes has a major impact on the enzyme dose.<br><br>Unripe grapes => very high pectin content;<br>Ripe grapes => moderate pectin content.<br>Overripe grapes (> botrytized) => high glucan content.<br><br>Doses should be increased for pectin-rich or small-berry and thick-skinned varieties.<br>In overripe grapes => β-glucans may lower the clarification of grape must. |
| pH | At ΔpH = 0.3, then up to ~ 50% difference in enzymatic activity:<br>pH = 3.2 => 60% enzymatic activity;<br>pH = 3.5 => 100% enzymatic activity;<br>pH = 3.8 => 160% enzymatic activity. |
| Temperature | ▪ The optimum temperature for clarifying musts = 50°C, but in practice of winemaking, this is usually between 10 and 20°C;<br>▪ The inactivation temperature of the enzymes is over 55°C and lower than 5°C<br>▪ Temperature increase by 10°C => a doubling of the enzymatic activity |
| Time/Concentration | Generally, doubling the enzyme dose reduces the reaction time by half |

# ORGANIC WINE PRODUCTION

## OENOLOGICAL PRODUCTS UNDER ORGANIC PRODUCTION

### Selected yeasts – parameters to consider depending on the desired wine

**Name/Origin:**

- **Commercial name:** ***eg.*** Zymaflore 011 Bio
- **Genus, species, variety:** ***eg.*** *Saccharomyces cerevisiae var. bayanus*
- **Origin:** ***eg.*** Pays de Bade (Germany)

**Technical properties:**

- **Oenological use:** white wine / rose / red / sparkling
- **Sensory impact, releasing aromas:** neutral / varietal character / esters / terpenes / thiols
- **Floculation capacity:** slow (>15 zile) / moderate (6-15 zile) / fast (3-6 zile)
- **Foaming properties:** low / moderate / high
- **Killer toxins:** neutral / sensitive / active ($K_1$ / $K_2$ / $K_{28}$ / Klus)
- **MLF compatibility:** inhibitory / neutral / recommended / very recommended
- **Cinnamoyl decarboxylase activity (formation of volatile phenols):** negative / low positive / positive
- **Nitrogen requirements (YAN):** low (150 mg N/l) / moderate (200 mg N/l) / high (250 mg N/l)
- **Reaction to $O_2$ addition:** low / moderate / strong
- **Minimum temperature (inoculare):** ex.: >12°C
- **Fermnentation temperature range:** ex.: 14-30
- **Anthocyanin adsorption:** low / medium / high

# ORGANIC WINE PRODUCTION

## OENOLOGICAL PRODUCTS UNDER ORGANIC PRODUCTION

### Selected yeasts – parameters to consider depending on the desired wine

French Wine and Vine Institute provide an useful online tool which provide a database with selected yeasts with their properties (https://www.vignevin.com/outils/fiches-levures/) :

Synthetic medium (1) TAP=12% vol. alc.

- Latency time: very short / short / medium / long / very long
- Fermentation kinetics: very slow / slow / moderate / fast / very fast
- Sugar / alcohol yield (g sugars / 1% vol. ethanol): range between 15 to18
- Malic acid degrading capacity, %: n.d. / low <15% / medium 15-30% / strong >30%
- Volatile acidity production (g/l acetic acid): low, <0,25; medium, 0,25-0,40; high, >0,40;
- $SO_2$ production, mg/l: n.d., 0; low, 1-20; medium, 20-40; high, 40-100; very high, >100.
- Acetaldehide production, mg/l: low, <30; medium, 30-50; high, >50;
- Pyruvic acid production, mg/l: low, <5; medium, 5-15; high, >15;
- Glycerol production, g/l: low, <4; medium, 4-6; high, >6;

Synthetic medium (2) TAP=16% vol. alc.

- Alcohol tolerance, % vol.: generally range between 12 to 18
- Residual sugars, g/l (after 40 days): 0-80
- Volatile acidity production (g/l acetic acid): low, <0,40; medium, 0,40-0,70; high, >0,70;

# ORGANIC WINE PRODUCTION

## OENOLOGICAL PRODUCTS UNDER ORGANIC PRODUCTION

### Selected yeasts – parameters to consider depending on the desired wine

**Other properties of yeasts:**

- Succinic acid production, mg/l: low; medium; high;
- Relative $H_2S$ potential production, mg/l (under nutritional deficiency, YAN = 60 mg/l): low; medium; high;
- Relative $H_2S$ potential production, mg/l (under moderate nutrition, YAN = 170 mg/l: low; medium; high;

# OENOLOGICAL PRODUCTS UNDER ORGANIC PRODUCTION

## Selected yeasts – proper rehydrating protocol of active dry yeasts

- Rehidrating active dry yeasts in a vessel with a large surface with water + grape must (80:20 parts) at a temperature ranging from 37 to 40°C, in a proportion of 1 part dry yeast at 10 parts liquid.

- The technique involves spreading of yeast on the surface of liquid and slowly stirring for 5 minutes only to facilitate moistening of the yeast and introducing oxygen to the slurry, then is recommended 15 minutes to rest. After this time, the yeast slurry should be homogenized, aerated and acclimatized gradually with grape must at room temperature, decreasing <5 °C at a time.

- Activated yeast is ready for being added to fermenter when $\Delta T < 8 - 10$℃, $where$: $\Delta T = T_{slurry} - T_{juice}$



# ORGANIC WINE PRODUCTION

## OENOLOGICAL PRODUCTS UNDER ORGANIC PRODUCTION

### Selected yeasts – proper rehydrating protocol of active dry yeasts

- Optimum rehydrating liquid needs 1-2% sugars, about 100 mg N/l yeast assimilable nitrogen (DAP is not recommended during rehydration of yeast) and a temperature of about 37-40°C.

- Using of protectants (lipids, vitamins and minerals) during rehydration helps yeasts to maintain the vitality.

- The presence of GSH and Cysteine during this phase may be interesting for thiol production in Sauvignon blanc or other varieties because of early $H_2S$ production as a consequence of enzymatic activity of cysteine desulfhidrase in yeasts (Winter *et al.*, 2011). Two C6 aldehydes, (E)-2-hexen-1-ol and (E)-2-hexenal, are thiol-precursors during early stages of fermentation in the presence of $H_2S$ (Harsch *et al.*, 2013).

