Changes in yeast available nitrogen (YAN) concentrations during alcoholic fermentation

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The importance of nitrogen as a nutrient for wine grapes, in the vineyard, and for wine yeasts, during fermentation, is now well accepted (e.g., Keller, 2005; Pambiancho, 2011; Nanaimo Winemakers "nanaimowinemakers.org"). In addition to serving as a nutrient, the use of at least one nitrogen source (diammonium phosphate or DAP) can also affect the flavour and nature of wine (Ugliano et al., 2007). When nitrogen is used as a nutrient however, its chemistry becomes important – and can be confusing! Yeasts are able to use nitrogen in an inorganic form – as ammonia or ammonium (e.g. diammonium phosphate or DAP) and when it is in an organic form – in amino acids. When nitrogen is in amino acids however, it is only available when it is in a relatively free chemical state (i.e., free amino nitrogen or FAN). In spite of that, FAN is the main source of nitrogen used by yeasts during fermentation.

Yeasts require nitrogen for the proteins used by the cell. This is true especially during fermentation when cells are rapidly increasing in number – the exponential phase of growth. At this time, the need for nitrogen is greater than when cells are adapting to a new must or when cell production decreases or stops – the stationary phase – or at the end of fermentation when cells are dying off. As a result of these changes, the provision of adequate nitrogen, in an available state (YAN), is essential during the exponential phase while lesser amounts are required or not needed during the later phases of alcoholic fermentation. In fact, adding nitrogen at the end of fermentation can be detrimental, potentially aiding in the growth of unwanted organisms.

Nutrient supplementation is done at intervals during alcoholic fermentation. Although interval timing is open to debate – often heated

debate – there are very good discussions of nitrogen usage and when to supplement in Leonardelli (2014). Timing can be critical – periodic supplementation may be a feast to famine situation – lots of nitrogen for a short time and then nothing – starvation. It is also important to consider the type of nutrient supplement to use. One needs to look beyond nutrient trade names – find out what is in the nutrient and why it is present. Keep in mind that nutrients are for yeasts – do they need just nitrogen (e.g., DAP) or do they need a range of minerals and vitamins as well as nitrogen (e.g., Fermaid-K[1])? The question that is being addressed in this document is what changes occur in the concentration of YAN during alcoholic fermentation? Information from the study will hopefully help in our understanding of YAN usage by yeasts during alcoholic fermentation.

Measurements of YAN concentrations were made almost daily during the alcoholic fermentation of a cabernet sauvignon must and two syrah musts. Data for degrees brix and YAN are compared for all three musts with greater details provided for the cabernet sauvignon must which started with a much higher YAN concentration and one syrah must which had a very low initial YAN concentration. YAN levels were measured with a formol titration technique that is specific for free amino nitrogen levels but also provides an estimate of the nitrogen available as ammonia. Even though the technique does not provide a complete analysis of YAN it does provide an estimate that can be used to trace changes that occur during alcoholic fermentation.

Each of the three musts was prepared from 500 pounds of Washington State grapes harvested in the Fall of 2014. Fermentation of each must was in a 50-gallon plastic drum. Potential overfill, anticipated for must

[1] Scott Laboratories Fermaid® K is a blended complex yeast nutrient that includes nitrogen as well as essential organic and inorganic agents.

expansion during peak fermentation, was placed in a 20L plastic bucket and partially exchanged with the drum daily. Syrah grapes from the first vineyard were picked on September 18, 2014, and then shipped to New Westminster where crushing and destemming occurred on September 20. Syrah grapes from the second vineyard were picked on September 29, crushed and destemmed on September 30, at the vineyard, then refrigerated in the 50-gallon fermentation drum until shipment to Burnaby, British Columbia for pickup on October 2. Cabernet Sauvignon grapes from the second vineyard were picked on October 21, crushed and destemmed on October 22, at the vineyard, then refrigerated in the 50-gallon fermentation drum until shipment to Burnaby, British Columbia for pickup on October 24.

