White and Rosé Wine Fermentation Protocol with Zyme-O-Stab Acid Protease for Unstable Protein Removal

Overview

This protocol outlines a standard white or rosé wine fermentation process enhanced with Zyme-O-Stab acid protease treatment to remove unstable proteins responsible for haze formation and improve long-term stability in white and rosé wines. Zyme-O-Stab hydrolyzes unstable proteins in musts and wines, preventing haze and other protein-related instabilities while improving filtration efficiency.

Enzyme Properties

- Zyme-O-Stab is active at a pH window of 1.5 – 5.3 and a temperature window of 50 – 140 °F / 10 – 60 °C.
- The enzyme will not be deactivated by normal ethanol or sulfur dioxide additions.
- The hydrolyzed proteins will not form a haze due to exposure to heat or cold, and will not reform into large, unstable proteins.
- Typical white and rosé juice (pH 3.0 – 3.6) falls well within the active pH window, so no pH adjustment is required for enzyme activity.

Grape Processing

Wine grapes can be crushed, pressed, clarified, and filtered per the winemaker's preferred method. Make any pre-fermentation amendments to the juice before adding the enzyme.

Zyme-O-Stab Addition

- Timing: Zyme-O-Stab is recommended for addition to the juice immediately before inoculation. It should be added separately from any other additions being made to the juice before inoculation.
- Dose rate: Add 10 – 15 mL/hL (380 – 570 mL/1000 gallons) to the treated tank. Use the upper end of the range for juice with a high baseline protein level, and the lower end for juice that is already close to stable.
- Preparation: Dilute the measured enzyme in 10 – 20 times its volume of clean, cool water, then add the diluted enzyme to the tank while mixing to ensure even distribution.
- Temperature: Zyme-O-Stab is active between 50 – 140 °F / 10 – 60 °C. For use with thermovinification, the must or juice must be cooled to below 140 °F (60 °C) before adding the enzyme.

Inoculation and Fermentation

- Zyme-O-Stab is compatible with all winemaking yeast strains and malolactic bacteria strains. It will not influence yeast choice or performance.

- It is expected that Zyme-O-Stab will complete protein hydrolysis in 6 – 10 days under normal fermentation conditions. The enzyme is intended to remain active in the tank throughout this period and is then removed by the addition of bentonite after fermentation (see Heat Stability Testing). Do not add bentonite to the fermentation before hydrolysis is complete, as bentonite binds the enzyme and removes it from suspension.

Trial Design and Controls

Run the treated and untreated wines in parallel from a single homogeneous juice lot, with Zyme-O-Stab as the only variable.

- After clarification, split the juice lot into two equal volumes: a treated tank and an untreated control tank.
- Record the volume of each tank. Both tanks must receive identical pre-fermentation amendments, the same yeast strain and inoculation rate, and the same fermentation temperature regime.
- Add Zyme-O-Stab to the treated tank only. Make no enzyme addition to the control.
- Where tank capacity allows, replicate each treatment to strengthen the result. A single tank per treatment is acceptable but yields no measure of variability.
- Label both tanks clearly and sample both at every point listed under Trial Sampling Points.

Trial Sampling Points

1. Juice Phase: After clarification and splitting the lot, record tank volume, Brix, pH, TA, & VA for both tanks. Analyze for total and soluble protein to establish a baseline protein level, if desired.
2. Fermentation: 3, 6, & 10 days after Zyme-O-Stab addition, record Brix, pH, TA, & VA, and analyze for total and soluble protein. Use the same protein assay method at every sampling point.
3. Post-fermentation: Record pH, TA, & VA, and analyze for total and soluble protein using the same method, in addition to regular post-fermentation analyses.
4. Sample the treated and control tanks at every point above and record results side by side using the data recording table at the end of this protocol.

Once the treated wine is heat stable, the enzyme has done its work and should be removed. Adding 12 g/hL (1 lb/1000 gallons) of bentonite to the treated wine will strip residual enzymes from the wine. This step is recommended if the wine will later be treated with CMC or acacia gum, as residual protease could interfere with the stabilization of these products.

Zyme-O-Stab Acid Protease Trial Data Recording

Varietal: _____ Vintage: _____
Vineyard: _____ Yeast strain: _____

Control tank — Tank ID: _____

Measurement	Juice (baseline)	Day 3	Day 6	Day 10	Post-ferm.
Tank volume					
Brix					
pH					
TA					
VA					
Total protein*					
Temperature					
Date sampled					
Initials					

Treated Tank — ID: _____ Enzyme lot: _____ Dose: _____ mL/hL

Measurement	Juice (baseline)	Day 3	Day 6	Day 10	Post-ferm.
Tank volume					
Brix					
pH					
TA					
VA					
Total protein*					
Temperature					
Date sampled					
Initials					

**not required*

1230 Shiloh Road, Windsor, CA 95492
Tel: (707) 836-6840 Fax: (707)836-6843

www.ATPGroup.com

2 Madison Avenue, Larchmont, NY 10538
Tel: (914) 834-1881 Fax: (914) 834-4611

Heat Stability Testing

After fermentation is complete, perform a heat stability test on the treated and control wines. Both wines must be tested with identical parameters on the same day.

Bentonite bench trial
A single bentonite rate will not quantify the difference between the two wines. Run a ladder on each wine and compare the lowest rate that achieves stability.

- Prepare a bentonite ladder for the treated wine and for the control wine across the same range, for example, 0, 1, 2, 3, 4, 5, and 6 lb/1000 gallons.
- Heat stability test every rung of both ladders using the parameters above.
- For each wine, record the lowest bentonite rate that achieves a change of less than 2 NTU. The difference between those two rates is the primary result of this trial.

Test parameters

- Filter or centrifuge the sample until clear and record the initial reading.
- Hold the sample at 80 °C for 2 hours, then cool to 20 °C and hold for 3 hours before reading.
- Record the final turbidity. A wine is considered heat stable when the change from the initial reading is less than 2 NTU.

Heat stability bench trial (ΔNTU; stable = <2 NTU). Record the lowest stabilizing rate for each wine in the final column; the difference is the trial result.

Initial Reading	NTU
Treated Sample	
Control Sample	

Bentonite rate	0 lb	1 lb	2 lb	3 lb	4 lb	5 lb	6 lb	Stable at
Treated ΔNTU								
Control ΔNTU								