WEST COAST
SMOKE EXPOSURE
TASK FORCE

Nano-Scale Fermentation Protocol

As an alternative to analyzing grape samples for their content of smoke related volatile phenols, grape samples can be fermented for use as a tool for the prediction of risk to wine quality from a smoke exposure in the vineyard. Grapes fermented in five gallon buckets will provide 3-4L of wine for sensory and chemical analysis, but it generally takes 4-7 days. Nano-scale fermentation was developed to shorten the fermentation time. The technique has been studied and results have been found to be comparable to fermentation in buckets and bins.

Nano-scale fermentation uses glass canning jars as the fermentation vessel, with the addition of a water bath for temperature control to help increase the fermentation rate. Some winery labs already have access to a heated water bath which will typically hold four to six 16 oz. canning jars. A water bath alternative is a plastic storage container, coupled with a sous vide cooker to circulate temperature controlled water in the container. Using this approach, fermentations can be completed in as little as 36 hours.

SAMPLE COLLECTION

With the smaller fermentation volume in the canning jars, it is critically important to obtain a representative sample of the vineyard block. In some cases, it is useful to collect multiple samples from a block to be fermented separately. This may be particularly useful if the vineyard has large elevation changes or in instances where a wildfire has burned near the vineyard and there may be a gradient of smoke exposure impact from the side of the block closer to the fire to the side of the block furthest from the fire. When collecting clusters for a micro-fermentation, it is important to preserve the identity and chain of custody of the grapes and resulting wine.

- 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 bucket or other container and remove any MOG.
- 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.
- Transfer enough crushed fruit to *three canning jars per block (24 oz. jars are recommended, but 16 oz. or 30 oz. are also acceptable) to fill the jars two-thirds full (*Note: research recommends doing in triplicate, but not necessary)
- Add 50 mg/L KMBS (for non-research purposes, this can be measured as a teaspoon of KMBS…or more accurately, make a solution of KMBS at 10g/L of water and add 4mL of the solution per jar).
- Collect an additional sample of the must for TA, pH and Brix analysis as well as an additional sample to freeze for later if required.

- Adjust YAN level to 250 mg/L (for non-research purposes, this can be measured as 1/2 teaspoon of DAP).

FERMENTATION

- To prepare a yeast slurry for inoculation, multiply the number of 24 oz. jars (1.5 pints) to be inoculated by 0.2. (if using 16 oz. (1.0 pint), multiply the number of pint jars to be inoculated by 0.14). This is the number of grams of yeast to rehydrate; if it's one jar, it's 0.2 grams of yeast. Suspend the yeast in 10x the weight of 105°F water. EC1118 is recommended for rapid fermentations. If desired, use the yeast strain that would be used in normal production practice for the grape variety.
- After 10 minutes, add an equivalent volume of grape must to the yeast slurry to bring the temperature of the yeast slurry down closer to that of the must. Repeat as necessary at 10 minute intervals until the temperature of the slurry is within 10 degrees of the temperature of the must.
- Divide the slurry across the set of canning jars to be inoculated. Mix the inoculation into must with a spoon or by putting a lid onto the jar and inverting it several times.
- Place the jars into an appropriately sized storage container, then fill the container with warm water until the level is about two-thirds of the way up the container.
- Following the manufacturer's instructions, set the temperature of a sous vide machine to the desired temperature (85-87°F is recommended), then place the sous vide machine into the water bath. Most sous vide machines have a clip that can be connected to the side of the water bath.
- Canning jar lids and rings should be placed on the jars loosely, to avoid a pressure build up in the jar during fermentation.
- 2-3 times daily, remove jars one by one, tighten the lid, then invert the jar several times to mix the cap with the fermenting wine. Remember to loosen the lids prior to returning the jars to the water bath.
- Most sous vide machines have a low water level safety system which turns the machine off if the water level drops below a set limit. These safety systems can sometimes be activated during "pump overs," so check to ensure the sous vide system is running after all the jars have been returned to the bath. Periodically add more warm water if the level drops through excess evaporation.
- Brix measurements and smelling the fermentation should be done daily during the mixing session.

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Figure 1 Under-the-bed storage box set up as a nano-scale fermentation water bath, with sous vide machine for temperature control. These are 16 oz. canning jars, with lids and bands loosely applied to allow CO_2 to escape.

PRESSING

- When fermentations have completed (typically ~48-60 hours), the canning jar fermentations can be poured into a stainless steel strainer and pressed gently by hand to extract as much liquid as possible.
- The pressed wine can be decanted to smaller canning jars or other appropriately sized bottles to finish fermentation if necessary or for chemical analysis/sensory evaluation.
- 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.
    - For a 750mL bottle 30ppm SO2 is 0.065g
    - 50ppm SO2 is 0.039g
    - 0.1ppm copper in 750ml is 0.003ml of a 2.5% copper solution
    - Whites: 30ppm KMBS solution.
    - Reds: 50 ppm 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

- 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 that may affect the evaluation of subsequent wines.

ANALYSIS

Use an accredited third-party lab, such as ETS. Use 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 by Dr. Tom Collins, Washington State University, 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. (7/26/24)

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