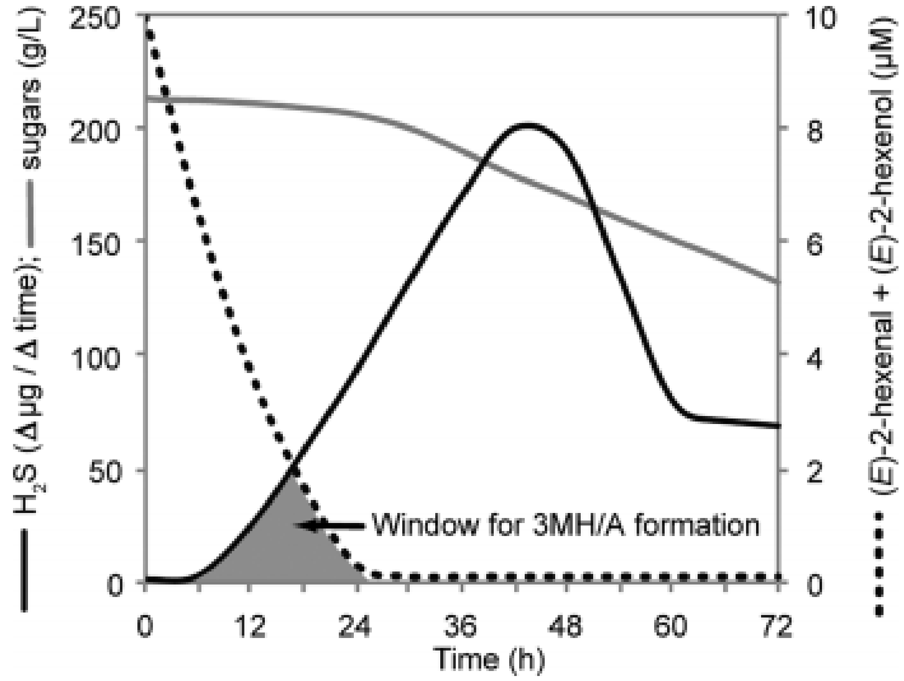



**Figure 5.** Kinetics of C6-thiol precursors, hydrogen sulfide, and sugars during a typical wine fermentation. The shaded area depicts the time window when C6-thiol precursors and hydrogen sulfide are present at the same time. (*E*)-2-hexenal, (*E*)-2-hexen-1-ol, and sugar kinetics are based on actual data at commercial scale. The estimated evolution of hydrogen sulfide is derived from data presented by Thomas et al.,[37] Mendes-Ferreira et al.,[38] and Ugliano et al.[39,44]

# ORGANIC WINE PRODUCTION

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## OENOLOGICAL PRODUCTS UNDER ORGANIC PRODUCTION

### Yeast derivatives – products to improve the technological and sensory properties in wines

**Yeast derivatives can have various uses in oenology: adsorbents, fining agents, nutrients or as stabilizing agents.**

***Inactivated yeasts***

**Product obtained by thermal inactivation of yeasts at temperatures over 60°C, being commercially available as inactivated dry yeasts or can be prepared from active dry yeasts, which can be inactivated by hydration 1/10 part water at temperature over 60°C.**

***Autolysed yeasts***

**Whole yeasts are killed (thermolysed) and then kept in favorable conditions to allow the autolysis process: incubated at 45°C, for a certain period of time, in the presence of endogenous enzymes from yeasts or by adding exogenous enzymes (β-glucanases). Cell walls, which contain glucans, are partially degraded, and the cell membrane and soluble constituents in the cytoplasm are exposed, thus being available for use by active yeasts or other microorganisms as nutrients.**

## OENOLOGICAL PRODUCTS UNDER ORGANIC PRODUCTION

### Yeast derivatives – products to improve the technological and sensory properties in wines

**Yeast derivatives can have various uses in oenology: adsorbents, fining agents, nutrients or as stabilizing agents.**

***Yeast cell walls (Yeast hulls)***

**Products *derived from autolysed yeasts*, which *contain only the yeast cell walls* (which are insoluble), being separated from the rest of the cellular constituents by centrifugation. Depending on how they are washed, the yeast cell walls may or may not contain certain parts of the cell membrane.**

**Generally, yeast hulls are used in stuck or sluggish AF or MLF because of their adsorptive properties being able to remove medium chain saturated fatty acids (C6-C8-C10), and pesticide residues which are inhibitors (Lafon-Lafourcade, 1984). In wines, yeast hulls can eliminate cork taint and moldy character, inactivate of Brettanomyces sp. Yeasts or eliminate of volatile phenols.**

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### *Yeast extracts*

Products derived from autolysed yeasts, which contain only the intracellular components of yeast (which are soluble), representing the supernatant resulting from separation by centrifugation process.

Depending on further separation process they are used as:

- organic nutrients: autolysis products containing only the intracellular components of yeast, they are used for amino acids, vitamins and micronutrients to increase the content of YAN in grape must;
- fining agents: specific yeast protein extracts (YPE) containing intracellular proteins;

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## Yeast derivatives – products to improve the technological and sensory properties in wines

**Yeast derivatives can have various uses in oenology: adsorbents, fining agents, nutrients or as stabilizing agents.**

**Mannoproteins (glycoproteins) extracted from yeast cell walls, being more or less purified used for stabilizing properties as protective colloids in wines.**

**The enzymatically obtained hydrolysate is purified into several specific types of mannoproteins:**

- **mannoproteins with a molecular mass of 31.8 kDa (called MP32) contain approx. 85% mannose and 15% protein, being intended to improve protein breakdown and reduce bentonite doses by 50%.**
- **highly glycosylated mannoproteins with a molecular mass of ≈40 kDa (called MP40) containing phosphoglycerides (glycosyl-phosphatyl-inositol anchors) in their structure are effective for tartaric stabilization of wines.**
- **mannoproteins rich in Hsp12 peptide fractions responsible for the perception of sweet taste contribute to improving the fineness of wines and balancing acidity and bitter taste.**

**The recommended doses are between 15 and 25 g/hl based on trials. Over 30 g/hl may be ineffective.**

## OENOLOGICAL PRODUCTS UNDER ORGANIC PRODUCTION

### MANAGEMENT OF NUTRIENTS DURING WINEMAKING

### YAN correction can be done in two or even three steps as follows:

**Step 1:** before inoculation with yeasts, the YAN deficiencies are corrected with organic nutrient up to 120 mg N/l, inorganic nutrients (DAP) are avoided at this stage because it limits the formation of thiols and fermentation esters. It should be noted that the $NH_4^+$ cation is used first by yeast to increase biomass and then the amino acids. Step 1 correction is necessary only in deficient grape musts.

**Stage 2:** after the latency period (≈48 hours after inoculation) at the onset of AF, it is possible to correct with 10-20 mg N/l (at this stage, DAP can be used up to 100 g/hl);

**Step 3:** until the yeast can still assimilate YAN, coincides with the maximum fermentation rate, until 1/3 of the sugars are consumed, with organic or inorganic nutrients;

