Yeast Assimilable Nitrogen Content of Juice and Production of Volatile Sulfur Compounds in Oregon Wine

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INTRODUCTION

The objectives of this research project are to gain a better understanding of juice/must nutrition and the production of hydrogen sulfide and other 'off' sulfide odors in Oregon wines in relationship to both viticulture and winemaking practices. Specifically, the objectives of this research project are:

- To analyze commercial juices/musts from specific vineyard blocks for yeast assimilable nitrogen content prior to fermentation
- To screen commercial wines from the specific vineyard blocks for 'reduced' sulfide character
- To analyze wines from the specific vineyard blocks for their content of volatile sulfur compounds
- To survey viticulture and winemaking practices of the juice and wine lots analyzed.

Commonly, winemakers experience fermentation problems with fruit from specific vineyard blocks often over the course of several vintages and may need to modify vineyard management practices and/or add supplemental nutrients in order to obtain timely, healthy fermentations. Nitrogen compounds are required by yeast for the production of cell biomass, the synthesis of DNA, RNA, and the proteins and enzymes necessary for the biochemical processes of fermentation. The readily fermentable nitrogen content in juice and musts is composed primarily of ammonia (NH_3) and the alpha-amino acids present. An approximation of the total yeast assimilable nitrogen content (YANC) is taken as the sum of the nitrogen available from ammonia and the nitrogen available from the amino acids present in the juice or must (Bisson 1991; Dukes and Butzke 1998; Jiranek, Langridge, and Henshcke 1995). If the levels of fermentable nitrogen are too low, the total cell biomass produced will be low, the yeast fermentation may be slow, and the fermentation may stop or 'stick' before all the fermentable sugar is utilized. Yeast under nutritional stress due to nitrogen deficiency may also produce hydrogen sulfide (rotten egg) and other sulfur compounds with 'off' odors such as rotten garlic and onions from degradation of sulfur containing amino acids present in grape juice proteins (Kunkee 1991; Jiranek, Langridge, and Henshcke 1995). Other undesirable flavors may develop in wines produced under drought and stressed conditions including those related to atypical aging syndrome (UTA) (Sponholz, 2000). Many of the amino acids are metabolized by yeast to produce important aromatic alcohols and esters which may affect wine quality either positively or negatively. Some researchers have reported an increase in fruity aroma intensity and wine quality with moderate nitrogen supplementation. Elevated levels of some aromatic alcohols known as 'fusel' oils, however, may be detrimental to wine quality (Rapp and Versini, 1995; Vos, 1982).

Recommended levels of fermentable nitrogen needed by yeast for healthy fermentations vary from 140 mg N/L to as high as 500 mg N/L or more (Butzke, 1998; Spayd, 1998). A total of 207 Oregon commercial juice samples taken at harvest were analyzed for fermentable nitrogen content during three vintages (1997-1999). The nitrogen available from ammonia ranged from 0 to 145 mg N/L. The nitrogen available from the alpha amino acids ranged from 35 to 380 mg N/L. The total estimated yeast assimilable nitrogen content (YANC) taken as the sum of the available nitrogen from ammonia plus the available nitrogen from alpha amino acids ranged from 38 to 500 mg N/L. The percentage of commercial Pinot noir juice samples with less than 140 mg N/L was 37% in 1997, 34% in 1998, and 8.6% in 1999. The percentage of commercial Chardonnay juice samples with less than 140 mg N/L was 80% in 1997, 79% in 1998, and 17% in 1999. The fermentable nitrogen content of commercial Oregon juice and must samples at harvest has been shown to often be lower than the minimal recommended levels and to vary with both variety and vintage (Watson, Hellman, Specht, and Chen, 2000).

