Estimation of Yeast Assimilable Nitrogen using the Formol Titration Technique

Successful fermentation of wine requires grapes with adequate level of yeast nutrients. The primary nutrient requirement of yeast is limited by the availability of fermentable nitrogen sources distributed in the form of ammonium ions (NH_4) and certain alpha-amino acids. Yeasts that are deprived of adequate nutrients show signs of stress including the production of hydrogen sulfide and other undesirable byproducts of fermentation. A shortfall of yeast nutrients could be handled by dumping lots of commercially available yeast nutrients (DAP, Fermaid, etc.) into all batches of juice or must. But such a solution is not optimal because (1) overfeed yeast populations can grow too quickly during the reproductive stage of fermentation, and (2) unused yeast nutrients can foster the growth of spoilage microorganisms following fermentation. The **formol titration technique** is a relatively simple procedure that can be used to provide a rough (but useful) estimate of the nutritional status of grape juices and musts expressed in terms of mg/L of **yeast assimilable nitrogen (YAN)**. When formaldehyde is added to a properly conditioned sample of grape juice or must it reacts with the amino acids that provide the major sources of nitrogen used in yeast metabolism. These reactions free-up hydrogen ions (H^+) in proportion to the available nitrogen and, as a result, yield a systematic decrease in the pH of the test sample. The amount of NaOH needed to neutralize the H^+ ions freed by the formaldehyde addition provides an estimate of the nitrogen content provided by amino acids; and, as a bonus, also reflects the nitrogen available as ammonia in the juice or must. Given the results of the formol titration and a determination of the target nutritional requirements of the grapes based upon varietal, climate and viticultural practices, the wine maker can add optimal levels of yeast nutrients.

Procedure

1. Filter a small, representative sample of juice/must using a 5-µm syringe filter or filter paper.
2. Pipette precisely 10 ml of filtered sample to a 25 ml volumetric flask and top-off with distilled water.
3. Pipette 10 ml of the diluted/filtered sample to a small beaker, insert a magnetic stir bar and pH probe. Add enough distilled water to ensure that the pH probe is sufficiently submerged.
4. Excessive levels of SO_2 (previously added to the juice/must) can introduce error into the YAN determination. If SO_2 > 150 PPM, add 1 ml of 3% hydrogen peroxide to neutralize the SO_2.
5. Turn on the magnetic stirrer and titrate the sample with 0.05 N NaOH until neutralized (pH=8.0).
6. Add 2 ml (nominal) of previously neutralized formaldehyde solution to the sample using a small graduated cylinder. The formaldehyde will react with the YAN sources yielding a proportionate release of H+ ions (thus, lowering pH).
7. Record the current level of 0.05 N NaOH in your burette.
8. Titrate the sample with 0.05 N NaOH until pH = 8.0 to neutralize the H+ ions released in Step 6.
9. Record the new level of 0.05 N NaOH in your burette and calculate the amount needed to neutralize the sample following the addition of the formaldehyde solution.
10. Estimate yeast assimilable nitrogen (YAN) level as follows:

YAN (mg N/L) = (ml of 0.05 N NaOH titrant) x 175 [Eq. 1]

Long version of YAN (mg/L) computational formula:

$$\frac{(\text{ml titrant}) \times (0.05 \text{ mmol } OH^-/\text{ml}) \times (14 \text{ mg N/mmol}) \times (1000 \text{ ml/L})}{(10 \text{ ml sample}) \times (10 \text{ ml}/25 \text{ ml dilution factor})}$$

Reagents

0.05 N NaOH [sodium hydroxide] solution (Prepared from a 2:1 dilution of stock 0.1 N NaOH solution)
37% formaldehyde solution
10% NaOH solution (for normalizing formaldehyde)
pH buffers for calibration of pH meter

Equipment (and purpose)

burette and lab stand (titration)
pH meter and probe (titration end point)
magnetic stirrer and small stir bar
30 ml beaker (for sample)
50 ml beaker (normalize formaldehyde)
10 ml volumetric pipette (measuring sample)
25 ml volumetric flask (diluting sample with water)
eye dropper (neutralize formaldehyde)
10 ml graduated cylinder (measure formaldehyde)
5-µm syringe filter (or filter paper)