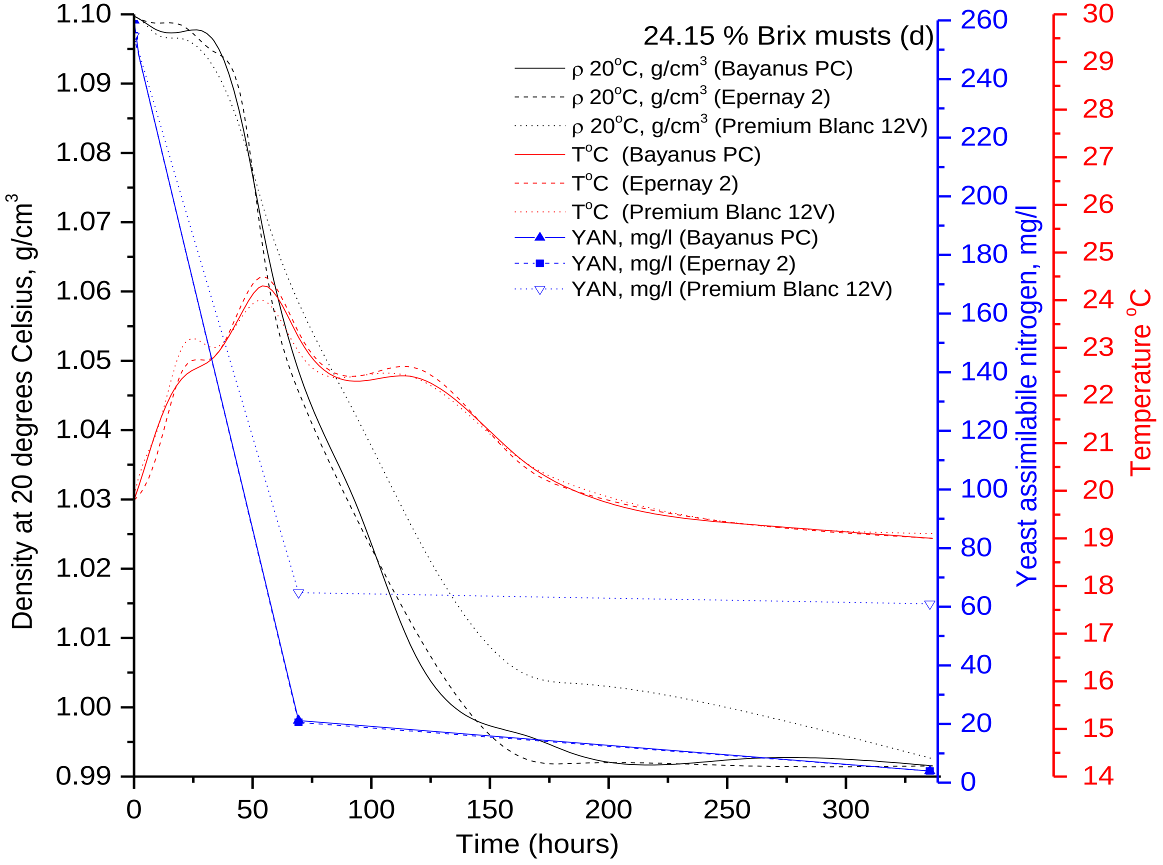
# ORGANIC WINE PRODUCTION

## OENOLOGICAL PRODUCTS UNDER ORGANIC PRODUCTION

### MANAGEMENT OF NUTRIENTS DURING WINEMAKING

Nutrients for fermentation are classified into:

- **inorganic nutrients:**
  - ✓ diammonium hydrogen phosphate - $(NH_4)_2HPO_3$ (provides ~21% N, which corresponds to 21 mg N/l using a dose of 10 g/hl in grape must) – permitted in organic wine production;
  - ✓ ammonium sulfate - $(NH_4)_2SO_4$ (provides ~ 21% N) – this is not permitted in organic wine.
- **organic nutrients:** extracts derived from autolyzed yeasts (they have about 4-8 mg N/l at each dose in must of 10 g/hl, but they are much more effective because they have minerals and vitamins.
- **complex nutrients:** are mixtures of inorganic and organic nutrients, to which is added cellulose, as an inert material, yeast support and vitamin $B_1$.



# OENOLOGICAL PRODUCTS UNDER ORGANIC PRODUCTION

## MANAGEMENT OF NUTRIENTS DURING WINEMAKING

### Factors to take in consideration when correct YAN

- Yeast strain nutritional requirement;
- Increased fermentation temperature => higher YAN requirement
- Large $O_2$ additions during AF => higher YAN consumption
- Increased sugar concentration in grape must => higher YAN requirement
- Low turbidity grape must => higher YAN requirement
- Late additions of YAN when >10% vol. alc. => residual YAN
- pH = 4.0 => about 90% of $NH_4^+$ can be used, while at pH = 3.0 => only 70% of $NH_4^+$ is used.
- Large YAN in grape must (>250 mg N/l):
  - ⇒ may increase the biomass, heat and hence speed of AF (need of a higher cooling capacity) – sometimes high YAN may be beneficial in red winemaking;
  - ⇒ may rapidly depleted by yeasts especially when is corrected with DAP and therefore lead to $H_2S$ production (Mendes-Ferreira *et al.*, 2009).
  - ⇒ if DAP is used instead organic nutrients, come up the risk of ethyl acetate production and ethyl carbamate production in wine.
  - ⇒ residual YAN is possible when the concentration of YAN was 300 mg N/l which can be a growing source for unwanted microorganisms.

# OENOLOGICAL PRODUCTS UNDER ORGANIC PRODUCTION

## MANAGEMENT OF NUTRIENTS DURING WINEMAKING

### Factors to take in consideration when correct YAN

- Generally grape juices with moderate concentrations of YAN tends to produce wines with more complex aroma than grape juices with elevated YAN concentrations which instead tend to produce fruitier wines.

- It is recommended to make successive corrections of YAN instead of large additions to have a moderate kinetic of fermentation and balanced biomass.

- Grape juices with high YAN deficiency (over 100 mg N/l), needs successive YAN corrections using an adequate management to deplete sugars.

# OENOLOGICAL PRODUCTS UNDER ORGANIC PRODUCTION

## MANAGEMENT OF NUTRIENTS DURING WINEMAKING

Nutritional requirements of yeasts vary quite widely between 0.5-1.5 mg / l AUA for 1 g/l fermented sugars, and are classify ed as follows:

- very low (<100-120 mg N/l) with a consumption of 0.50 mg N / g fermented sugars;
- low (120-150 mg N/l) with a consumption of 0.75 mg N / g fermented sugars;
- moderate (150-200 mg N/l) with a consumption of 0.90 mg N / g fermented sugars;
- high (200-250 mg N/l) with a consumption of 1.25 mg N / g fermented sugars;
- very high (> 250 ~ 350 mg N/l) with a consumption of 1.50 mg N / g fermented sugars;

Assessment and classification of YAN deficiency. The minimum YAN concentration recommended for the completion of fermentation AF is generally 120 mg N/l, even if the musts have very low sugar concentrations. To evaluate the type of deficiency is necessary to analyze YAN anc calculate expected YAN depending on yeast strain used.

$$\Delta\text{YAN}, mg/l = YAN(\text{lab result}) - YAN\ (\text{expected})$$

- non-existent ≤ 0 mg N/l;
- weak < 30 mg N/l;
- average < 80 mg N/l;
- strong > 100 mg N/l.

# OENOLOGICAL PRODUCTS UNDER ORGANIC PRODUCTION

## MANAGEMENT OF SURVIVAL FACTORS DURING WINEMAKING

The term "survival factors" came from the interpretation of the impact of sterols and long-chain unsaturated fatty acids on yeasts and fermentation kinetics.

Oxygen addition or aeration prior-AF is consumed by oxidases (tyrosinases or laccase) and during AF by yeasts. The oxidase enzymes decrease the availability of oxygen for yeasts. Supplementing about 4 mg/l $O_2$ during the yeast multiplication period is of great importance for the synthesis of sterols and unsaturated fatty acids. Deprivation of yeasts from $O_2$ inhibits the synthesis of sterols which in turn:

- decrease the resistance of yeast to stress factors (alcohol tolerance, temperature extremes, osmotic pressure).
- decreases the protein synthesis of the cell membrane (role in the transport of compounds)
- decreases the consumption of YAN;
- decreases the viability of yeasts towards the end of the AF and even stuck the AF;
- increase the concentrations of short and medium chain saturated fatty acids (growth inhibitors).

