ACCUVIN, LLC

AV – Malic Acid

Cat. no. 223

Intended Use

AV-Malic Acid is intended for measuring the Malic Acid level of wine that is undergoing or has undergone malolactic fermentation. It can also be used for grape juice and must analysis.

Methodology

AV-Malic Acid is based on the change in color exhibited by a tetrazolium color indicator during a reaction involving malic acid and nicotine-adenine dinucleotide (NAD) in the presence of the enzyme malate dehydrogenase.

$$\text{L-malate + NAD} \xrightarrow{\text{MDH}} \text{oxaloacetate + NADH}$$

$$\text{NADH + Color Agent (oxidized)} \xrightarrow{\text{Diaphorase}} \text{NAD + Color Agent (reduced)}$$

Sample

Samples of wine may be used as is. Grape juice and must samples should be diluted 1:20 prior to analysis if the expected value is greater then 500 mg/L. Use ACCUVIN Quick Dilute tubes. The ACCUVIN AV-Malic Acid patent pending test strip removes the usual interferences from colored and turbid samples. Samples do not have to be pre-filtered or treated with color removing substances such as activated carbon or polyamide powder. Sample temperature may be from 0°C - 35°C (32°F - 95°F).

Procedure

1. Squeeze upper sampler bulb once. Dip sampler tip into wine, must or grape juice sample, then release to aspirate sample. (If you prefer to use an air displacement pipette, set sample volume at 20 μL.)
2. Transfer sample to rectangular absorbent layer on back of test strip by squeezing sampler bulb. ***Apply slight pressure with sampler tip*.** Allow sample droplet to absorb into absorbent layer. Note that only sample present in the sampler tip will be dispensed. Wait 4 min. for color development.
3. Determine sample malic acid level in mg/L by comparing the developed color to the color chart on the test strip container. If test strip color falls between two color chips select an intermediate value for the sample malic acid level. Note that if a sample was diluted prior to analysis, the sample malic acid level is 20 times the level obtained from the color chart. (***Since fluorescent lights have a green cast, color matching is best under incandescent or natural lighting**.)*

Storage

Store away from direct sunlight at temperatures below 80ºF. Keep dry. Product is satisfactory until the date printed on the test strip container label.

ACCUVIN, LLC
P.O. Box 5328
Napa, CA 94581
Phone, fax: (707) 255-2029

www.ACCUVIN.com

for technical inquiries: email: techinfo@accuvin.com

Summary Interpretation for Most Wines

(Because of varietal & stylistic differences, growers & winemakers should make final interpretations.)

Malic acid levels in grape juice vary widely from 10 g/L early in the season in grapes for white wines grown in cool climates to 3 g/L in red and white wine varieties grown in warmer climates. Malic acid levels tend to decrease faster than tartaric acid levels as the period of optimum ripeness approaches, especially in warmer growing regions. Malic acid levels, in conjunction with titratable acidity, pH and sugar, can be used to determine the best time to harvest. Note that some varieties have higher percentages of malic acid at optimum ripeness than others.

Malolactic fermentation is a method for reducing total titratable acidity and raising pH by adjusting the relative concentrations of L-malic and L-lactic acids, thus softening the wine and, for a red wine, allowing it to develop mellowness and full-bodiedness."[1] In addition, a number of other changes occur in wine during this process. Diacetyl, a compound that adds complexity at concentrations between 1 and 4 mg/L, is increased, as are the concentrations of acetoin, acetaldehyde and 2,3-butanediol and other sensory-enhancing compounds. A final benefit of malolactic fermentation is enhanced microbiological stability.[2] In fact, for better control of quality a winemaker usually desires to have the secondary, malolactic, fermentation completed as soon after alcoholic fermentation as possible to allow for finishing operations to be completed, and thus to protect stored wine from the jeopardy of harmful microorganisms.[3] It is also important to know promptly that MLF has been completed, since spoilage bacteria can continue to grow until the winemaker intercedes through clarification or the addition of sulfur dioxide.[7, 8]

The presence of L-lactic acid is not sufficient proof of the occurrence of malolactic fermentation since it may also be produced from other compounds in the wine. Paper chromatography is sometimes used to monitor MLF, however this method is basically qualitative and suffers from a sensitivity threshold of 100 mg/L.[4, 6] Detection levels down to at least 30 mg/L are considered necessary for ensuring malolactic fermentation is complete.[2, 9]

A.	Juice Sample (diluted)	≥ 3 g/L	- consider harvesting if levels are falling and/or if titratable acidity is below 7 g/L.
B.	Wine or Must Sample (diluted)	≥ 2 g/L	- consider malolactic fermentation, especially if red wine titratable acidity (TA) is above 7 g/L (as tartaric acid). This information can also be used to estimate final titratable acidity levels post-MLF.
C.	Wine Sample (undiluted)	≤ 30 mg/L	- malolactic fermentation is complete.
		≥ 75 mg/L	- malolactic fermentation is <u>not</u> yet complete.

References

1. Peynaud, E., *Knowing and Making Wine,* John Wiley and Sons, New York, **1984**. pp. 120-131.
2. Hennick-Kling, T. and T. E. Acree, "Modification of Wine Flavor by Malolactic Fermentation," Vignavegni, **1998**
3. Boulton, R. B., V. L. Singleton, L. F. Bisson, and R. E. Kunkee, **Principles and Practices of Winemaking**, Chapman and Hall, New York (1996)
4. Cooke, G. M., and H. Berg, A re-examination of table wine processing practices in California. I. Grape standards, grape and juice treatment and fermentation, *Am. J. Enol. Vitic*. **34**, 249 – 256 (1983)
5. Zoecklein, B. W., K. C. Fugelsang, B. H. Gump, and F. S. Nury, **Wine Analysis and Production**, Chapman and Hall, New York (1995)
6. Gilis, M., H. Durliat and M. Comtat, "Electrochemical biosensors for assays of L-Malic and D-Lactic acids in wines, Am. J. Enol. Vitic., 47 (1): 11 – 16 (1996)
7. T. Hennick-Kling, T. E. Acree, S. A. Krieger, M-H. Laurent, and W. D. Edinger, Modification of wine flavor by malolactic fermentation, *Wine East*, 4: 8 – 15, 29 – 30 **1994**.
8. C. R. Davis, D. J. Wibowo, T. H. Lee and G. H. Fleet, Growth and metabolism of lactic acid bacteria during and after malolactic acid fermentation in wines at different pH," Appl. Environ. Microbiol., 51: 539 – 545 **1986**.
9. K. C. Fugelsang, **Wine Microbiology**, Chapman & Hall (1997)

223C, July 2006