TRACKING A MALO-LACTIC FERMENTATION

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Monitoring MLF

- MLF – easily monitored by paper or thin-layer chromatographic separation
  - absence of a malic acid spot

- Visual resolution for malic acid limits at approximately 100 mg/L
  - most winemakers prefer 15 to 30 mg/L malic acid to consider their wine “safe”

PAPER CHROMATOGRAPHY

- Whatman No.1 chromatography paper
- Chromatography developing tank
- Separatory funnel
- Micropipettes (20 mL)

PAPER CHROMATOGRAPHY

- Wine acid standards (0.3%) purchased as part of kit or made up
- Chromatography solvent – can be purchased commercially
- 100 mL n-butanol
- 100 mL de-ionized water
- 10.7 mL stock formic acid
- 15 mL indicator solution prepared by dissolving 1 g of water-soluble bromocresol green in 100 mL of de-ionized water.
- Shake solvent mixture thoroughly by repeated inversion of separatory funnel. Allow for phase separation, and discard lower phase.
- NOTE!! Solvent should be prepared fresh on a weekly basis.

PAPER CHROMATOGRAPHY

- Taking care to handle chromatography paper only by the edges, cut a piece of appropriate size to fit into developing tank.
- Using a pencil, draw a line parallel to, and approximately 2.5 cm from the bottom edge of the paper.
- Using micropipettes, spot standard acids and wine samples at equal intervals along baseline. Spots should be of as small a diameter as possible (less than 1 cm). Re-spot at least twice in order to achieve this goal.
- Each spot should be at least 2.5-3.0 cm apart. A hair dryer can be used to assist in drying the spots between applications.

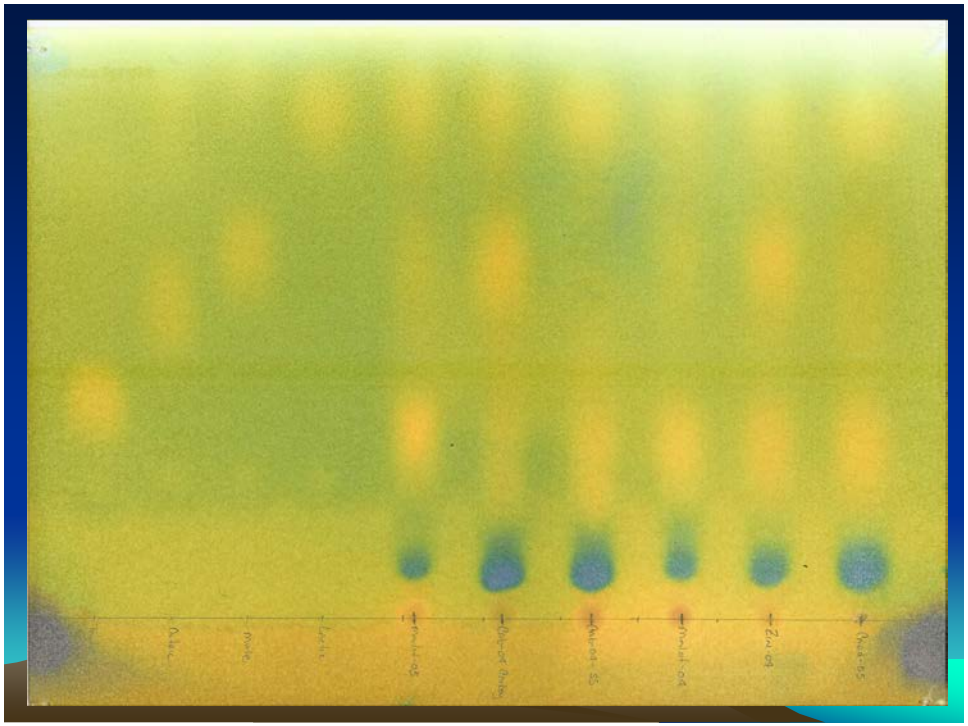
PAPER CHROMATOGRAPHY

- Transfer solvent to developing tank, allowing at least 30 min for vapor saturation to occur (shake tank).
- A minimum depth of 0.75 cm of solvent is required for adequate development.
- Immerse baseline side of paper into tank, taking care that solvent moves uniformly up the paper.

- When the solvent has ascended to near the upper edge of paper, chromatogram may be removed and allowed to dry.