Musts were treated with potassium metabisulfite and lysozyme shortly after pickup. Scottzyme Color Pro[2] and Lallzyme-EX[3] were added and pH adjustments were made with tartaric acid before inoculation, to bring the pH in each must to between 3.6 and 3.7[4]. Must samples drawn for specific gravity measurements, and degrees brix calculations, were through a fine metal screen, which means the degrees brix values were slightly higher than if the must samples had been filtered or centrifuged. Portions of the same samples were allowed to settle or were filtered for pH and YAN measurements. Nutrient supplementation was with GoFerm[5] (added during yeast rehydration), DAP and Fermaid-K. (A small

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[2] Scottzyme® Color Pro is a specialty pectinase with pro-tease side-activities.

[3] Lallzyme EX is a blend of pectinase and hemicellulase specially formulated to improve color stability and enhance mouthfeel in red wines.

[4] The choice to reduce pH to between 3.6 and 3.7 was to minimize the amount of potassium metabisulfite necessary to achieve an 0.8 molecular SO_2 level after alcoholic fermentation; pH adjustment should be done prior to yeast inoculation.

[5] Scott Laboratories Go-Ferm® is a natural yeast rehydration nutrient containing a balance of micronutrients.

amount of yeast energizer and two small amounts of Bi-Activ[6] were used with the #1 syrah.) Both degrees brix and YAN concentrations were used to indicate times for supplementation and choice of nutrient types, and levels, for supplementation. 50 grams of Lalvin ICV D-21 yeast was used for each of the three musts. Analysis of YAN followed the techniques in http://www.moundtop.com (Schreiber, MoundTop Microvinification) using a Hanna Instruments HI 2210-1 benchtop pH meter. (The formol titration technique is a modification of the technique in Zoecklein et al. (1995) and Gump et al (2000).) Analysis duplication was not possible so technique error is unknown; the moundtop description does provide a means of checking techniques using a known sample – which was used to insure the accuracy of the technique was with 10% of the predicted value. Values of initial specific gravity, degrees brix, pH, and initial YAN level are provided in table 1. Changes that occurred during alcoholic fermentation, and levels and nature of added nutrients, are shown in figures 1-5.

The three musts provided a wide range of initial YAN levels, from 70 mg/L for the #1must (syrah) to over 300 for the #3 must (cabernet sauvignon). As shown in figure 1, the rapid drop in degrees brix after inoculation for all three musts, followed the characteristic inverted S-shaped curve for alcoholic fermentation. In contrast, the decrease in YAN after inoculation was abrupt (figure 2), more evident in the cabernet sauvignon (#3), where initial YAN concentration was high, but also evidenced in the two syrah musts (#s 1 & 2) by the decrease after inoculation in the #2 must and the decrease after addition of GoFerm in the #1 must. Especially with the #1 must, the available nitrogen, as well as other nutrients in the GoFerm used in yeast rehydration, may have been important during yeast acclimation and the beginning of active

[6] Laffort Bi-Activ is a specific formulation based on yeast cellular envelopes (yeast hulls).

fermentation.

When comparing the changes in degrees brix and YAN in the cabernet sauvignon must (#3, figure 3), it is apparent that even with the high initial YAN, nitrogen supplementation needed to be considered early; waiting for a 10% drop in brix before supplementation may have lead to unwanted fermentation problems. One obviously needs to consider the unique needs of each type of yeast as well as the must YAN value; in addition to nitrogen rich nutrients like DAP, complex nutrients like Fermaid-K provide the range of organic and inorganic agents that with nitrogen, will enable normal alcoholic fermentation.

When comparing total amounts of YAN – initial plus added (figures 4 & 5) – keep in mind that even with the high amounts of YAN added to the #1 must, the low daily YAN values (figure 2) suggest a need rather than an excess. Although total amounts of supplemented YAN were quite different, the ratio of DAP to GoFerm was similar in both the #1 (DAP = 58%) and #3 (DAP = 62%) musts. In considering these values now, it would probably have been better to have a higher ratio of DAP in the #1 must due to the very low initial YAN value. This is a strong indication that designing a supplementation pattern really needs both the benefit of experience and measurement as well as more than a little luck!