In a research trial conducted at Benton Lane Vineyards in the Willamette Valley (1999-2001) the vineyard management practices were shown to significantly affect the juice fermentable nitrogen content at harvest. The greatest effect was observed with tilling of alternate rows compared to no tilling. Tilling was done in early spring to encourage nitrogen utilization and to reduce nutrient and water competition with the vines. In 2001 the tilled treatments averaged a 39% increase in juice YANC at harvest compared to untilled treatments. Wines produced from the tilled treatments also fermented more rapidly than the no tilled treatments. Overall during ripening, the juice ammonia content decreased steadily from veraison to harvest while at the same time the amino acid content of juice increased. The net result was an observed decrease in YANC in the early stages of ripening followed by a net increase in YANC at the later stages of ripening (Figures 1-3). The YANC was also observed to vary considerably with vintage. The YANC of Pinot noir at harvest was much higher in 1999 than in either 2000 or 2001 (Figure 4). Yeast assimilable nitrogen content at harvest appears to be affected by vintage, by degree of fruit maturity, as well as by soil nitrogen availability and water availability during ripening (Watson, et al.2002).

MATERIALS AND METHODS

Sample Collection

Juice samples from specific vineyard blocks were taken after processing prior to inoculation and frozen for nutrient analysis. New wine samples from the specific vineyard blocks are being collected at the end of fermentation for sensory screening and for analysis for volatile sulfur compounds. A survey of viticultural and winemaking practices is being compiled to better understand the relationship of juice nutritional content and the production of volatile sulfur compounds in wines.

Analysis of yeast assimilable nitrogen content

Ammonia content of juice is being determined with a Sigma enzymatic diagnostic kit. The alpha amino acid content is being determined with the NOPA spectrophotometric assay (Dukes and Butzke 1998) using isoleucine (ile) as the standard. The yeast assimilable nitrogen content (YANC) is estimated as the sum of the assimilable nitrogen from ammonia plus the assimilable nitrogen from alpha amino acids expressed as mg N/L.

Analysis of Amino Acid Profiles
Juice amino acid profiles are being analyzed by high performance liquid chromotography (HPLC) with the Waters Acc.Q. Tag Amino Acid Analysis System using a pre-column flourescent derivatization technique. The amino acid analysis is being conducted in the laboratories of Alan Bakalinsky and Jim Kennedy.

Analysis of Volatile Sulfur Compounds
The analysis for volatile sulfur compounds in the wines is being conducted in the laboratory of Michael Qian. Sulfides analysis will include hydrogen sulfide, ethyl and methyl mercaptan (ethanethiol and methanethiol, respectively), and polysulfides including dimethyl disulfide and diethyl disulfide.

Sensory Evaluation
The wines will undergo sensory evaluation in the Sensory Science Laboratory in collaboration with Mina McDaniel and the sensory data will be correlated with the analysis of the volatile sulfur content of the wines.

RESULTS AND DISCUSSION

During the 2002 vintage approximately 200 juice samples were collected from 20 cooperating Oregon wineries. The samples represent several varieties and numerous specific vineyard block/sites. The juice samples frozen at harvest are currently being analyzed for ammonia content, alpha amino acid content by the NOPA assay, and for complete amino acid profiles by HPLC. To date, approximately 80 samples have been analyzed. Wine samples from the specific vineyard blocks are currently being collected and will be analyzed for volatile sulfur compounds.

Vineyard and winery information is being collected on each vineyard block and on the wine lots produced. Vineyard factors which may affect juice nutritional composition include fruit maturity, condition of fruit at harvest, yields, irrigation and fertilization practices, rootstocks, soil type/depth, vine age, trellis type, etc. Winemaking factors which may affect the content of volatile sulfur compounds include the yeast(s) strains used, 'spontaneous' fermentation practices, nutritional supplementation, juice clarification, fermentation management practices, temperature regimes, fermentor size, cap management, pre and post fermentation maceration, exposure to air, and post fermentation practices such as settling, racking, and barrel aging regimes.

