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INSTRUCTIONS FOR CO-INOCULATION

Preliminary Considerations

Objective of this protocol is to promote malolactic fermentation in conjunction with alcoholic fermentation.

1. Work within a temperature range from 59 to 66°F for white and <77°F for reds.
2. Control the SO_2 levels at the beginning of primary fermentation.
3. In order to obtain maximum aromatic expression it is important to ensure a reductive environment before and after fermentation.
4. Select yeasts that can facilitate the development of bacteria such as **EnartisFerm Vintage Red** and **ES 488.**
5. For high alcohol wines select high alcohol tolerant yeast like **EZ Ferm 44** or **Top 15**.

   Select yeast strains with moderate or long lag phase in order to avoid inhibition of bacteria growth.

6. Inoculate with bacteria at the beginning of fermentation and in any case at least 12 hours after the last addition of SO_2 or 48 hours after yeast inoculation if there was a cold soak
7. Several reasons to inoculate bacteria at early stages of fermentation:
   - There is a greater concentration of nutrients
   - Less alcohol and harsh conditions for the bacteria
   - Ideal temperature conditions

Most important factors influencing the development of malolactic bacteria:

Parameters	Influence
Free and total SO_2	Negative
High Tannins	Negative
Low temperature (<59 °F)	Negative
pH <3	Negative
Total acidity high	Negative
Cu^+	Negative
Alcohol (high %)	Negative
Lack in nutrients	Negative
pH> 3.3	Positive
Mannoproteins	Positive

Table 1 *- Limit negatively influencing parameters to one. If more than one negative parameters is present (2 or 3) the performance of bacteria will be reduced.*

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Co-Inoculation Protocol

Moderate SO_2 addition: 50 ppm maximum

Healthy grapes: do not make co-inoculation if grapes are moldy or affected with *Botrytis* (risk of stuck or sluggish alcoholic fermentation and risk of oxidation).

CASE 1: JUICE WITH POTENTIAL ALCOHOL CONTENT LOWER THAN 15%

Grapes Destemming/Crush

- Add 50 ppm SO_2 to avoid undesirable microorganism development, prevent the oxidation of color compounds and fatty acids in the skin (which lead to vegetal character).

Cold Soak (if used)

- Add 100g /ton of sacrificial tannin (Enartis Tan Fermcolor) at the crusher, to reinforce SO_2 antioxidant protection.
- Do not prolong cold soak over 2 days (this help to minimize wild yeast growth and to guarantee selective yeast dominance).
- Keep the temperature at about 53-55°F (do not go above 55°F in order to minimize wild yeast growth)
- At the end of cold soak, check YAN content.

Yeast Inoculation

- Follow yeast rehydration protocol as described in the Technical Data Sheet, inoculate the yeast at the rate of 3 lb/1000gal (this rate is higher than the usually recommended to be sure that the yeast prevail over the spontaneous flora that develops during cold soak). In case there is no cold soak is possible to apply the standard dosage of 2 lb/1000gal.
- Avoid temperature shock.
- At the time of yeast inoculation, add 200 g/ton of Nutriferm Energy (reintegration of vitamins and YAN).
- After 24 hours add the quantity of DAP if necessary to increase the initial YAN content up to 200 mg/L.
- Keep the fermentation temperature between 59 and 77°F (too low or too high temperatures are difficult for bacteria growth).

Bacteria Inoculation

- 48 hours after the yeast inoculation (12 hours if no cold soak was used), if the fermentation has started, check free SO_2:
  - Free SO_2 < 10 mg/L: proceed with the inoculation of Enartis ML Silver or MCW.
  - Free SO_2 < 10 mg/L: wait to inoculate until the free SO_2 content is lower.

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Alcoholic And Malolactic Fermentation

- Keep the fermentation temperature between 59 and 77°F (too low or too high temperatures are difficult for bacterial growth)
- At 1/3 sugar depletion, add 300 g/ton of Nutriferm Advance (complex nutrient that helps a steady and complete alcoholic fermentation).
- Monitor alcoholic fermentation by measuring alcohol and sugar contents and check the malolactic fermentation by measuring the malic acid and volatile acidity.
- If malolactic fermentation finishes before primary fermentation, check volatile acidity more frequently and in case of abnormal values add 20 mg/L of SO_2 or 300 ppm of lysozyme.

CASE 2: JUICE WITH POTENTIAL ALCOHOL CONTENT HIGHER THAN 15%

Attention: Potential alcohol > 15%: higher alcohol content can make end of the primary fermentation difficult and increase the risk of the development of sourness.

GRAPES DESTEMMING/CRUSH

- Add 50 ppm SO_2 to avoid undesirable microorganism development, prevent the oxidation of color compounds and fatty acids in the skin (which lead to vegetal character).