Since measurement of YAN is demanding and the formol titration technique can be dangerous, measurement is not something to be done by every wine maker. At present, there is no BRIX:YAN algorithm that can provide what is needed although there is information on nitrogen demands of yeast strains during fermentation (e.g., Manginot et al., 1998). It is important to realize that measuring YAN before the start of fermentation does not indicate how much the yeasts will need; it only provides an indication of how much available nitrogen is present. The one thing that has become obvious from the present study is that supplementation – especially slight supplementation with DAP – should

be considered very early in alcoholic fermentation. In providing supplementation through the entire period of alcoholic fermentation however, it is essential to provide the broad range of nutrients, minerals and vitamins that are necessary for the normal growth of yeast cells.

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Table 1. Grape must details				
Grape	Initial pH	pH at inoculation	Brix at inoculation	YAN (mg/L) prior to inoculation
#1 - Syrah	3.94	3.59	26.10	70
#2 - Syrah	4.01	3.73	26.70	131
#3 - Cabernet Sauvignon	3.83	3.61	26.30	350

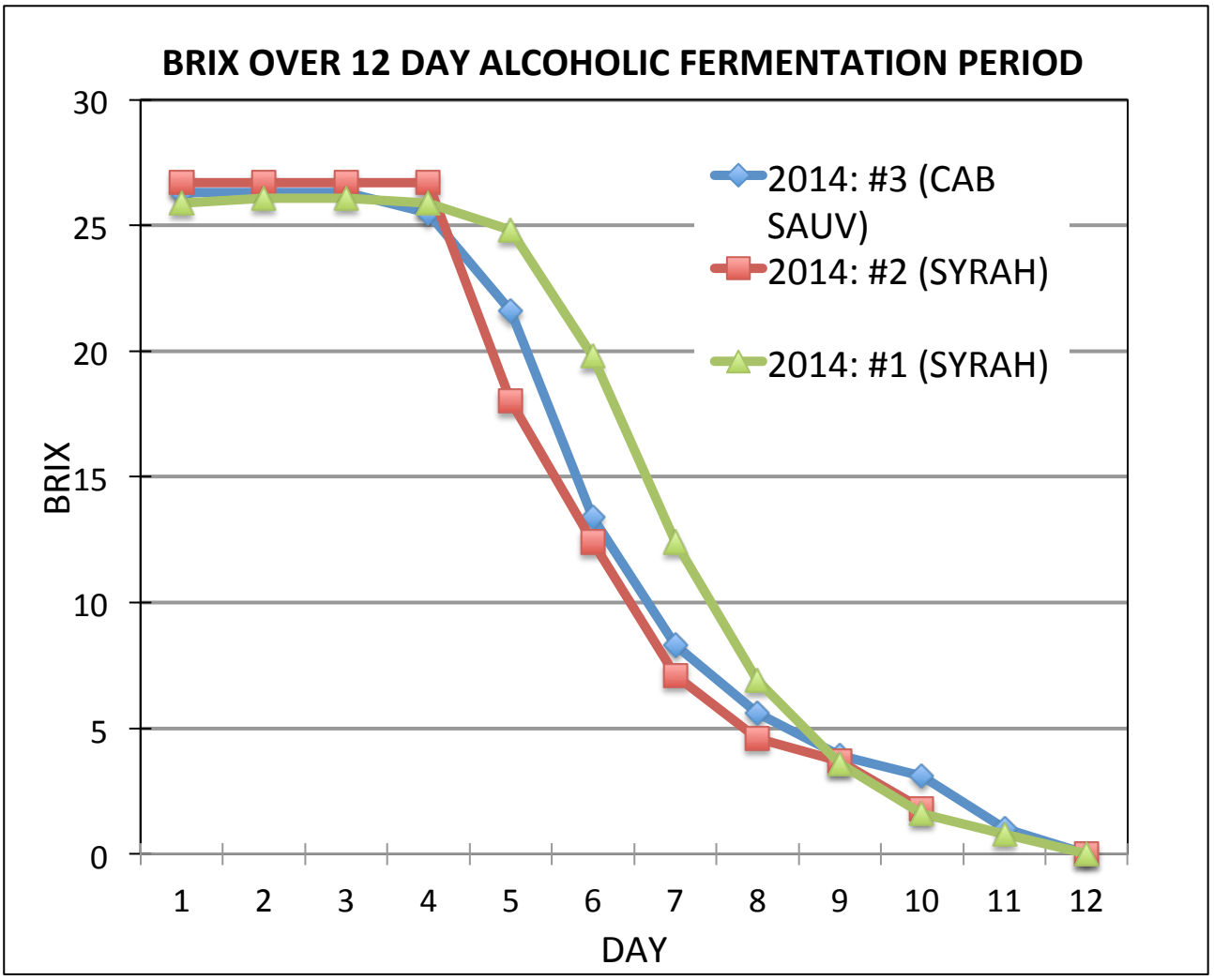

Figure 1. Degree brix values during the alcoholic fermentation period of three grape musts. (Yeast inoculation occurred on either day 3 or day 4.)