Of the 2002 juice samples analyzed to date (80), the ammonia content at harvest ranged from 24 mg/L to 140 mg/L with an average of 66 mg/L. The corresponding mg N/L of available nitrogen from ammonia ranged from 20 to 115 with an average of 54 mg N/L. The available nitrogen from ammonia is calculated by multiplying the mg/L of ammonia by 0.8235 which is the ratio of the weight of nitrogen to the molecular weight of ammonia (14/17=0.8235). The mg/l of alpha amino acids at harvest as estimated by the NOPA assay ranged from 581 mg/L to 2260 mg/L with an average of 1205 mg/L. The corresponding mg N/L of available nitrogen from the alpha amino acids ranged from 62 to 240 with an average of 128 mg N/L. The available nitrogen from alpha amino acids as estimated by the NOPA assay is calculated by muliplying the alpha amino acid content by .1069 which is the ratio of the weight of nitrogen to the molecular weight of isoleucine (14/131=1069) (Table 1). The yeast assimilable nitrogen content (YANC) in mg N/L is calculated as the sum of the mg N/L available from ammonia plus the mg N/L available from the alpha amino acids present. The YANC at harvest ranged from a low of 95 mg N/L to a high of 374 mg N/L with an average of 176 mg N/L. Of the 2002 juice samples analyzed to date, the percent which were at or below about 150 mg N/L was 29.5% (Table 1).

The total amino acid content as analyzed by HPLC ranged from a low of 745 to a high of 2517 mg/L with an average of 1531 mg/L (Table 1). Table 2 shows the complete amino acid profiles of Pinot noir juice samples from two different vineyard sites representing the range from low to high in amino acid content. Figure 5 shows the range from low to high and the average concentration of the major amino acids present which included L-arginine, L-proline, L-glutamine, L-alanine, L-glutamic, gamma-amino-n-butyric acid, and L-serine. L-arginine and L-proline were present in the greatest concentration. L-proline content averaged 309 mg/L, however, the nitrogen from L-proline is not considered to be utilizable by yeast during normal fermentation conditions. L-arginine content ranged from as low as 95 to as high as 672 mg/L and averaged 260 mg/L. L-arginine has four nitrogen atoms per molecule, three of which are considered to be utilizable by yeast during fermentation. The NOPA assay only measures alpha amino acids or primary amines so two of the utilizable nitrogen atoms from arginine are underestimated by the NOPA assay. On the average the NOPA assay underestimated the assimilable nitrogen content from the amino acids present by about 20% (on average 128 mg N/L as measured by NOPA compared to 153 mg N/L as measured by HPLC) (Table 1).

The total concentration of ammonia and amino acids in juice at harvest varied considerably in fruit from different vineyard sites in 2002. The average contribution of ammonia to the yeast assimilable nitrogen at harvest was 26% (54 mg N/L) while the average contribution of amino acids was 74% (153 mg N/L).

SUMMARY

The primary objective of this research is to provide winegrowers with information to better understand how juice/must nutrition at harvest and vineyard and winemaking practices may effect sulfides levels in commercial wines. A survey of volatile sulfur compounds in Oregon wines related to juice/must nutrient analysis has not been previously done. This survey and research will help provide winemakers and researchers with additional valuable information to help develop more effective vineyard and winemaking strategies for managing the production of healthy, sound fermentations. A newly purchased detector for amino acid analysis and a newly purchased purge-trap gas chromatograph and sulfur specific chemiluminescence detector will now make it possible to have amino acid analysis and volatile sulfur analysis capability in the Department of Food Science and Technology.

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Figure 1. Juice nitrogen from ammonia during ripening.
Benton Lane Vineyards, 2001.

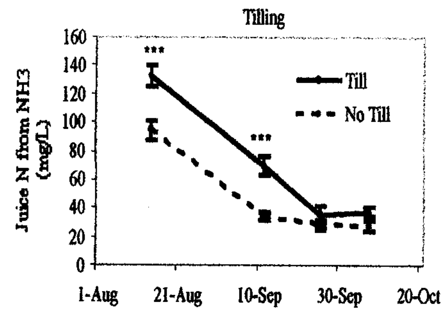

Figure 2. Juice nitrogen from amino acids during ripening.
Benton Lane Vineyard, 2001.