COLD SOAK (if used)

- Add 100g /ton of sacrificial tannin (Enartis Tan Fermcolor) at the crusher, to reinforce SO_2 antioxidant protection.
- Do not prolong cold soak over 2 days (this help to minimize wild yeast growth and to guarantee selective yeast dominance).
- Keep the temperature at about 53-55°F (do not go above 55°F in order to minimize wild yeast growth)
- At the end of cold soak, check YAN content.

YEAST INOCULATION

- Follow yeast rehydration protocol as described in the Technical Data Sheet, inoculate the yeast at the rate of 3 lb/1000gal (this rate is higher than the usually recommended to be sure that the yeast prevail over the spontaneous flora that develops during cold soak). In case there is no cold soak is possible to apply the standard dosage of 2 lb. /1000gal.
- Avoid temperature shock.
- At the time of yeast inoculation, add 200 g/ton of Nutriferm Energy (reintegration of vitamins and YAN).
- After 24 hours add the quantity of DAP if necessary to increase the initial YAN content up to 200 mg/L.
- Keep the fermentation temperature between 59 and 77°F (too low or too high temperatures are difficult for bacteria growth).

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BACTERIA REHYDRATION & ACCLIMATIZATION

- Rehydrate the bacteria as described in the Technical Data Sheet
- At the end of the rehydration, add an equal volume of a wine with alcohol content about 15-16%, free SO_2 lower than mg/L, total SO_2 lower than 50 mg/L, pH equal or higher than 3.5. acclimatize and build up the culture for at least 24-48 hours before adding it to the juice

BACTERIA INOCULATION

- 48 hours after the yeast inoculation (12 hours if no cold soak was used), if the fermentation has started, check free SO_2:
    - Free SO_2 < 10 mg/L: proceed with the inoculation of Enartis ML Silver or MCW.
    - Free SO_2 < 10 mg/L: wait to inoculate until the free SO2 content is lower.

ALCOHOLIC AND MALOLACTIC FERMENTATION

- Keep the fermentation temperature between 59 and 77°F (too low or too high temperatures are difficult for bacterial growth)
- At 1/3 sugar depletion, add 300 g/ton of Nutriferm Advance (complex nutrient that help to assure a steady and complete alcoholic fermentation).
- Monitor alcoholic fermentation by measuring alcohol and sugar contents and check the malolactic fermentation by measuring the malic acid and VA.
- If MLF finishes before primary fermentation, check volatile acidity more frequently and in case of abnormal values add 20 mg/L of SO_2 or 300 ppm of lysozyme.

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SUGGESTED PROTOCOL OF CO-INOCULATION F BACTERIA AND YEAST:

Wine style: Young and fruity red wine (without cold soak)

Objectives:

1. Vibrant and intense color
2. Aromatic enhancement with EnartisPro Blanco
3. Young red wines for early consumption

Reception of grapes	• 100 g/ton of Winy • 100 g/ton of sacrificial tannin (ENARTIS TAN FERMCOLOR) Controls: Brix, total acidity, pH, YAN
Management of oxidations	If possible, avoid contact with air. Use dry ice to saturate the tank and avoid oxidation.
At beginning of maceration	• 30-50 g/ton of **Enartis Zym COLOR.** (High dose due to low temperature) The enzymatic treatment is justified for the extraction of aromas, tannins and polysaccharides.
Yeast Inoculation	• 2 lb./1000 gal yeast **Enartis Ferm ES 488.** • 2 lb./1000 gal of **Nutriferm Arom** (reduces the risk of hydrogen sulfide production and ensures completion of fermentation). • 2 lb. /1000 gal of **Enartis Pro Blanco** (reduces astringency and bitterness and the loss of color).
Bacteria Inoculation **(at least 12 hours after last addition of SO_2)**	The temperature should be between 59°C and 66°F. Avoid temperature shocks higher than 10°F between the colture and the must. Add **Enartis ML Silver or MCW** rehydrated with **Nutriferm ML.**
Fermentation	Keep the temperature **< 77°F** (not lower the 59°F) At 1/3 of fermentation: • 2 lb./1000 gal of **NUTRIFERM ADVANCE** (only if necessary, according to the YAN), the minimum number of applications. • 1 lb./1000 gal of **Enartis Tan RED FRUIT** (reinforces the structure of the wine and improves fruit).Dose 6 mg/L oxygen delivered twice during fermentation.
After pressing	• Check Malic acid and Lactic acid. • Check that Glucose and Fructose <0.3 (minimum possible). • If you have finished alcoholic fermentation, rack, separate gross lees and apply Winy. It is recommended to split the addition in two to eliminate the action of bacteria. After 15 days, apply the final dose of Winy. • It is the best time to apply tannin for color stabilization: **Enartis Tan Fruitan** 0.4 lb./1000 gal (increase the dose according to the style of wine desired).

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