# OENOLOGICAL PRODUCTS UNDER ORGANIC PRODUCTION

## MANAGEMENT OF SURVIVAL FACTORS DURING WINEMAKING

### Vitamins

The must obtained from healthy grapes generally has sufficient concentrations in vitamins, but certain vinification practices considerably reduce the concentration in vitamins, even to exhaustion. Yeasts need vitamins to properly support the fermentation process. The vitamins necessary for the development of healthy fermentation processes are: vitamin B1 (thiamine); vitamin B7 (biotin); vitamin B9 (folic acid); inositol; vitamin B3 (niacin or nicotinic acid); vitamin B5 (pantothenate); vitamin B6 (pyridoxine); vitamin B2 (riboflavin).

Practices and treatments that lower the content of vitamins are: cold settling of grape must; filtering; clarification with fining agents; sulphiting; certain conditions for uncontrolled multiplication of non-Saccharomyces yeasts and lactic acid bacteria.

### Minerals

They have multiple roles, as co-factors of some enzymes, are part of some proteins (cell wall), stimulate protein synthesis, maintain cellular integrity and respectively increase resistance to stress factors (temperature variations, ethanol concentrations).

## GRAPEVINE DISEASES AND WINE QUALITY

There are three main diseases need to be controlled in vineyards which may change the yield and composition of grapes:

- Grapevine powdery mildew (*Erysiphe necator*)
- Grapevine downy mildew (*Plasmopara viticola*)
- Grapevine bunch rot (*Botrytis cinerea*)

Generally when processing grapes with over 1-5% infected berries, chemical composition of must and resulted wine change, lowering the perceived quality.

Harvesting of infected grapes:

- The grapes can be selectively hand harvested and infected grapes avoided.
- For machine-harvested grapes, hand pickers can remove the worst-affected bunches prior to machine harvesting.

# ORGANIC WINE PRODUCTION

## IMPLICATION OF POWDERY MILDEW IN WINE

Compositional and sensory changes were detected in juice and wines made from Chardonnay grape batches with as little as 1–5% of bunches affected by powdery mildew (Stummer *et al.*, 2008).

Processing infected grapes leads to low quality wines, off flavors, and difficult to manage the cleanliness of aroma. Clarification of must before alcoholic fermentation is difficult.

Stummer (2005) and Steel *et al.* (2013) indicate that powdery mildew can cause:

- off-flavors difficult to manage (mushroom, earthy, wet fur and cooked tomato characters);
- increased oiliness and viscosity in grape juice that affect the gravitational settling of dispersed particles and microorganisms which leads high loads of microbial populations;
- an increased fermentation duration due to the lack of nutrients and vitamins for yeasts.
- reduced color intensity in red wines and increased phenolics and bitterness;
- increased pathogenesis-related protein expression and as a consequence increased doses of bentonite to stabilize white wines;
- increased titratable acidity;

## MANAGING POWDERY MILDEW - Technical approaches to improve *white wine quality* from infected grapes

- It is recommended the addition of $SO_2$ at higher doses than normal vinification in the range of 60-100 mg/l depending on severity and to limit the skin contact and extraction as much as possible using shorter pressing cycles.

- A common technique applied in EU is whole bunch pressing of affected grapes. This technique may aid juice draining. Testing the fractions during pressing for negative attributes and off-flavors is recommended. Generally first pressing fraction should be separated, as is more affected by off flavours.

- If negative attributes are detected, either discard the product if the negative attributes are significant, or conduct fining trials with bentonite, casein, or other proteinaceous fining agents permitted in organic wine production.

- Cold settling or flotation of grape must aided with pectolytic enzyme and fining agents to achieve low turbidity and a good particle separation. Removal of lees as soon as possible.

- It is necessary to avoid spontaneous fermentations and is important to use a selected yeast inoculum at a higher rates to reduce growth of undesirable microorganisms.

- It is reccomanded to test and manage yeast assimilable nitrogen (YAN) during alcoholic fermentation because can be lowered by powdery mildew infection or by other microorganisms growth.

- Protein stabilization of white wine may require higher doses of bentonite than wines resulted from healthy grapes.

# ORGANIC WINE PRODUCTION

## MANAGING POWDERY MILDEW - Technical approaches to improve *red wine quality* from infected grapes

- • It is recommended the addition of $SO_2$ at higher doses than normal vinification in the range of 60-100 mg/l depending on severity and to limit the skin contact and extraction as much as possible using shorter pressing cycles.
- Generally is recommended to reduce as much as possible the time between destemming and and yeast inoculation and to avoid cold soaking.
- It is necessary to avoid spontaneous fermentations and is important to use a selected yeast inoculum at a higher rates to reduce growth of undesirable microorganisms.
- It is reccomanded to test and manage yeast assimilable nitrogen (YAN) during alcoholic fermentation because can be lowered by powdery mildew infection or by other microorganisms growth.
- The pressing of marc should be done earlier than normal protocol, when the fermentation is still active and to reduce contact time with skins and to avoid post-fermentation maceration or extended lees contact.
- It is recomanded to rack off heeavy lees as soon as possible and to fine the resulted wine with gelatine or other proteinaceous fining agents if showing excessive coarseness or lack of balance.

# ORGANIC WINE PRODUCTION

## IMPLICATION OF DOWNY MILDEW IN WINE

- Downy mildew infections lead to significant crop loss (premature berry drop) and low quality grapes.
- Vines severely affected by this disease lose their leaves early exposing the immature berries to direct sun.
- Generally, late infections leads to immature berries which does not soften, with a green character perceived in the resulted wines.
- If a vine loses its leaves before the fruit ripens, the berries do not mature normally and the resulted grapes are withered and of inferior quality with an atypical green aroma.
- The overall quality of wine produced from infected berries is reduced.

## IMPLICATION OF DOWNY MILDEW IN WINE

- **Red wines produced from withered grapes were marked by intense odor reminiscent of green, herbaceous notes but also figs and cooked fruit.**
- **The compounds responsible for atypical aroma were identified by means of GC-O and GC-MS in Merlot and Cabernet Sauvignon wines.**
- **Lactones, aldehydes and methoxypyrazine were associated with these flavors .**
- **The threshold of 5% infected berries was determined to alter the wine characteristics and lower the perceived quality (Pons *et al.*, 2017).**

Cooked fruit notes - 3-methyl-2,4-nonanedione, γ-nonalactone and γ-decalactone.

Herbaceous and green aromas - (Z)-1,5-octadien-3-one and 3-isobutyl-2-methoxypyrazine

Pons, A., Mouakka, N., Deliere, L., Crachereau, J.C., Davidou, L., Sauris, P., Guilbault, P., Darriet, P., Impact of Plasmopara viticola infection of Merlot and Cabernet Sauvignon grapes on wine composition and flavor, Food Chemistry (2017), doi: http://dx.doi.org/10.1016/j.foodchem.2017.06.087

# ORGANIC WINE PRODUCTION

## MANAGING DOWNY MILDEW - Technical approaches to improve *wine quality* from infected grapes

- The specific protocols should be similar as for powdery mildew.