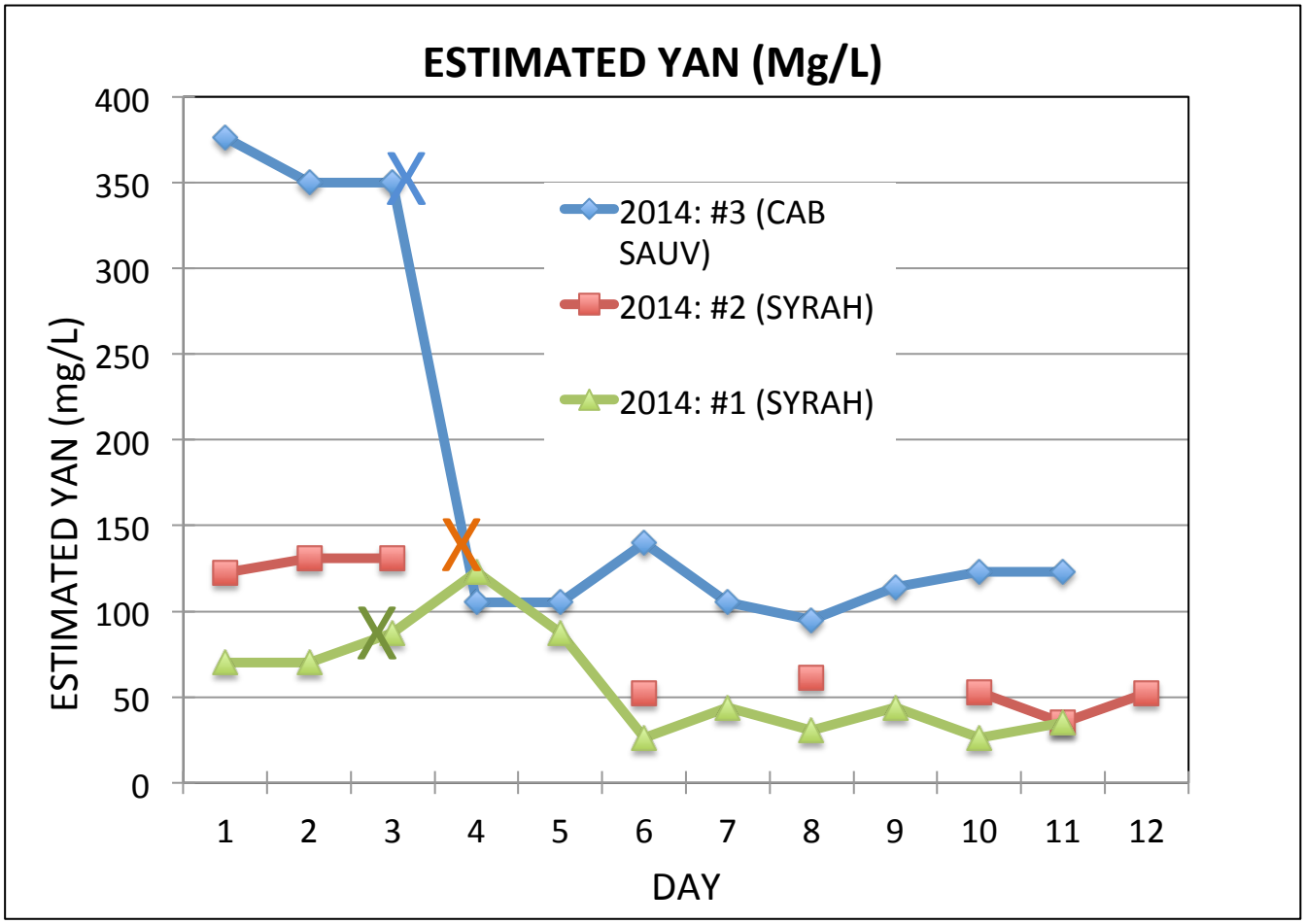

Figure 2. Estimated YAN values during the alcoholic fermentation period of three grape musts. (X indicates yeast inoculation date; lack of connecting lines indicates missed estimate dates.)

