ACCUVIN, LLC

AV – D-Lactic Acid

Cat. no. 280

Intended Use

AV-D-Lactic Acid is intended for measuring the D-Lactic Acid level of grape juice, must, and wine as an indicator of Lactic Acid Bacteria contamination.

Methodology

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

$$\text{D-lactate + NAD} \xrightarrow{\text{D-LDH}} \text{pyruvate + NADH}$$

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

Sample

Samples of grape juice, must and wine may be used as they are. The ACCUVIN AV-D-Lactic 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

Step 1 Quick Dilution

1. Squeeze sampler bulb. Dip sampler tip into juice, must or wine, then release bulb to fill. Wipe sampler tip to remove excess droplets.
2. Open tube cap, place sampler tip into dilution mixture and squeeze sampler bulb ***only once***. Withdraw sampler before releasing bulb. Replace cap and shake.

Note: Step 1 dilutes the sample 1 to 4, and reduces the concentration of interfering substances.

Step 2 D-Lactic Acid Quick Strip Test

1. Squeeze upper sampler bulb. Dip sampler tip into the diluted 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 the rectangular absorbent layer on back of test strip by squeezing sample 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 D-Lactic 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 lactic acid level. (***Since fluorescent lights have a green cast, color matching is best under incandescent or natural lighting.)***

Note: Multiplying the answer by 4 has been done. The color chart reflects the concentration of D-Lactic acid in the original sample.

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.

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Summary Interpretation for Most Wines

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

Lactic acid bacteria are important in wine production. They are responsible for the malolactic fermentation that occurs in many wines, reducing the wine's total titratable acidity, softening the wine,[1] producing a broader palate of flavors, and enhancing the wine's microbiological stability.[2] Unfortunately, these same bacteria may inhibit primary alcoholic fermentation,[9] and can also cause development of a range of off flavors known as lactic taint, *piqûre lactique* in France, and *spunto lattico* in Italy.

How does this occur? It's related to bacterial metabolic pathways. Under preferred conditions of moderate wine pH, sufficient nutrients, warmer temperatures, and low carbohydrates (sugars), lactic acid bacteria (LAB) convert L-malic acid to L-lactic acid, and deliver the improvements listed above. However, under many other conditions they deliver excess lactic acid, produce acetic acid (volatile acidity), generate bitter acrolein from glycerol, cause "ropy" wines by metabolizing residual sugars, produce excess levels of buttery diacetyl, cause "mousy" wines, and produce ethyl carbamate from arginine. They also can form biogenic amines like histamine and tyramine, chemicals which cause allergic reactions in susceptible individuals.[3, 13, 14]

Where do LAB come from? Many strains of LAB have been found at low levels on grapes. Where fruit damage has occurred, levels are considerably higher. In one study, LAB were found on 9 of 21 batches of undamaged grapes, but on 16 of 22 batches with damaged grapes.[4] In another study, when sampled during fermentation, 31% of Oregon wines were found to contain detectable levels of Lactobacillus sp., with one winery showing a contamination rate of almost 80%.[10] LAB can also be introduced from inadequately sanitized pumps, valves, and transfer lines, as well as from almost-impossible-to-sterilize cooperage.[3] Given the conditions at crush of high sugars, warmer temperatures, and higher than desired pH levels, LAB can multiply rapidly. They generally decrease during primary fermentation as a result of their susceptibility to higher alcohol levels, but LAB populations can reemerge at troublesome levels during malolactic fermentation, or during aging and storage, if protections are inadequate.[11, 12] Monitor often! Grapes themselves do not produce lactic acid. ***D-Lactic Acid,[3, 13, 14] at levels above 300 mg/L,[13] is related to contamination!***

How do we control them? LAB generally do not grow in lower pH wines, exhibiting reduced growth in wines with a pH below 3.5, and essentially no growth when the pH is below 3.2, a corrective action available with white wines but typically not reds.[2, 7] Higher alcohol levels (>13%) slow their growth, but are not lethal. The LAB are susceptible to SO2, especially at total SO2 levels above 70 ppm. Lysozyme has been found useful for control of LAB at levels of 100 – 250 mg/L, especially during crush, cold soak, and primary alcoholic fermentation when malolactic fermentation is planned.[5, 6, 8] Since grapes do not produce lactic acid, regular monitoring throughout winemaking and aging, i.e., regular measurement of D-Lactic Acid levels, can be used as an indicator of the onset of bacterial contamination and allows for minimally invasive yet effective control of lactic acid bacteria infections.

References

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