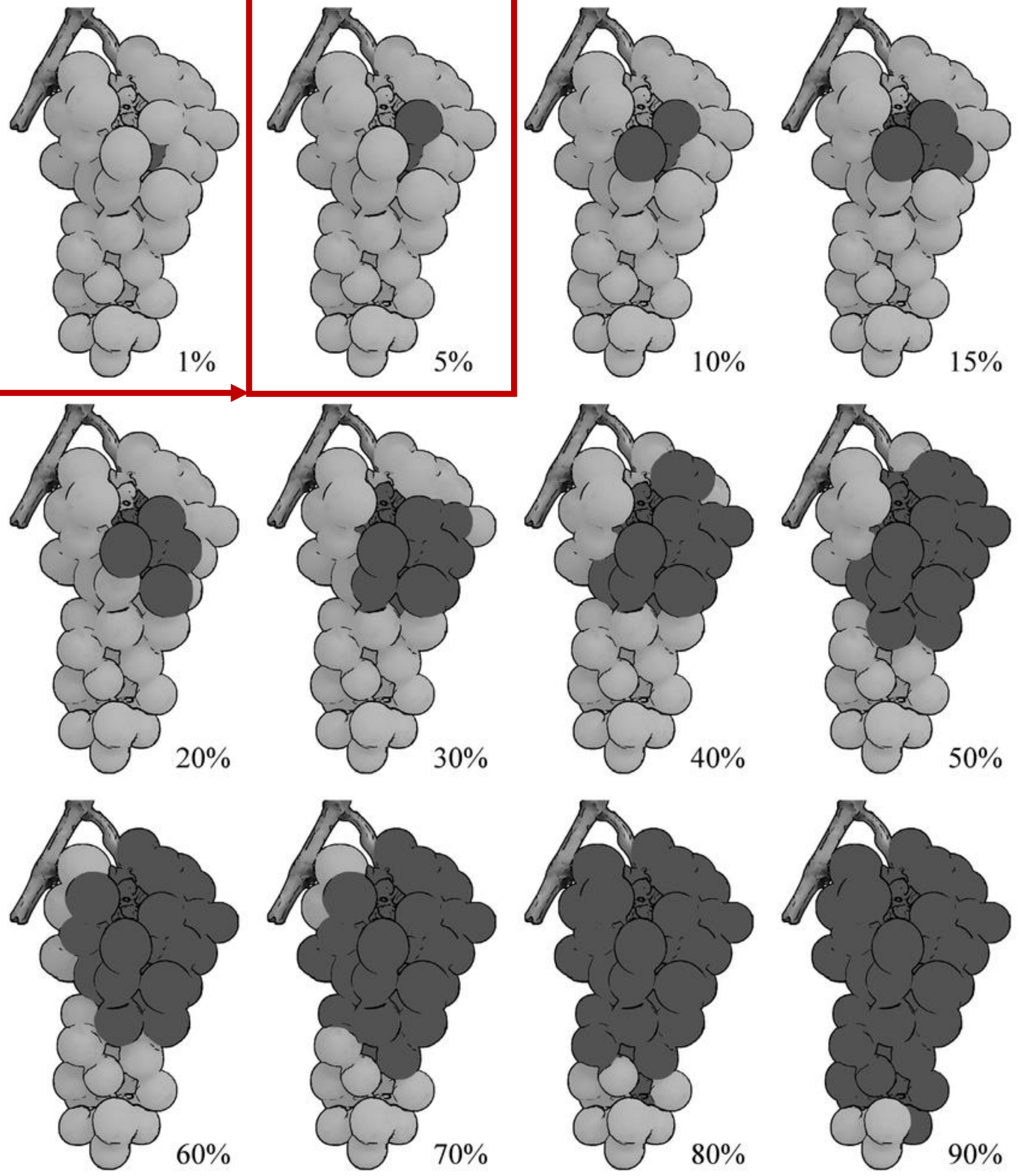
# ORGANIC WINE PRODUCTION

## IMPLICATION OF BOTRYTYS IN WINE

**The loss in quality of white wine resulting from the use of grapes at various stages of rot injury (Loinger *et al.*, 1977):**

- **0% produced wine of best quality.**
- **5-10% rot injury => reduction in wine quality, though still acceptable.**
- **20-40% rot injury => decrease in quality was more marked.**
- **80% rot injury => totally rejected wines.**

**Threshold for perceptible lowering the quality of wines (3-5%)**




**Botrytis bunch rot severity assessment key.** Lighter areas represent healthy berries; darker areas represent diseased berries. Numbers indicate the percentage of the visible side of the bunch occupied by diseased berries (image analysis software was used to calculate percentages to within ±1%).

(Hill *et al.*, 2010)

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## IMPLICATION OF BOTRYTYS IN WINE

- Key compounds associated with *Botrytis* infections: gluconic acid (resulted via glucose oxidation) and glycerol.
- *Botrytis* and bacteria populations produce acetic acid and lactic acid.
- The infected berries presents risk of nitrogen and thiamine deficiency due to the microbial populations growing in turbid grape must and slow settling process.
- The presence of *Botrytis* on grapes can also lead to off flavours (mouldy, earthy, mushroom) in resulted wines.
- The presence of secondary microorganisms such as *Penicillium* (blue-green mould), especially found in cool climates, produce geosmin, a compound that causes strong earthy off flavour in wine.
- **These changes affect sensorial characteristics of wines, depending on severity off-flavors may occur, difficult to manage under organic production rules.**

## IMPLICATION OF BOTRYTYS IN WINE

### Impact of Botrytis cinerea on must and wine (Claus, 2014)

| Increasing | Decreasing |
|---|---|
| Glucose, Fructose, Galactose, Arabinose | Glucose/Fructose |
| Polyols (Glycerol, Mannitol, Sorbitol, Inositol, Erythrol) | |
| Gluconic acid, Malic acid, Acetic acid, Citric acid, Galactaric acid, Galacturonic acid , 2-Ketoglutaric acid, Pyruvic acid | Tartaric acid |
| Polysaccharides (β-Glucan), Viscosity | Filtrability |
| Laccase, Glucosidases, Esterases, Pectinases | Polyphenol content<br>Colour (Anthocyanins) |
| Proteases | Nitrogen content (Amino acids, Proteins);<br>Foaming properties of sparkling wine |
| Ethanol, Methanol<br>Botrycins<br>Osmolarity | Vitamins (Thiamin) |
| Microbial diversity<br>Osmotolerant and fructophilic yeasts<br>Acetic- and Lactic acid bacteria | *Oenococcus oeni* |
| $SO_2$ demand | |

## IMPLICATION OF BOTRYTYS IN WINE

- Laccase enzymatic activity + $O_2$ => grape juice browning and oxidative spoilage (polyphenol oxidation, color loss in red wine)
- The increased pectolytic activity of the enzymes secreted by the fungus, hydrolyse pectins found in healthy grapes and for this reason an increased risk of over-extraction during grapes processing.
- Other enzymes secreted by fungus (glycosidase, esterase) => loss of aroma;
- *Botrytis* infection cause clarification and filtration issues - production of *β*-glucans (protective colloids) leads to an increased microbiological risk due to the improper settling of microorganisms and dispersed particles.
- Botrytis is known to produce at least three substances with antimicrobial properties: oxalic acid, botrycine (high molecular weight polysaccharide which inhibit yeasts in grape musts) and botrydial (a bicyclic non-isoprenoid sesquiterpene active against fungi and Gram-positive bacteria, as is *Oenococcus oeni* or *Lactobacillus sp.*).

