Oregon Wine Advisory Board Research Progress Report

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Amino Acid and Amine Content of Northwest Wines and Strategies to Limit Amine Formation

Mark A. Daeschel, M.B.A. Gloria, L.Simon-Sarkadi and Barney T. Watson Department of Food Science and Technology

Problem

Wines, specifically those in which lactic acid bacteria can grow can cause transient toxic effects due to the formation of biogenic amines such as histamine and tyramine. Some people specifically avoid red wines because they are more often associated with headaches and other discomforts. An understanding of the origin, occurrence and formation of amines in grape and berry wines will lead to developing approaches to preventing their formation.

Justification and Literature Review

The amounts and proportions of free amino acids in juices and wines are important from several perspectives. Amino acids provide a significant part of the nutritional requirements of both yeast and malo-lactic bacteria. Sluggish fermentations can result from amino acid deficiencies. Secondly, amino acids can serve as substrates for the microbial production of volatile aroma compounds in wine, primarily higher alcohols and esters. Lastly, amino acids can also serve as substrates for the bacterial production of biogenic amines (histamine, tyramine, etc.). High amounts of amines in wines can elicit symptoms such as rashes, blood pressure increases, headache and nausea.

Spoilage type lactic acid bacteria such as pediococci and lactobacilli can form amines whereas Leuconostoc *oenos* does not. Whether or not yeast can form amines is not completely resolved since data exists that suggests they can and cannot form amines. However, what evidence is available suggests that they are not involved in the formation of significant amounts of amines from a health standpoint. Histamine and other amines are formed in wines by decarboxylation of amino acids such as histamine coming from histidine. The toxic level of biogenic amines in wine is not precisely known due to the presence of other types amines such as putrescine and cadaverine which can potentiate the action of the monoamines such as histamine by interfering with the bodies detoxification mechanism. A toxic amount is usually considered to be 5-8 ppm or more depending on the amount of food or beverage consumed.

No information is available concerning the incidence of biogenic amines in grape and berry wines from the Northwestern United States. Red wines are more prone to amine formation, likely because they are more susceptible to growth by naturally occurring lactic acid bacteria. We all have heard people remark 'Oh! I can't drink red wine, it gives me a headache". Amines are partially if not primarily responsible for this situation. However, individuals vary greatly in respect to their sensitivity to amines. We believe that controlling the occurrence of spoilage type lactic acid bacteria or limiting their sources of amino acids

are valid approaches to reducing the likelihood of amine formation.

Specifice Objectives

(*Year one*) To analyze the amine and amino acid contents of representative grape must and finished wines produced in the Northwest. To determine whether the amine contents are such that may warrant concern from a health standpoint.

Procedures

We will analyze finished wines. Enough samples will be procured for a rigorous statistical treatment in order draw inferences as to the frequency of amine occurrence as well as concentrations. Similarly, corresponding fresh juices will be obtained to determine native amine concentrations as well as amino acid contents.

We have developed High Pressure Liquid Chromatography (HPLQ procedures that will detect and quantify nine different amines simultaneously. In addition we are able to detect and quantify the major amino acids (Histidine, arginine, tyrosine and tryptophan) that serve as substrates for amine production. The HPLC system consists of a C-18 reverse phase column coupled with a fluorescence detector. We use a post-column derivitization procedure that allows quantification at the parts per billion level. One possible pitfall is that may be too many interfering compounds in the juices and wines. These may mask the amines as they come off the column. Extraction procedures will most likely need to be modified and optimized for each of the different berry types.

Results to Date

We have thus far completed analysis of 41 finished wines for amine content. We plan to analyze at least another 25 wines. Our sampling design consisted of evaluating wines from the 1991 and 1992 vintages since these varied greatly in terms growing season climatic conditions. Secondly, we sampled only the two varieties; Pinot Noir and Cabernet Sauvignon. Lastly, we obtained wines from 5 major Northwest appellations with a bias toward the Willamette valley appellation. The amine data is summarized in table. For Pinot noir histamine levels ranged from none to 24 ug/ml (ppm), tyramine from none toto 8.3 ppm and putrescine from 1.2 to 203.1 ppm . For Cabernet Sauvignon, Histamine ranged from 0.2 to 4.3 ppm, tyramine from none to 2.62 and putrescine from 4 to 15.4 ppm. At this point in the amine survey it appears that Pinot noir has significantly higher levels of amines than Cabernet Sauvignon. One third of the Pinot Noirs had histamine levels exceeding 10 ppm which is a level that may elicit histamine reaction symptoms in some individuals.