Neutralizing the 37% formaldehyde solution

During storage, the pH level of formaldehyde solution tends to rapidly drop and can reach a level as low as 3.5 or so. In order to maintain the validity of the formol titration technique, the formaldehyde must be neutralized to a pH level of 8.0 sometime shortly before the procedure is performed. This can be accomplished by pouring a small (e.g., 40 ml) sample of formaldehyde into a beaker, inserting a pH probe and then adding 10% NaOH using an eye dropper until the pH reaches (and holds) a level of 8.0. This neutralized formaldehyde should then be transferred to a sealed storage jar and used within 24 hours for optimal results. NOTE: Formaldehyde is nasty stuff. You need to make sure that you avoid any skin contact and also must avoid breathing its fumes. Needless to say, good ventilation is a must.

Formol Titration Practice Run

When learning a new analytical technique it's always helpful to have a "known" sample with which to experiment. In the case of practicing the formol procedure, you probably already have a quantifiable source of yeast assimilable nitrogen that you can use for such purposes. Almost all home wine makers keep a supply of diammonium phosphate (aka DAP) on hand as a source of yeast food for their juices and musts. Since DAP is 21.2% nitrogen by weight, it follows that an aqueous solution of 1 g/L of DAP contains a nitrogen concentration of 212 mg/L. Hence, DAP and distilled water can be used to create (unbuffered) solutions with known nitrogen concentrations that can be used to test the accuracy of your formol titration technique.

Case Study:
Create an aqueous test solution with a 212 mg/L nitrogen concentration by adding 1g of DAP to 1 liter of distilled water. Use this sample to run a test of the formol titration procedure.

Results:
A total of 1.25 ml of 0.05 N NaOH was required to neutralize the sample following addition of the formaldehyde. Using equation 1, the YAN level was estimated as 1.25 x 175 = 219 mg/L Nitrogen. This estimate is within 3% of the known nitrogen concentration of the aqueous DAP test solution. This suggests that the formol titration technique was performed correctly since its margin of error is typically in the vicinity of 10%. Since all of the prep is done, try performing the test a few more times to see if your results are reliable.

Supplementing YAN Levels in Juice or Must

The primary technique used for increasing YAN levels in juice or must is the addition of yeast nutrients containing **diammonium phosphate** – $(NH_4)_2\ HPO_4$ – better known as DAP. The amount of nitrogen added per unit DAP supplement is typically reported in one of two ways: (1) 1g DAP/L increases yeast assimilable NH_3 (ammonia) by 258 mg/L, or (2) 1 g DAP/L increases yeast assimilable nitrogen by 212 mg/L. These values are derived as follows:

1g DAP/L = NH_3 mol. wt./DAP mol. wt. = $2(NH_3)/132 = 2\ (14+3)/132 = 34/132 = 0.258$ g NH_3/L
1g DAP/L = Total N mol. wt./DAP mol. wt. = $2N/132 = 2(14)/132 = 28/132 = 0.212$ g N/L
DAP is 25.8% ammonia (NH_3) by weight or 21.2% nitrogen (N) by weight.
Note: **Fermaid-K** is 13% Nitrogen by weight…i.e., 1 g/L of Fermaid K boosts the YAN by 130 mg/L.

What is the Optimal YAN Level

There is no consensus answer to this question. Optimal YAN values depend upon the grape cultivar, viticultural practices, Brix level, yeast variety and other factors. The Nanaimo Winemaker's web page has an excellent set of guidelines for estimating YAN target values and instructions for using DAP or Fermaid K to achieve these values (see reference below).

References

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Nitrogen: Estimate of FAN by Formol Titration
http://www.fst.vt.edu/extension/enology/downloads/FermNitro.pdf

Adding Nitrogen to Fermentations. Nanaimo Winemakers of Vancouver Island, British Columbia.
http://www.nanaimowinemakers.org/Winemaking/General/AddingNitrogen.htm