- When dry, results may be interpreted by noting the positions of yellow spots (acids) on blue background. Identification of various wine acids may be made by comparison to standard acids

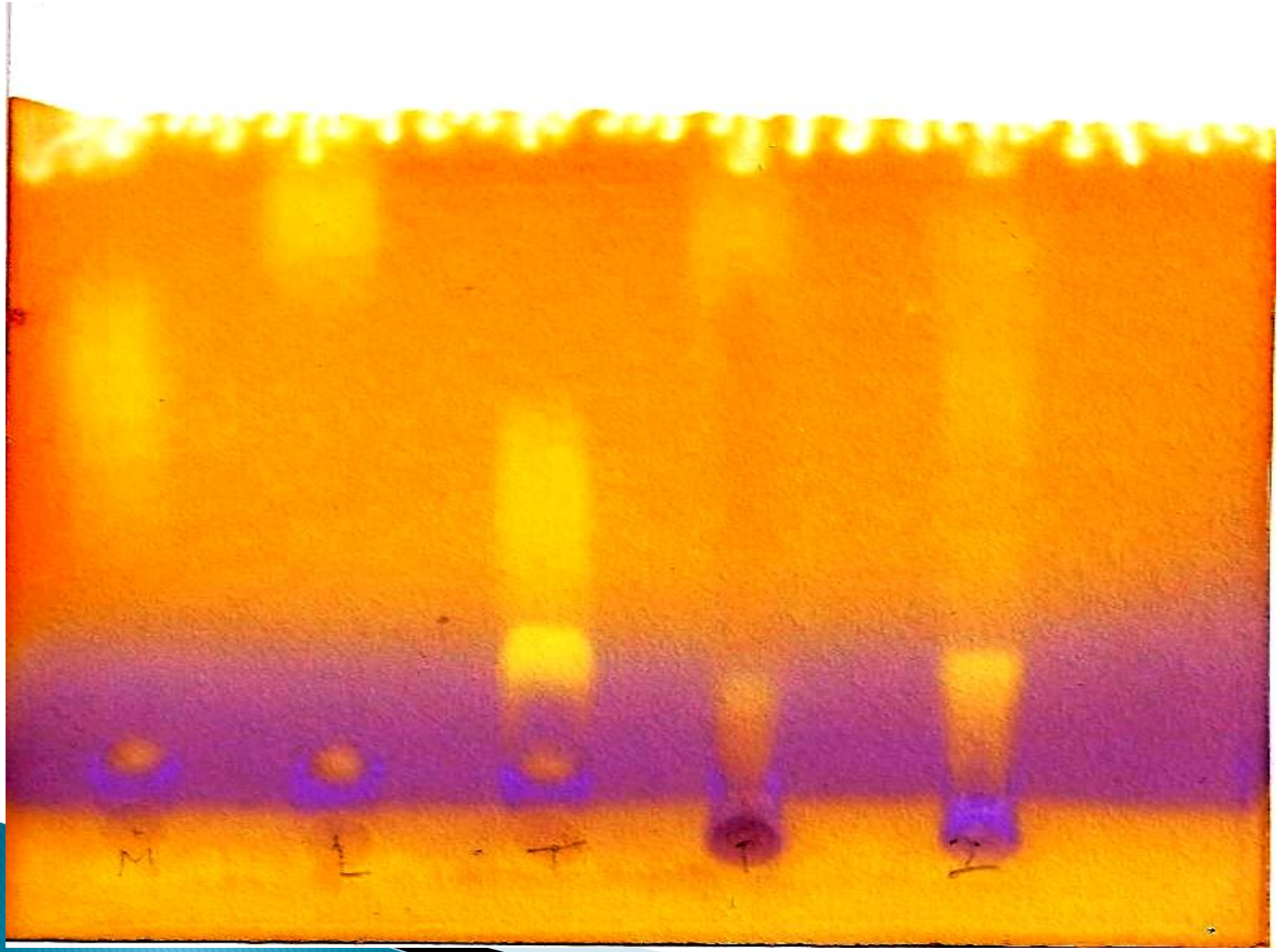
Results



TLC – MALIC ACID

- I. J. T. Baker pre-coated plastic chromatography sheets with microcrystalline cellulose (J. T. Baker 4480 20x20 cm or equivalent)
- Chromatography developing tank
- II. Wine acid standards (0.5%): malic, lactic, and tartaric acids
- III. Chromatography solvent: In a separatory funnel, mix the following:
- 100 mL n-butanol 100 mL deionized water
- 10 mL stock formic acid 0.1 g bromphenol blue indicator

- IV. Spot 10 µL standard acids and wine samples at equal intervals along baseline. Spots should be of as small a diameter as possible. Let spots dry and re-spot another 10 µL. A hair dryer can be used to assist in drying the spots between applications


M
1
T
T
2

Enzymatic Malic Acid

- Limitations on paper chromatographic method ~ 100 mg/L

- Available kits
  - Production of NADH
  - Small sample volumes
  - Need to run standards