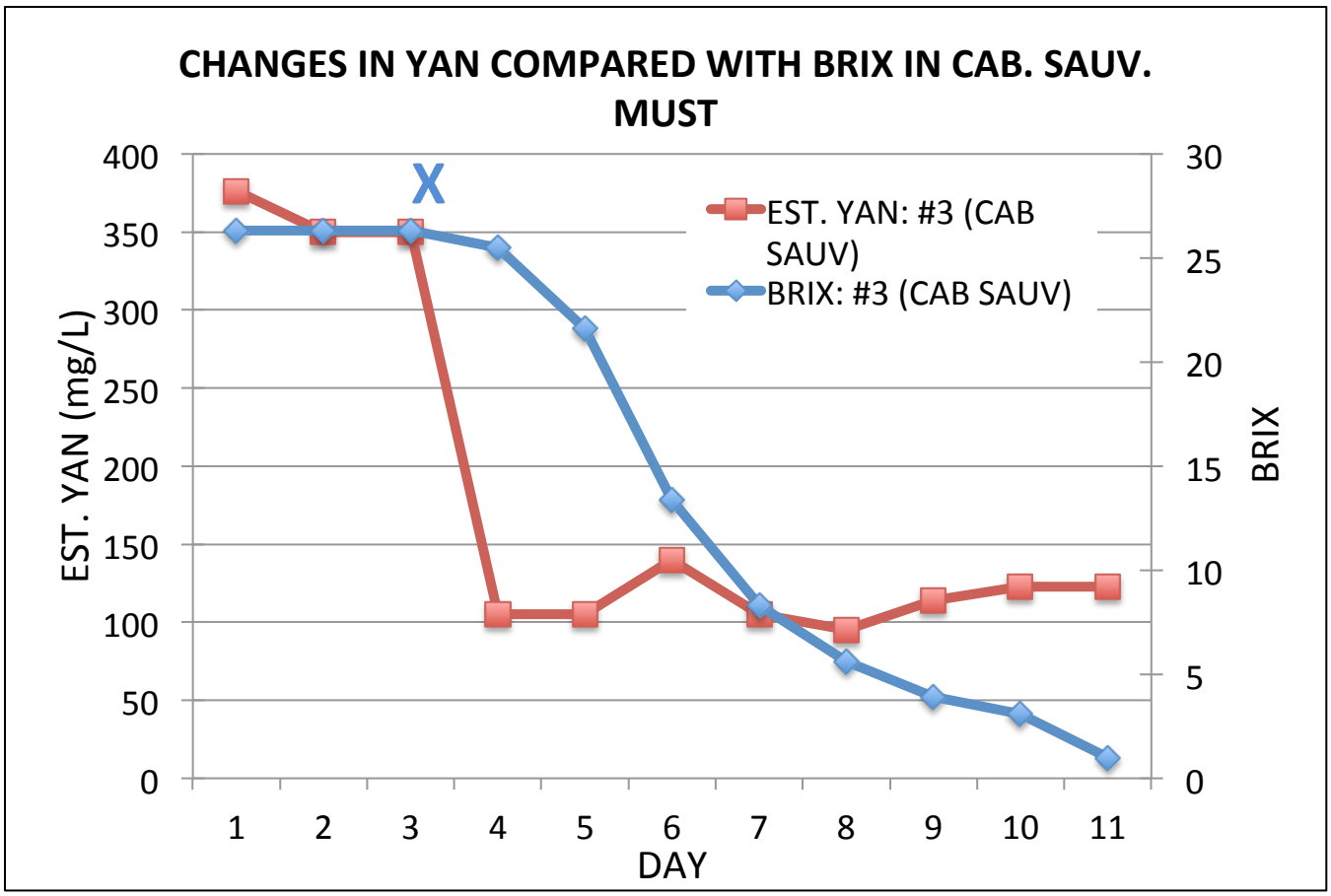

Figure 3. Changes in YAN compared with degrees brix in cabernet sauvignon must (#3) during alcoholic fermentation.

