COLLEGE OF AGRICULTURE AND LIFE SCIENCES
FOOD SCIENCE AND TECHNOLOGY
VIRGINIA TECH

WINEMAKERS RESEARCH EXCHANGE

Acids Education Series:
Monitoring Malic Acid Depletion using Paper Chromatography

Materials/Equipment

- Chromatography paper
- Chromatography chamber
- Capillary tubes or pipette tips for sample application
- Wine acid standards: tartaric, malic, lactic & citric
- Chromatography solvent
- Sample(s) to be tested

Procedure *Note: This procedure can take up to 24 hours from start to finish (including passive running and drying time). Throughout the process, avoid touching the chromatography paper directly. Always handle the paper by the edges to avoid depositing oils that will impede sample progress during the chromatography run.*

Setup:

1. Pour enough solvent into the chromatography chamber to fully cover the bottom, approximately 0.5-0.75 cm depth. The solvent should be deep enough that it will wet the chromatography paper, but not so deep that the sample application line will be submerged. Close the lid to allow vapor pressure to build up. Allow 30 minutes to pass before loading paper into the chamber.

Chromatography:

1. Using a pencil, draw a line parallel to the bottom edge of the chromatography paper 2.0 – 2.5 cm from the bottom.
2. Mark positions for sample deposition along the parallel line at 2.5 cm increments. Number each mark and record which sample will be loaded at each mark. Include positions for standards as well as samples. (For example, Lane 1 = malic acid standard, Lane 2 = tartaric acid standard, Lane 3 = Chardonnay.)
3. Place a glass rod or pencil under the bottom edge of the paper so that it is lifted off the lab bench at a slight angle.
4. Dip a capillary tube or clean pipette tip into the first sample. Liquid will be pulled up into the tube or tip by capillary action.
5. Gently place the loaded tube/tip onto the designated mark on the chromatography paper. Capillary action will draw the liquid from the tube/tip onto the paper.
    a. Use a separate capillary tube or pipette tip for each sample to avoid cross contamination.

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   b. Apply enough liquid so that the spot is visible but not so much that the spots bleed into one another.
6. Allow the spots to fully dry, then repeat the procedure (to deposit more of the sample onto the same mark on the paper). Spot a minimum of 4 times to ensure enough sample is present for detection, allowing spots to dry between applications.
7. Allow the paper to dry for at least 30 minutes after the final sample application.
8. Handling the paper only by its edges, roll the paper into a standing tube with the sample spots located along the bottom of the tube. Line up the edges of the paper without overlapping, then staple the sides of the tube together. Make sure the bottom of the tube is flat and even against the workbench so that it will be evenly submerged in solvent once added to the chamber.
9. Position the chamber in a spot where it can run for 8-12 hours without being disturbed. Place the tube into the chamber and ensure all edges of the tube are evenly submerged in chromatography solvent and that the paper is not touching the sides of the chamber.
10. Place the lid on the jar. Capillary action will draw the solvent up the paper. Allow the chromatography to run undisturbed until the solvent reaches the top of the paper. Do not move or jostle the chamber while chromatography is running. The time required for this step is variable depending on the paper being used and usually takes about 8 hours but can be extended over night.
11. When the solvent is within a couple of centimeters from the top of the paper, remove the paper from the chamber.
12. Allow the paper to dry for several hours in a well ventilated area.
13. When dry, yellow spots should appear on a blue/green background.

Interpreting results

1. Use the standards to identify the position of tartaric, malic, lactic and citric acids.
2. Compare spots for each sample with the position of the standards to determine which acids are present and which are absent.
   a. Tartaric acid should be present in all samples and serves as a positive control indicating enough sample has been loaded to be detected.
   b. Lactic acid may be produced by yeast, so the presence of lactic acid does not indicate malolactic fermentation *per se*.
   c. The limit of detection for malic acid by chromatography is near 0.3 g/L. If subsequent winemaking operations require 0 g/L malic acid, results should be validated via enzymatic analysis or by a trusted service lab.

Frequently Asked Questions

I noticed that the last time I ran this sample, the malic acid spot was much darker in color. Does this indicate that malolactic fermentation is happening?
Not necessarily. There may be differences in detection between chromatography runs due to the amount of sample loaded or the freshness of the solvent. This test can determine presence or absence only, not amount.

What if I don't see any spots?
The indicator that is used for detection is pH sensitive. If yellow spots do not appear on a blue/green background after the paper is dried, it is likely the air is too acidic. To expose the spots, open an ammonia-based product near the developing chromatogram to allow for proper development. (A paper tower that has been spritzed with glass cleaner and allowed to dry can be crumpled up and placed near the chromatography paper. The alkaline fumes from the paper towel are usually sufficient to develop the indicator and reveal acid spots.)

What if the solvent runs off the paper? Do I have to re-run the whole thing?
No. As long as the standards have not migrated off the paper, the results are accurate.

The spots are streaky and not localized. What does that indicate?
Poor separation of samples indicates that the solvent needs to be replaced. To prevent this, solvent should be replaced about once per month.

Help! My marker spots and line migrated up the paper, too! What happened?
The pigments in most pens are soluble in the solvent, so they, too will migrate up the paper. Use only pencil to mark the chromatography paper.

Malolactic fermentation seems to be taking forever! I keep testing and I keep seeing malic acid. What does this mean?
Malic acid bacteria will not consume all forms of malic acid. If malic acid has been added for acidulation, full conversion through malolactic fermentation will not occur and there will be residual malic acid.