UCDAVIS COOPERATIVE EXTENSION

CONCENTRATION OF PRIMARY AMINO ACIDS IN GRAPE JUICE

NOPA Procedure

Butzke & Dukes 1998[1]

JUICE PREPARATION

1. Clarify the juice by centrifugation or cold settling.

OPA/NAC REAGENT PREPARATION

The reagent solutions, sufficient for about 300 analyses, are a modification of that used by Medina-Hernandez et al., 1990[2].

1. REAGENT SOLUTION A consists of 0.671 g OPA dissolved and made to 100 mL with 95 %vol ethanol. This OPA solution is then added to a 1000 mL volumetric flask containing an aqueous solution of 3.837 g NaOH (s), 8.468 g of boric acid (s) and 0.816 g NAC (s). The solution is made to volume with deionized water.
2. REAGENT SOLUTION B consists of 100 mL 95 %vol ethanol added to an aqueous solution of 3.837 g NaOH (s), 8.468 g of boric acid (s) and 0.816 g NAC (s). The solution is made to volume with deionized water.

The two reagent solutions can be stored in a refrigerator for up to two weeks.
When used, the solutions should be at room temperature.

ASSAY METHOD

Blank

1. 50 µL of water are pipetted into a standard UV-grade methyl acrylate or quartz cuvette using a digital pipette.
2. 3000 µL of REAGENT SOLUTION A are added using a Repipet®II dispenser.
3. Zero the UV spectrophotometer at 335 nm with this "BLANK".

Sample

4. 50 µL of the juice sample are pipetted into a cuvette. A dilution of the juice may be needed if its nitrogen concentration grossly exceeds the range of the standard curve (>200 N mg/L).
5. 3000 µL of REAGENT SOLUTION A are added.
6. Stir mixture well. This solution is the "SAMPLE".
7. Record gross sample absorbance A_{335} of the sample after 10 minutes at 335 nm.

Juice Blank

8. 50 µL of the (diluted) juice sample are pipetted into a cuvette.
9. 3000 µL of REAGENT SOLUTION B are added.
10. Stir mixture well. This solution is the "JUICE BLANK".
11. Record absorbance A_{335} of the sample after 10 minutes at 335 nm.
12. Calculate net **ABSORBANCE = "SAMPLE" – "JUICE BLANK"**

Duplicate determinations are suggested. Record and store all data generated for future analysis!

STANDARD CURVE AND CALCULATION OF PRIMARY AMINO NITROGEN CONCENTRATION

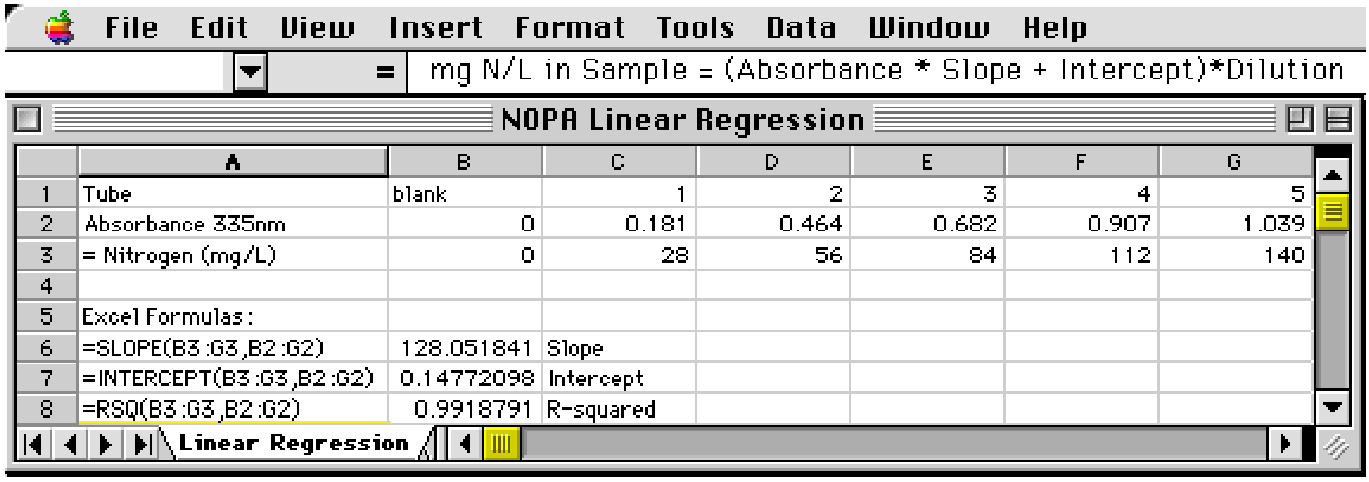
The standard curve is prepared from a 10 mM isoleucine stock solution which is made by dissolving 0.328 g of ile (s) in deionized water in a 250 mL volumetric flask. (2 mL aliquots of this standard can be frozen in plastic microcentrifuge tubes for later use.) The suggested standard concentrations range from 2 to 10 mM ile, which corresponds to 28 – 140 mg Nitrogen/L.
If the sample concentration exceeds this range, a dilution of the sample is suggested.
Any dilution is later compensated for by multiplication with a dilution factor.