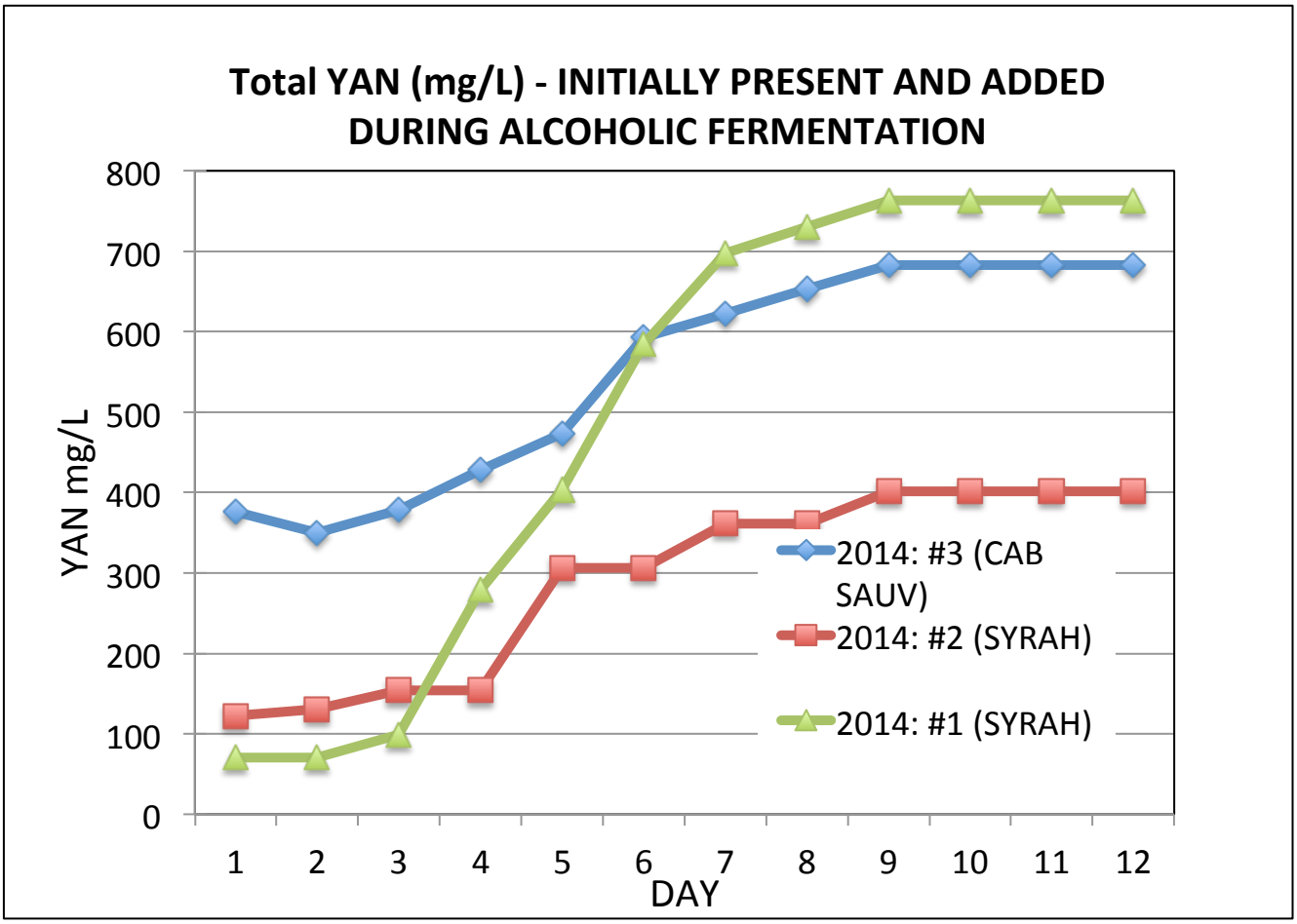

Figure 4. Total YAN (mg/L) initially present and added during alcoholic fermentation of three grape musts. For each day, the value is the sum of the initial value plus everything added up through that day.