## IMPLICATION OF BOTRYTYS IN WINE

- Percentage of berries with disease of both non-wounded and wounded inoculated berries increased significantly with increasing sugar content (Figs a & c).

- There was also a significant increase in % diseased berries with increasing YAN, although the relationship was much weaker than for sugar content (Figs b & d)

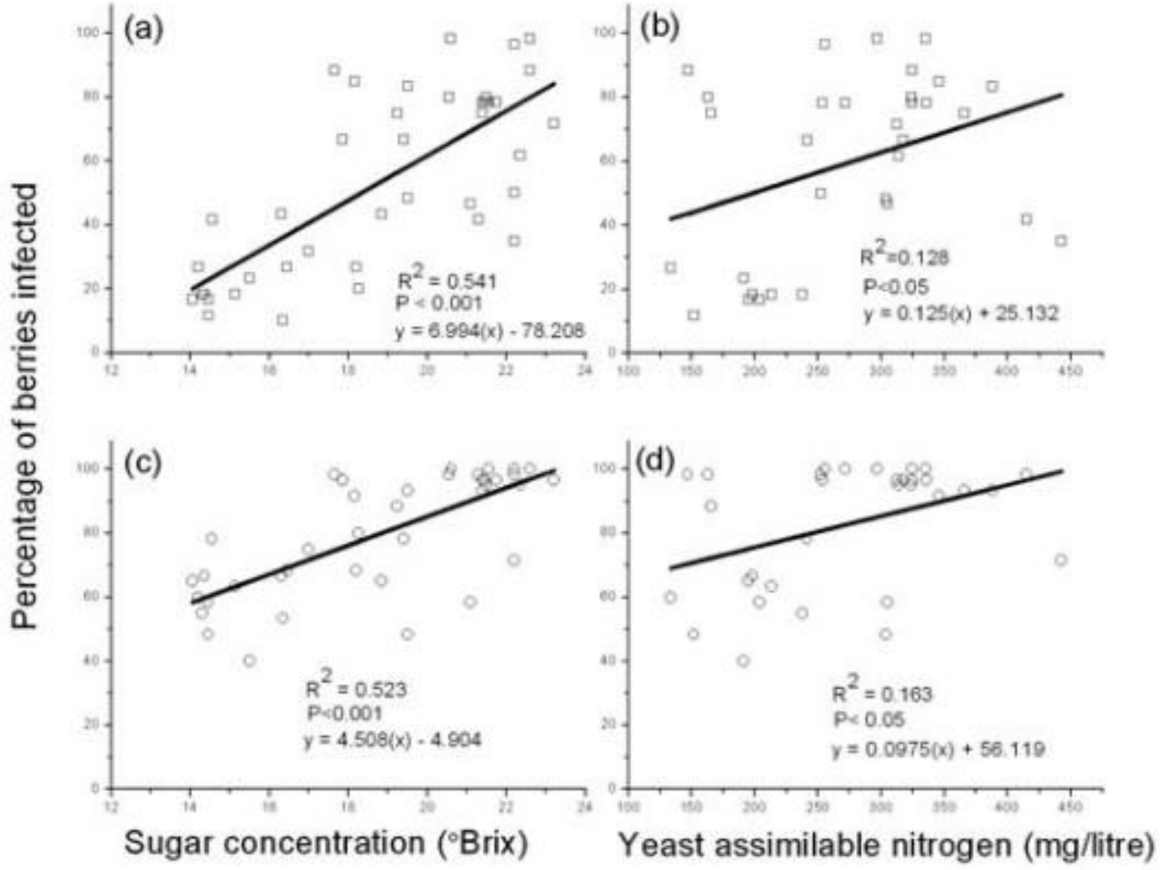
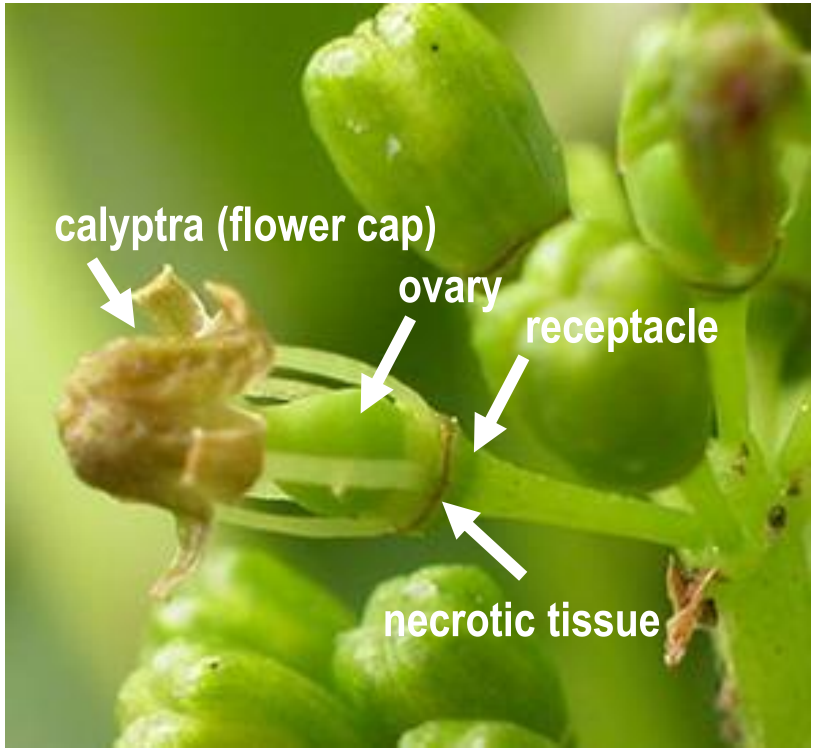



FIGURE 1: Mean percentage (%) of berries with *B. cinerea* sporulation after incubation, in relation to sugar concentration (a and c) and yeast assimilable nitrogen (b and d) for inoculated/non-wounded (a and b) and inoculated/wounded (c and d) treatments.

Mundy and Beresford, 2007

## MANAGING BOTRYTIS

- Preventive treatments in vineyard starting with the flowering for Botrytis (when about 80% caps fall).
- Flowering is a critical point for fungus to infect the necrotic plant tissue (the cap scar).
- The fungus infects this tissue but it is inhibited from colonising the surrounding green tissue because it contains a high concentration of stilbenes (natural antimicrobials).
- When the berry ripens, the stilbene concentration declines allowing the fungus to resume its growth and rot the berry.
- Other infections occur when natural openings or wounds created by insects, birds, rain, hail, frost, sunburn or mildew fungi.




Grape flower cap-fall with necrotic tissue (cap scar) that is common site for *Botrytis* infection in a young berry

- Another critical time to apply protective fungicides is at pre-bunch closure. This is the last chance to spray coverage inside the bunch where latent infections often emerge.