Biogenic amines in Pinot Noir

Sample	Amine (ug/ml)										
	Put	Cad	Tym	Him	Ser	Agm	Spd	Phe	Trm	Spm	
Willame	tte Valley										
1-2	6.89	0.44	2.29	6.66	0.59	nd	0.72	nd	nd	0.13	
1-3	20.86	0.44	0.01	9.91	1.06	0.22	1.03	nd	nd	0.16	
1-4	7.25	0.39	1.98	7.66	0.49	nd	0.78	0.20	nd	nd	
1-6	7.22	0.48	0.19	3.96	1.34	0.41	0.42	0.07	0.22	0.06	
1-7	203.12	1.35	0.17	0.47	0.52	0.51	0.61	0.29	nd	0.26	
2-1	67.13	0.72	nd	13.37	0.62	0.11	0.73	0.13	nd	nd	
3-1	1.28	0.25	nd	0.15	0.33	.034	0.66	0.51	nd	nd	
4-1	28.28	0.54	nd	6.11	1.38	1.03	0.69	nd	nd	0.31	
4-3	47.10	0.34	4.90	15.83	2.23	2.21	1.63	nd	nd	nd	
4-4	11.15	0.79	1.92	5.20	1.37	0.99	0.69	0.20	nd	nd	
4-5	3.00	0.77	3.75	0.20	1.19	1.01	0.83	0.22	nd	0.42	
5-1	33.53	1.55	1.61	1.95	0.98	0.39	0.32	0.25	nd	nd	
5-2	29.83	0.46	2.31	10.48	1.81	0.92	1.04	0.30	nd	0.42	
5-3	19.84	0.39	2.84	8.43	nd	0.16	1.18	nd	nd	nd	
5-4	22.96	2.07	nd	1.33	0.50	1.58	0.74	0.19	0.16	nd	
5-5	34.60	1.01	3.82	9.68	0.28	0.66	0.69	nd	nd	nd	
5-6	28.35	nd	nd	17.85	nd	1.50	2.35	nd	nd	1.49	
5-7	7.47	0.70	nd	23.98	0.24	nd	1.74	0.23	0.93	nd	
5-8	38.00	nd	8.31	15.92	nd	nd	nd	0.50	5.51	nd	
5-9	8.43	nd	6.50	2.63	nd	nd	nd	nd	nd	nd	
5-10	2.43	nd	nd	0.96	nd	nd	0.33	nd	nd	nd	
5-11	10.80	nd	nd	2.97	nd	nd	0.54	nd	nd	nd	
5-12	12.20	nd	7.50	1.45	0.25	nd	nd	nd	0.37	nd	
Umpqua	Valley										
1-5	10.70	0.13	0.06	nd	0.84	0.27	0.98	0.80	nd	0.22	
Rogue Va											
1-1	30.09	0.27	6.88	16.32	1.12	8.37	1.39	0.89	0.03	0.1	

Biogenic amines in Cabernet Sauvignon

Sample	Amine (ug/ml)									
	Put	Cad	Tym	Him	Ser	Agm	Spd	Phe	Trm	Spr
Willame	tte Valley		1. 1. 1.	1.44			900 J. C. C.	~ 1,10		1.8.1
1-9	4.59	0.45	nd	0.65	0.11	nd	0.34	nd	nd	nd
2-2	10.35	0.95	nd	2.85	0.39	0.28	0.79	0.14	nd	0.23
4-2	9.00	0.06	nd	0.18	0.49	0.39	1.68	nd	nd	nd
Rogue Va	alley									
2-3	8.50	1.51	nd	1.60	nd	0.17	0.44	0.13	nd	nd
3-4	9.31	1.10	nd	3.50	0.08	0.41	nd	nd	nd	nd
Umpqua V	Valley									
2-4	19.81	0.39	nd	0.83	0.37	0.43	0.62	0.07	nd	nd
3-2	11.69	0.77	nd	2.47	0.35	0.44		nd	nd	1.17
3-5	17.62	0.46	0.64	1.42	0.33	0.42	1.32	nd	nd	0.36
? Valley	Y									
2-5	6.00	1.34	nd	2.35	0.96	0.49	1.57	0.13	nd	0.47
Columbia	a Valley									
1-8	6.21	0.93	0.19	0.16	0.47	0.39	nd	nd	nd	nd
1-10	8.20	0.96	0.22	0.93	0.72	0.70	1.62	nd	nd	0.48
3-3	4.54	0.96	nd	0.38	0.42	2.40	1.55	0.11	nd	0.66
3-7	7.19	0.80	nd	0.38	nd	nd	0.86	nd	nd	nd
Yakima V	Valley									
2-6	8.64	1.26	0.93	0.57	0.99	1.89	1.46	0.20	nd	0.36
Red Tab	le wine									
3-6	4.08	0.93	0.18	0.84	1.12	1.15	1.51	0.19	nd	0.19
3-8	15.42	1.07	2.62	4.33	nd	1.10	1.25	0.15	nd	nd