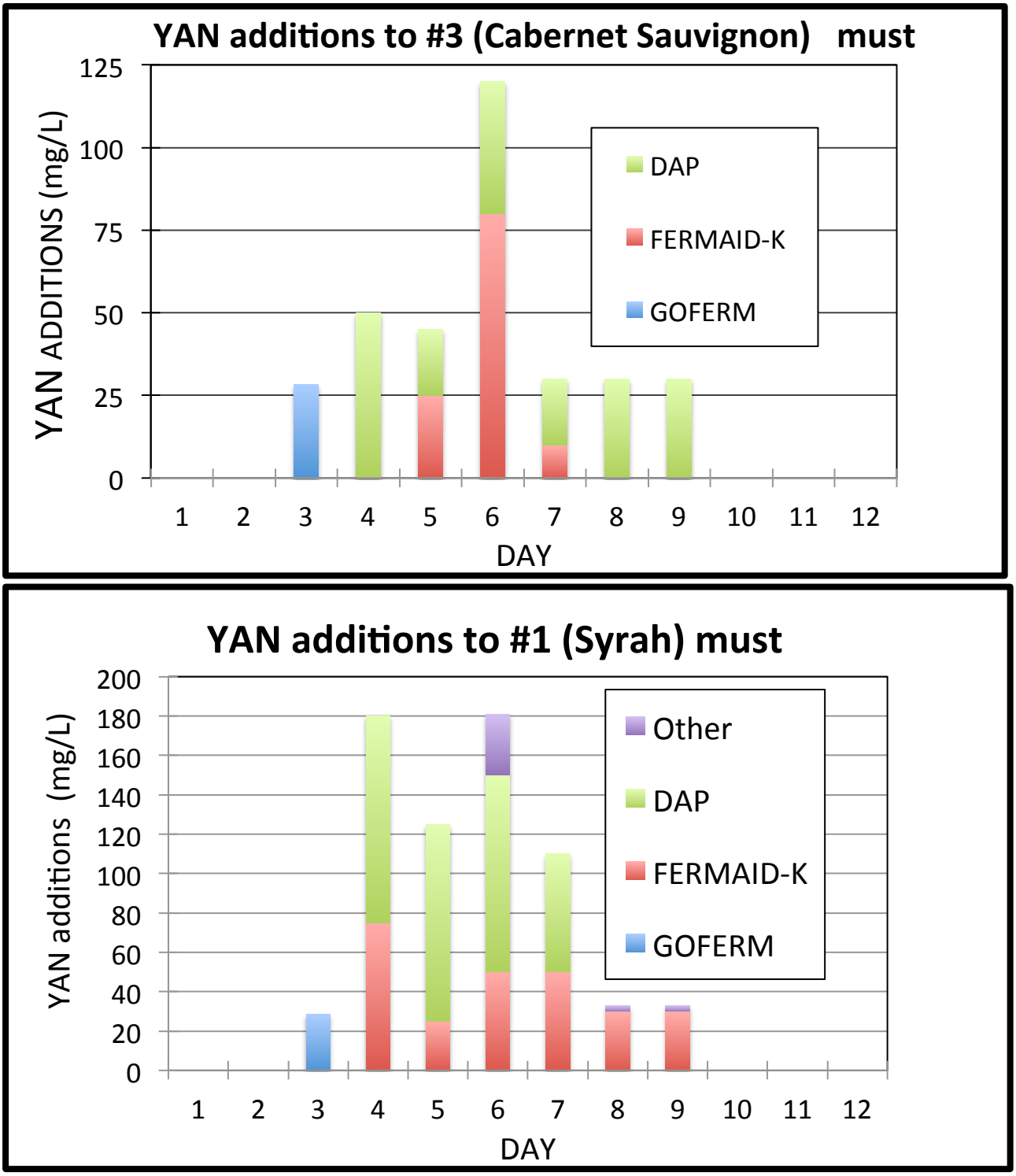

Figure 5. YAN additions (mg/L), nature of addition, and day of addition – to cabernet sauvignon must (top) and Sagemoor syrah must (bottom) during alcoholic fermentation.