## MANAGING BOTRYTIS – Guidelines to improve wine quality from infected grapes

- Limit the quantity of infected grapes during harvesting, sorting table or other strategies to keep only clean grapes.

- Assessment of infected grapes per vine and determine the percentage infected berries
  - ✓ <1% normal processing protocol
  - ✓ 1-5% sorting and normal processing protocol
  - ✓ 5-20% sorting and specific protocol for processing of infected grapes
  - ✓ >20% sorting required and specific protocol for processing of infected grapes

- Increasing the $SO_2$ concentration in grape juice:
  - to limit the oxidation risk from laccase;
  - to prevent unwanted microorganisms to grow.
- The use of inert gases and reductive winemaking techniques, limit the dissolved oxygen and the enzyme activity.

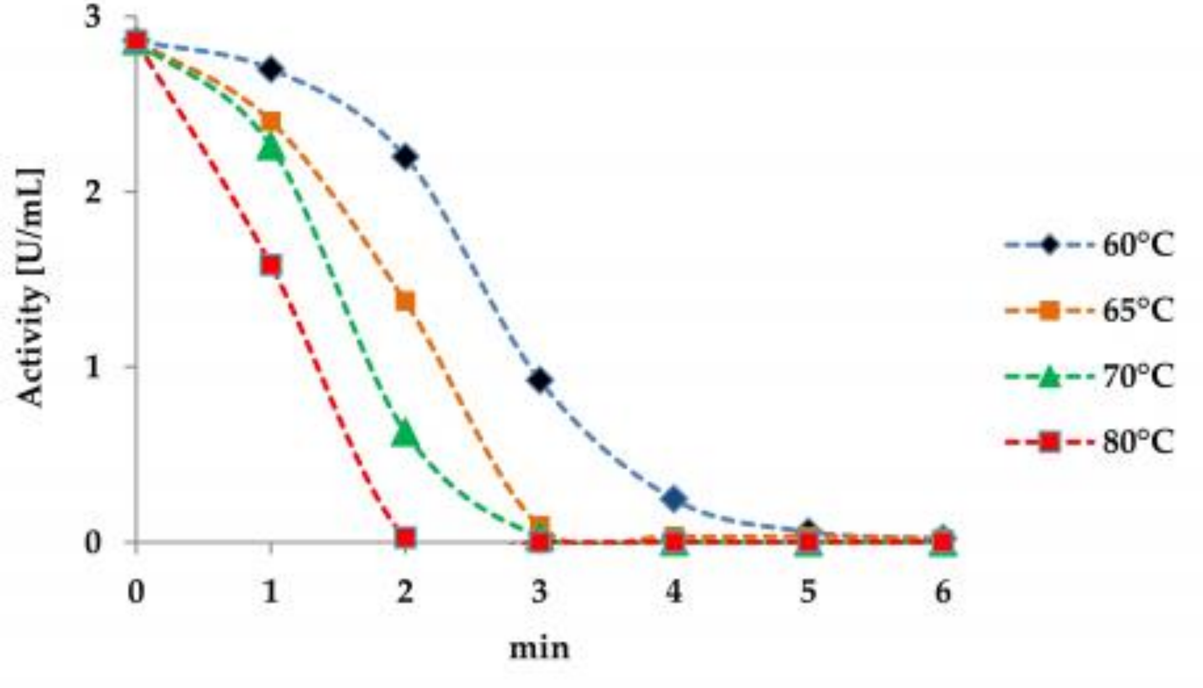
- Increased $SO_2$ concentrations to maximum allowed in organic wine does not inactivate enzyme.

- Early assessment for laccase - many direct or indirect tests: gluconic acid, colorimetric – with syringaldazine, glucans, test of grape juice in presence of air.

- Heat treatment to deactivate laccase enzyme, the temperature shall not exceed 70 °C - (EC) No 606/2009;

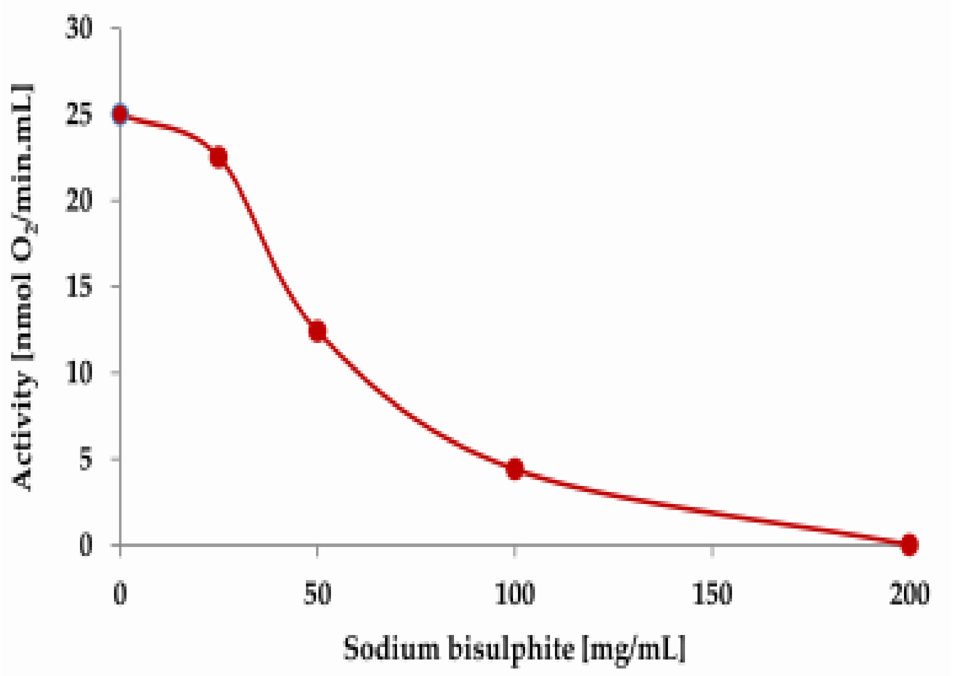
# ORGANIC WINE PRODUCTION

## MANAGING BOTRYTIS – Guidelines to improve wine quality from infected grapes




Influence of a heat treatment on must laccase

(Claus, 2020)




Influence of sulphite additions on the activity of a must laccase

(Zivkovic *et al.*, 2011)

# ORGANIC WINE PRODUCTION

## MANAGING BOTRYTIS – Guidelines to improve wine quality from infected grapes

- Pectolytic enzymes are allowed for organic wine production, but are not very effective for clarification of grape juice affected by Botrytis.

- The use of β-glucanase is not authorized under the EU organic regulation. Consequently, the clarification of grape must under organic wine production is more difficult.

- Poor clarification of grape juice allow the wild microorganisms to grow and to consume nutrients and vitamins necessary to yeasts for completely ferment sugars. Therefore, proper nutrition in clarified grape must for yeasts is recommended.

