NITROGEN: ESTIMATE OF FAN BY FORMOL TITRATION

This is rapid method for estimation of the fermentable nitrogen in juice, wine, or vinegar adapted from Zoecklein et al., 1999 and Gump, Zoecklein and Fugelsang, 2002 The method measures alpha-amino acids and ammonia.

I. Equipment

pH meter sensitive to 0.05 pH unit Electrode (Orion, epoxy body, sure flow, semi-micro or equivalent) 5µm syringe filters, or equivalent 2-mL and 10-mL pipets, or equivalent 25-mL volumetric flasks 10-mL buret (± 0.05 mL) 30-mL beaker mini stirbars

II. Reagents

Calibration buffers for the pH meter Sodium hydroxide (NaOH) 1 N Sodium hydroxide (NaOH) 0.05 N standardized against potassium hydrogen phthalate or equivalent Formaldehyde, reagent grade 37% (vol/vol or 40% wt/vol), neutralized to pH 8.0 with 1N NaOH

III. Procedure

- 1. Check pH of formaldehyde and if not at pH 8.0 neutralize with 1N NaOH
- 2. Clarify the wine sample with a 5μ m syringe filter or equivalent.
- 3. Transfer 10.0 mL of the clarified sample into a 25-mL volumetric flask. Bring to volume with deionized water, and mix well.
- 4. Transfer a 10.0 mL sample of the clarified and diluted wine into a 30-mL beaker, place calibrated pH/reference electrodes and a stirbar into the solution, mix, and adjust the pH of the sample to 8.0 with 1 N sodium hydroxide.
- 5. Add 2.0 mL of the previously neutralized formaldehyde (pH 8.0) to the aliquot, mix, and titrate back to pH 8.0 with the 0.05 N NaOH.
- 6. The concentration of fermentable nitrogen is given as follows:

Fermentable Nitrogen (mg N/L) = (mL of 0.05 NaOH titrated) x 0.05Nx(25/10)x(1000/10)x14

Fermentable Nitrogen (mg N/L) = (mL of 0.05 NaOH titrated) x 175

- IV. Supplemental Notes
- 1. The full equation for calculating assimilable nitrogen is:

 $Mg/L N = (mL of NaOH) \times (0.05meq OH/mL) \times (1mmol N/meq OH) \times (25mL/10mL- dilution factor) \times 1000/10$ (to convert to liters) x 14 mg/mmol N

If a different concentration of base is used, the equation requires one to use the correct normality instead of the 0.05N term. The dilution factor in the equation (25 mL/10 mL) is changed if one uses a sample volume other than 10 mL and dilution to 25 mL.

- 2. Formaldehyde is carcinogenic and a bronchial irritant so handle with proper ventilation.
- 3 The pH of formaldehyde gradually shifts downward. Its pH should be checked and readjusted to 8.0 prior to each use.
- 4. The Formol titration only titrates one nitrogen of arginine, it also titrates approximately 14% of the proline present. These two errors are, at least, partially offsetting.
- 5. A reduced volume Formol Titration procedure and additional information is posted on my web site at www.vtwines.info