

Micro-Fermentation (Bucket) Protocol

Fermenting a grape field sample is one of the best tools currently available to predict smoke exposure markers (both free and bound volatile phenols) that may be present in a wine post-fermentation. A winemaker can use the finished micro-fermented lot to evaluate the presence of off-aromas and ashy flavors and submit a wine sample to a lab for volatile phenol and glycoside analysis to understand the potential risk of smoke damage. *This method is most accurate as a predictor of risk when used closer to harvest.* This method is adapted from the AWRI Fact Sheet Small-lot fermentation method.

SAMPLE COLLECTION

- Collect samples as close to harvest as possible to provide a wine matrix similar to what would be expected at harvest
- Collect samples using your normal protocol or a minimum of 40-50 clusters per block (or sub-block if sampling for the presence of a gradient)
- Destem clusters into a 5 gal stainless or food grade bucket and remove any MOG, dispose of green material as you would normally going into a fermenter.
- Record weight of fruit (tare scale before filling bucket with fruit). A minimum of 10 pounds of fruit for fermentation is recommended. At 175 gal per ton (330ml/pound) this should result in 3.3L of must.

Note: You can always pick a bigger sample and use a larger fermentation vessel as it may be more representative.

- Crush the fruit with a manual crusher if available or with a potato masher; Red and white grapes follow the same recommended fermentation methods to evaluate highest risk potential, despite typical white winemaking practices.
- Add 50 ppm KMBS (0.165g for 10 pounds, corresponding to 3.3L) to crushed grapes and mix in thoroughly.
- Take sample of crushed grapes to lab for brix, pH/TA, YAN.
- Adjust must to 3.30-3.50 pH (in some cases this may be a K2CO3 addition) and 250 YAN.*.
 (For ease its recommended to add 2#/kgal DAP if you don't check YAN)
- Add pectinase enzyme to buckets and mix thoroughly (your normal rate of addition). For example:
 - For whites: Add 0.01ml/# (15ml/ton) of Cinn-Free to each bucket.*
 - For reds: Add 0.04ml/# (70ml/ton) of Color-Pro to each bucket.*

*Note: Adjusting pH and enzymes may more accurately reflect your normal fermentation, however it may not be necessary.

INOCULATION

- Rehydrate with 1g/gal (2.2#/kgal) of Dynastart rehydration nutrient (dissolved in 10x weight of 110F water). When water cools to 104F add 1g/gal (2.2#/kgal) EC1118, or any suitable yeast you would normally use for the variety.
- Warm grape must to 60-70°F. Industrial heaters and fish tank heaters are all useful items.
- After 10 minutes, stir in 50ml of grape must. Wait an additional 10 minutes, then stir in another 50ml of grape must. Continue until yeast mixture is within 10 degrees of juice temp.
- Add yeast slurry to fermentation vessel.
- Punch down bucket to ensure yeast is mixed in well.

FERMENTATION

- Fermentation temp should be maintained at 60-70°F for whites and 70-80°F for reds.
- Be sure to use loose lids and store in an area without smoke.
- Stir and punch down mixture four times per day to break up and submerge cap.
- Check brix/temp and smell/taste daily to ensure fermentation is healthy and at correct temperature.
- When mixture is at 1/3 sugar depletion (approx. a brix of 15), rehydrate 1g/gal (2.2#/kgal) of fermentation nutrient by dissolving in 10x weight of water. Add nutrient mixture to bucket. If H2S forms before 1/3 sugar depletion, add nutrient earlier.

PRESSING

- Press after five full days (5 x 24 hours increments) of being on skin contact. This is important for consistency.
- Place strainer over empty stainless or food grade bucket and drain ferment through strainer.
- Pour all skins into strainer and press grapes, extracting as much liquid as possible.
- Pour decanted wine into 1-gallon jugs and cover opening with cheese cloth. Confirm ferments are RS Dry (<2g/L sugar), using Clinitest or enzymatic assay before moving forward (additional time may be required to achieve RS Dry conditions).
- Once RS Dry, rack off lees, add SO2 and copper sulfate, mix well, and transfer into 750 ml bottles for storage, reserving some for analysis. Settle before evaluation; storing in your refrigerator can help with clarification.
 - o For a 750mL bottle 30ppm SO2 is 0.065g
 - o 50ppm SO2 is 0.039g
 - o 0.1ppm Copper in 750ml is 0.003ml of a 2.5% copper solution
- Whites: 30ppm KMBS solution.
- Reds: 50ppm KMBS solution.
- A subsample of each fermentation should be frozen for later use to support insurance claims or for later analysis if lab capacity is constrained during a large smoke event.

SENSORY EVALUATION

Sensory analysis, when coupled with post-fermentation analysis for the presence of smoke markers, is the best method for detecting smoke damage in grapes. While sensory analysis may prove more

revealing and useful than lab analysis alone, it's important to remember that even with experienced experts, taste and smell remain subjective experiences. So, a significant number of wine analyses may fall within a grey zone of uncertainty. Taste each sample at least twice within a short period of time. Test that evaluators are sensitive to smoke damage by screening, using both heavily smoke impacted and non-impacted wines as controls. Many people are not sensitive to smoke damage.

- 2-3 people should evaluate each wine separately
- Wines should be smelled for any off aromas related to smoke
- After smelling, wines should be tasted, with emphasis on the evaluation of the aftertaste of the wine, which may be bitter or there may be smoke related flavors that develop after the wine has been tasted.
- If multiple people are tasting, it may be useful to evaluate the wines in a different order to manage the carryover effect.
- During a tasting session, tasters should use a 4 g/L sugar (or glucose) rinse in between samples and wait 90 seconds before tasting the next sample to minimize the carry-over effect from smoke damaged samples.

ANALYSIS

Use an accredited third-party lab, such as ETS. Using an ETS tube – marked for *smoke volatile markers wine* and *bound or glycosylated smoke markers* and submit for analysis. When looking at micro-fermentations, nano-fermentations or grapes analyzing for bound compounds is necessary.

Note: An analysis from an accredited third-party lab will ensure conformity to standard methodology, provide an objective basis for assessing the status of a wine's source grapes and can be used to support a grower's crop insurance loss claim. USDA's Risk Management Agency (RMA) has not established specific threshold levels for the presence of smoke compounds in grapes or wine for purposes of determining smoke damage, except such lab results must support a finding of "elevated levels" of guaiacol and 4-methylguaiacol.

 Lab analysis will deliver results for guaiacol ug/L and 4-methylguaiacol ug/L. Most labs consider a value greater than 0.5 ug/kg (ppb) in grape samples or 1.0 ug/L (ppb) in wine or juice as an elevated level for these compounds.

Note: Most of these smoke marker compounds are naturally present in grapes without smoke exposure, so absent baseline data for a grape or wine variety, positive results don't necessarily correlate to damage.

This document was developed under the direction of the West Coast Smoke Exposure Task Force Research Committee chairs Alisa Jacobson and Melissa Hansen, with contributions from Task Force members. 7/26/2024 (v.5)