# ORGANIC WINE PRODUCTION

## MANAGING BOTRYTIS - Technical approaches to improve *white wine quality* from infected grapes

- Use of inert gases (eg. $CO_2$) in press and during white grape processing.
- Whole bunch press may aid juice draining. Testing the fractions during pressing for mouldy taint and off-flavors is recommended.
- Adding pectic enzyme even at the higher dosage may not help too much, but cold settle at low temperature is mandatory to achieve the desired turbidity. Low temperatures limit microorganisms growth, enzymatic activity and and thereof the oxidation of grape juice, allowing dispersed particles to settle out even the viscosity of juice is higher than in normal juices.
- Rack under protection of inert gases and remove the heavy lees.
- Trial additions with bentonite and other proteinaceous fining agents (eg. pea protein) permitted in organic wine production to remove off flavours and settle for 24 hours prior alcoholic fermentation. Usually bentonite doses are over 0.5 – 1 g/l and proteinaceous agents are in the range of 10-100 g/hl.
- Sometimes is better to use fining agents after 1-2 hours after pectolytic enzyme addition during cold settle in order to reduce the number of operations and thereof the oxidation of must.
- Racking off under inert gases and start alcoholic fermentation with selected yeasts.

# ORGANIC WINE PRODUCTION

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## MANAGING BOTRYTIS - Technical approaches to improve *red wine quality* from infected grapes

- Generally is recommended to reduce as much as possible the time between destemming and and yeast inoculation and to avoid cold soaking.

- It is important to start the alcoholic fermentation as early as possible, allowing yeast to quickly consume all the available oxygen to limit the laccase activity which can oxidize anthocyanins and many other phenolics.

- At first, must be considered a 'sacrificial yeast inoculum' (about 5-10 g/hl) to aid binding the excessive free $SO_2$. After few minutes of microbial activity, the normal yeast inoculum should be added as in normal protocols.

- It must be considered adding 30-80 g/hl of an oenological tannin at crushing to bind to some extent and inactivate the laccase protein.

- The pressing of marc should be done earlier than normal protocol, when the active fermentation will scavenge oxygen introduced during pressing, and secondly, the $CO_2$ released will provide a degree of protection from the ingress of oxygen.

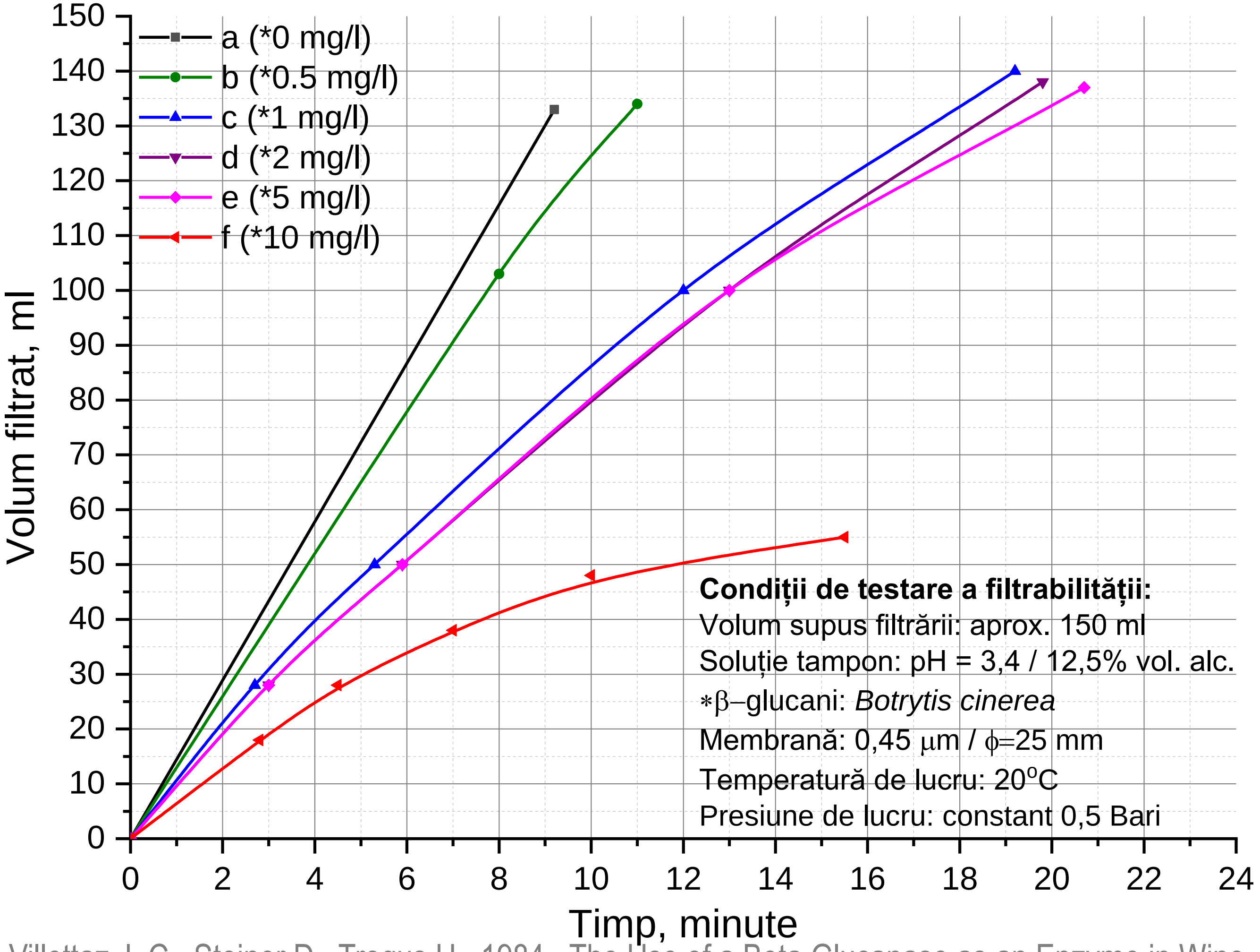
# ORGANIC WINE PRODUCTION

## MANAGING BOTRYTIS - Technical approaches to improve *red wine quality* from infected grapes

- At the end of AF, most of the laccase is bound to grape solids and will be partially removed with the heavy lees.
- It is recommended to rack off heavy lees after 24 hours, under the protection of inert gases to limit the oxygen ingress, and to test for laccase activity in the resulted wine. It must be considered to storage the resulted wines in stainless steel tanks and to avoid oak barrels and ullage.
- If the lab results detect laccase activity, tannin addition, settling and racking off must be considered. If laccase activity is still detected, heat treatment for 3 minutes at 70°C might be necessary.

## MANAGING BOTRYTIS - Technical approaches to improve *red wine quality* from infected grapes

- Red wines made from Botrytis-affected grapes are often difficult to clarify and filter due to the presence of long chain polysaccharides (*β*-glucans).
- The clarification and filtration take more time than normal red wines especially that glucanases are not permitted in organic wine production.
- Ethanol, acidity and cold storage will help to slowly precipitate glucan polysaccharydes.
- To aid the clarification, trials with colloidal sillica and gelatin may help settling.




**Influence of β-glucans in hydroalcoholic buffered solutions on filtrability using 0.45 µm membrane** (adapted: Villettaz *et al.*, 1984)

Villettaz J. C., Steiner D., Trogus H., 1984. The Use of a Beta Glucanase as an Enzyme in Wine Clarification and Filtration. Am. J. Enol. Vitic., Vol. 35, No. 4.