Recommended Protocol for Heat Stability Testing and Bentonite Trials
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This protocol for a fast heat test measures the change in turbidity after heating as an indicator of protein stability. Bentonite trials are run concurrently with a non-treated control to determine the dose of bentonite needed to achieve protein stability.

Make a 5% stock solution of pre-hydrated bentonite: The bentonite used for the trial should be from the same manufacturer, brand, and batch as the bentonite that will be used in the wine. Make up a new stock solution in the event a new bag of bentonite is opened.

1. To make a 5% Bentonite stock solution(50g/L)(1:20 dilution), weigh out the mass of bentonite you will need. For 1 L of stock solution, you will need 50 g of bentonite.
2. Measure 80% of the volume of water needed into a beaker with a stir bar. If you are making 1L of stock, you will need 800 mL or water, here. Use the same water as will be used in the winery to rehydrate bentonite for addition to wine. Adjust the stir plate to a moderate speed and slowly add the bentonite to avoid clumping.
3. If a stir plate is not available, bentonite can be mixed in a close-topped container by inverting the container several times. It may take time to suspend all of the bentonite. Check carefully for any clumps that may remain.
4. Heat can be used to encourage the bentonite to dissolve. Some bentonite products require heat. Follow the manufacturer's recommendation for water temperature.
5. Once the bentonite is fully dissolved, pour the solution into a graduated cylinder or volumetric flask. Add winery water until the target volume is reached (1 Liter).
6. Transfer the stock solution to a container with an airtight lid and store it in the refrigerator. To aid in resuspension before use, store with a stir bar in the container.

Heat Stability Test/Bentonite trial

1. Resuspend the 5% stock solution of bentonite by placing the storage container on a stir plate. If the bentonite has settled to prevent the stir bar from moving, gently shake or stir the solution manually. Once the stir bar can move, allow it to mix while you prepare the wine. The bentonite stock solution must be fully mixed prior to addition to wine for bench trials.
2. Determine the amount of 5% stock solution you will need to add to 100mL of wine. A useful technique is to realize that 5% solution has 5 g/100 mL of solution.
    a. For example, if you plan to test 10 g/hL, 20 g/hL and 30 g/hL of bentonite in a volume of 100ml wine, you will need to add 200 uL, 400 uL and 600 uL of stock solution, respectively.
    b. For wines that have a suspected high rate of instability (such as Sauvignon Blanc, Pinot Gris, Traminette and Petit Manseng), you may choose to first test a wide

range (25, 50, 75, 100 g/hL), then do a second test within a smaller range to determine in the precise addition. For example, if stability is reached between 50 and 75 g/hL in the first trial, test 50, 60, 70 g/hL in the second trial.

3. Measure 100mL of wine with a graduated cylinder into a small labeled bottle for each treatment plus control. (You will need 4 bottles total for the example given above.) Use a micropipette to add the well-mixed bentonite stock solution and mix. Mixing should mimic the rate of mixing that will occur in the tank. Practically, this is best determined by eye. If no stir plate is available, mixing can be done by inverting the closed bottle (ensure the closure is secure before inverting). Allow the bentonite to settle overnight after mixing.
    a. The volume of the sample can be adjusted if needed. Re-calculate the volumes of bentonite additions to accommodate a change in base wine volume. However, be aware of the final volume needed for the NTU meter. Allow for a 10% loss of volume during filtration to determine the minimum volume needed.
4. After settling, turn on a water bath to 80°C. If a water bath is not available, an immersion circulator (available commercially for kitchen use) can be used. Make sure the temperature gauge is set to Celsius.
5. Decant the wine off the settled bentonite into a separate container, then filter. A 0.45 um filter should be used to determine protein stability for wines that will be sterile filtered. Following are two possible methods for filtration:
    a. If using a syringe filter, insert a 0.45 um filter into the filter housing and secure it. Pull up the wine into the syringe, then screw on the filter and gently depress the plunger. Go slowly, as the filter can burst.
    b. If using a vacuum pump attached to a side arm flask, attach the Buchner funnel and place a 0.45um filter inside the funnel. Wet the membrane with distilled water and start the vacuum to clear the membrane. Discard the water in the flask. Re-wet the filter with a small amount of the wine sample, start the vacuum to rinse the filter. Discard this wine as well (it is still diluted with residual water from the rinsing). Then, filter the full wine sample.
6. Prior to heating, measure the NTU of each sample individually. Each NTU meter will be slightly different, so follow manufacturer's instructions for use.
    a. Generally, check the standards to make sure the meter is calibrated. (If the meter is not calibrated, follow the manufacturer's instructions for calibration.)
    b. Make sure the sample vials are clean prior to use. If needed, clean the vials with distilled water and a lint-free, scratch-free cloth. Avoid leaving fingerprints on the sides of the vial as this interferes with measurement.

    c. Rinse the sample vial with a small amount of filtered wine and discard. Then, fill the sample chamber with filtered wine. Read and record the initial NTU for each sample. Return wine the wine to the sample bottle after reading the NTU.
    d. The initial NTU of filtered wine should be less than 2.0. If the turbidity is higher than this, check the filter and filter again.
7. Transfer samples to glass bottles or tubes that can be heated in the water bath. Take care to record the position of each sample in the bath, as markings on glass containers may be removed by hot water and steam.
8. Incubate the samples in a warm water bath (80°C) for 30 minutes (or 120 minutes, if you prefer). Make sure the water level goes as far up as the sample in the bottle or tube but does not enter the tube. Use parafilm or tube covers if possible. It is likely the temperature of the bath will drop when the samples are put into the bath. Wait until the bath returns to 80°C before starting the timer.
9. After 30 (or 120) minutes, remove tubes from the water bath and allow them to cool to room temperature. Tubes should be allowed to cool for 6 hours to overnight.
10. After samples have cooled to room temperature, measure the turbidity again using the same procedure as above. Record the final turbidity.
    a. Important! Make sure to resuspend any particulates that have settled to the bottom of the tube prior to reading NTU. These are proteins that have come out of solution that should be included in the turbidity number.
11. Calculate the change in turbidity by subtracting the initial NTU from the final NTU. It is expected turbidity will rise as a result of heating. Generally, a change of less than 2.0 NTU is considered heat stable.
12. To clean, rinse the vials several times with hot water, then several times with distilled water. Dry the vials thoroughly before putting them away.

Measuring for CMC stability

CMC products (like Celstab or Claristar) are commonly used to achieve tartrate stability without having to cold stabilize large tanks of wine. These products require that the wine be protein stable prior to treatment because their addition can trigger protein haze formation. (CMC is a carbohydrate that can complex with protein to cause haze.) Though CMC instability has been reported in rare cases to take as long as 48 hours (with a single reported case at 4 months)(Eglantine Chauffour, personal communication), most instability occurs immediately.

If you plan to use a CMC-based product and want to first test to determine your wine is fully protein stable for this addition, the following protocol is recommended:

1. Treat the wine with bentonite to the determined rate. Allow bentonite to settle.
2. Take a sample of the bentonite-treated wine. Filter the wine through a 0.45 um filter.
3. Measure initial NTU. This number should be less than 2.0.
4. Add 1 ml/L CMC product.
5. Perform the heat test as described above (80°C for 30-120 minutes).
6. Measure the change in NTU from the initial test.
7. A difference of less than 2.0 indicates stability.

References

The above protocol was adapted using information from the following sources

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(3) Weiss, K. C.; Bisson, L. F. Effect of Bentonite Treatment of Grape Juice on Yeast Fermentation. *American Journal of Enology and Viticulture* 2002, *53* (1), 28–36.

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