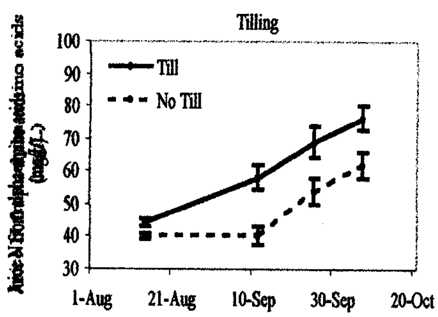

Figure 3. Juice yeast assimilable nitrogen content during ripening. Benton Lane Vineyard, 2001.

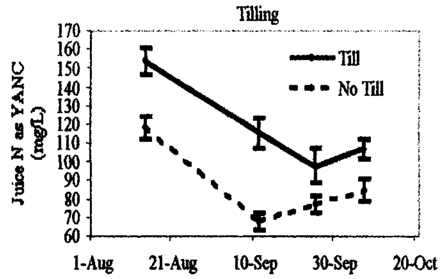

Figure 4. Ammonia, NOPA at veraison and harvest and YANC at harvest. Benton Lane Vineyard, 1999-2001.

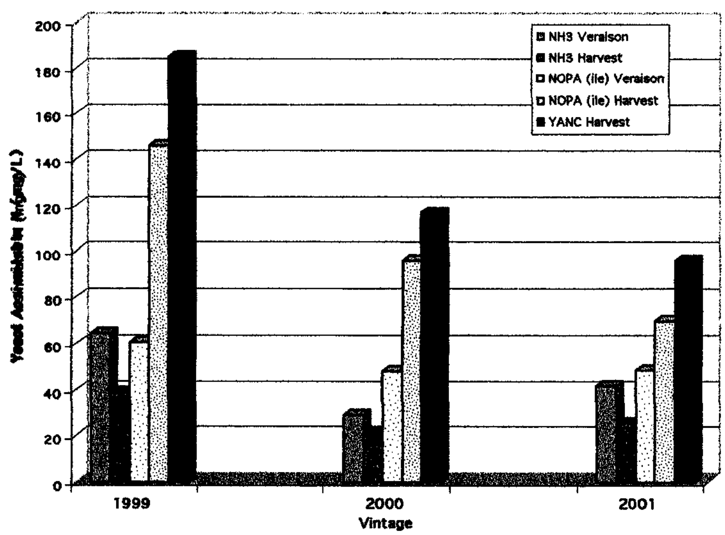

Figure 5. Juice amino acid content by HPLC, 2002

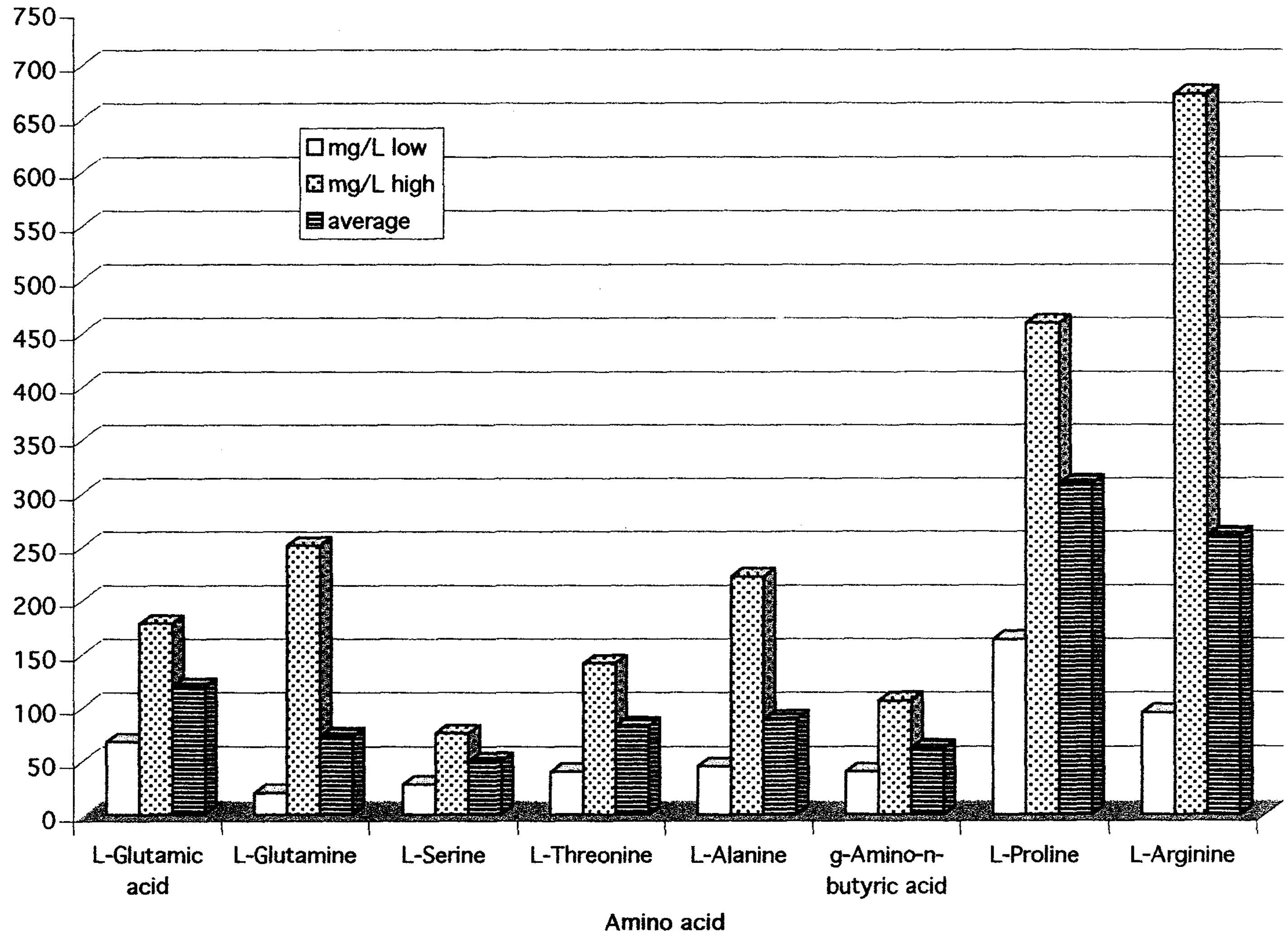

Table 1. Yeast assimilable nitrogen content and amino acid content of 2002 juice samples.

	Low	High	Average
YANC mg N/L	95	374	176
NH3 mg N/L	20	115	54
NOPA mg N/L (Ile)	62	240	128
Alpha amino acids mg/L (Ile) by NOPA	581	2260	1205
Amino acids by HPLC, mg/L	745	2517	1531
mg N/L amino acids by HPLC	73	350	153

Table 2. Amino acid profiles of Pinot noir from two vineyard sites in 2002

2002 Vintage
mg/L Amino acids

Sample description	Pinot noir Site 14	Pinot noir Site 75
Amino Acid		
L-Aspartic Acid	38.5	54.6
L-Glutamic Acid	94.8	166.2
Hydroxy-L-proline	2.7	4.32
L-Asparagine	1.9	9.9
L-Glutamine	20	242.9
L-Citrulline	1.4	17.6
L-Serine	29.7	100
L-Histidine	7.7	29.3
Glycine	6	14.1
L-Threonine	44.5	165.8
L-Alanine	45.1	222
g-Amino-n-Butyric Acid	43.1	106.1
L-Proline	234	459
L-Arginine	94.7	672.7
L-Tyrosine	20	25.3
L-Valine	17.5	55.4
L-Methionine	9.8	27
L-Isoleucine	13.1	41.9
L-Leucine	12.3	52.5
L-Phenylalanine	6.2	39.9
LOrnithine	0.9	4.4
L-Lysine	1	6.4
L-Hydroxylysine	nd	nd
Total: mg/L AA	745.3	2517.4