Tube	blank	1	2	3	4	5
10 mM ile (µL)	0	10	20	30	40	50
DI water (µL)	50	40	30	20	10	0
OPA reagent (µL)	3000	3000	3000	3000	3000	3000
Absorbance $_{335nm}$						
= Nitrogen (mg/L)	0	28	56	84	112	140

The net ABSORBANCE of the juice sample is used to calculate the concentration of primary amino nitrogen using the linear regression equation of the standard curve and the appropriate dilution factor of the sample:

mg N/L in Sample = (Absorbance * Slope + Intercept) * Dilution Factor

An MS Excel™ spreadsheet can be created for the standard curve:



	A	B	C	D	E	F	G
1	Tube	blank	1	2	3	4	5
2	Absorbance 335nm	0	0.181	0.464	0.682	0.907	1.039
3	= Nitrogen (mg/L)	0	28	56	84	112	140
4							
5	Excel Formulas:						
6	=SLOPE(B3:G3,B2:G2)	128.051841	Slope				
7	=INTERCEPT(B3:G3,B2:G2)	0.14772098	Intercept				
8	=RSQ(B3:G3,B2:G2)	0.9918791	R-squared				

NB: The NOPA procedure measures primary amino acids only.

The second main source for yeast assimilable nitrogenous compounds (YANC) are ammonium ions which have to be measured separately since both sources are not correlated[3]. Ammonia can be determined using an ion selective probe, or an enzymatic test kit (Sigma Diagnostics Kit 171-B $84.00/100).

Suggested COMBINED YANC concentrations:
200 mg N/L at 21 Brix
250 mg N/L at 23 Brix
300 mg N/L at 25 Brix
350 mg N/L at 27 Brix

(These are preliminary recommendations; Analysis of more NOPA data generated over time will provide a more accurate deficiency assessment.)

EXCESS nitrogen supplementation is undesirable !
Legal limit for addition of diammonium phosphate (DAP): 8 lb/1000 gal = 203 mg N/L

CHEMICALS & SUPPLIES

• N-acetyl-L-cysteine (NAC)	Sigma	A 9165	5 g	$11.15	Sigma Chemical
• Ortho-phthaldialdehyde (OPA)	Sigma	P 1378	1 g	$ 9.45	1-800-325-3010
• L-Isoleucine (ile)	Sigma	I 2752	1 g	$ 5.45	www.sigma.com
• Boric acid	Sigma	B 0252	500 g	$14.10	
• Sodium hydroxide	Sigma	S 5581	500 g	$17.25	
• Ethanol, denat. (HPLC Grade)	Sigma	27,074-1	1 L	$24.15	
• Cuvettes (methyl acrylate)	Fisher	14-385-996	4.5 mL (500)	$95.38	Fisher Scientific
• Pipette tips	Fisher	21-197-8E	200 µL (960)	$47.58	1-800-766-7000
• Pipette tips	Fisher	21-197-8A	1000 µL (1000)	$48.71	www.fishersci.com

(List prices)

EQUIPMENT

- Spectrophotometer capable of detection at 335 nm (UV range)
- Analytical balance to 0.000 g
- Rainin Pipetman® digital pipettes P-200/P-1000
- Repipet®II dispenser

LITERATURE CITED

1. **Dukes B.C. and Butzke C.E.**
   Rapid Determination of Primary Amino Acids in Must Using an OPA/NAC Spectrophotometric Assay.
   American Journal of Enology and Viticulture. 49(2):125-133 (1998).
2. **Medina-Hernandez, M.J., Camanas, R.M., Cuenca, E.M. and Alvarez-Couque, M.C.**
   Determination of Protein and Free Amino Acids Content in a Sample Using OPA and NAC.
   Analyst. 115:1125-1128 (1990).
3. **Butzke C.E.**
   Survey of Yeast Assimilable Nitrogen Status in Musts from California, Washington and Oregon.
   American Journal of Enology and Viticulture. 49(2):220-224 (1998).

ABSTRACT

Low levels of yeast assimilable nitrogenous compounds (YANC) in grape juice have been associated with sluggish or stuck fermentations. In contrast, excessive levels may lead to the increased formation of ethyl carbamate. Wine yeasts mainly consume primary amino acids and ammonium during vinification. While the ammonium fraction is easily determined by the winemaker, no winery-suitable analytical method has been available to estimate the primary amino nitrogen fraction. This research evaluated a spectrophotometric procedure to measure the primary amino nitrogen fraction. The assay was based on the derivatization of primary amino groups with an *o*-phthaldialdehyde/N-acetyl-L-cysteine (OPA/NAC) reagent. The resulting isoindole derivatives formed rapidly and were stable at a wavelength of 335 nm. The procedure used a juice blank to account for the absorbance of non-derivative forming compounds in the juice, particularly phenolics. The assay was insensitive to proline, showed a 3.5% response to ammonium ions, and correlated with HPLC estimates (R^2 = 0.988). The relative standard deviations for replicated analyses ranged from 0.27 to 3.20%. Estimates made using this procedure were within the systematic errors for model solutions, and known additions of isoleucine to grape juices had recoveries of 97.6 to 101.3%. The relative accuracy, precision, rapid analysis times and use of low-toxic reagents suggest that this procedure, dubbed NOPA for 'nitrogen by OPA', is appropriate for use in wineries.

During the 1996 harvest on the US West Coast, the overall variation in combined YANC ranged from 40 to 559 mg/L with an average of 213 mg/L. Primary amino nitrogen concentration ranged from 29 to 370 mg N/L, that of ammonium ions from 5 to 325 mg N/L.