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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. Beatriz A. Gloria, L. Simon-Sarkadi, and Barney T. Watson
Food Science and Technology, Oregon State University

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: *eonos* 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 of amines such as putrescine and cadaverine which can potentiate the action of the monoamines such as histamine by interfering with the body's 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.

SPECIFIC OBJECTIVES

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. To establish if lactic acid bacteria associated with wine fermentation's can produce amines.

PROCEDURES

We will analyze finished wines. Enough samples will be procured for a rigorous statistical treatment in order to 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 dirivitization procedure that allows quantification at the parts per billion level. One possible pitfall is that there 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.

We will analyze commercial strains of Malolactic starter cultures for their ability to produce biogenic amines. In addition, we will analyze indigenous strains of lactic acid bacteria isolated from wine for amine forming capability. The detection method for histamine producing bacteria is based on the reaction of the formed histamine with diamine oxidase. One of the reaction products is hydrogen peroxide which is then reacted with the leucobase form of crystal violet in the presence of horseradish peroxidase. The end result is the formation of a deep purple indicating the presence of histamine.

RESULTS

We have completed analysis of 41 finished wines for amine content. Our sampling design consisted of evaluating wines from the 1991 and 1992 vintages since these varied greatly in terms of 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 1 and the associated graphs. For Pinot noir histamine levels ranged from none to 24 ug/ml (ppm), tyramine from none to 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.

The majority of commercial MLF cultures were capable of producing histamine (Table 2) under defined laboratory conditions, however, this does not necessarily mean they can produce histamine in wine. Approximately half of the wine lactic acid bacteria isolates produced histamine.

Table 1

TABLE . Levels of biogenic amines in Pinot Noir and Cabernet Sauvignon wines

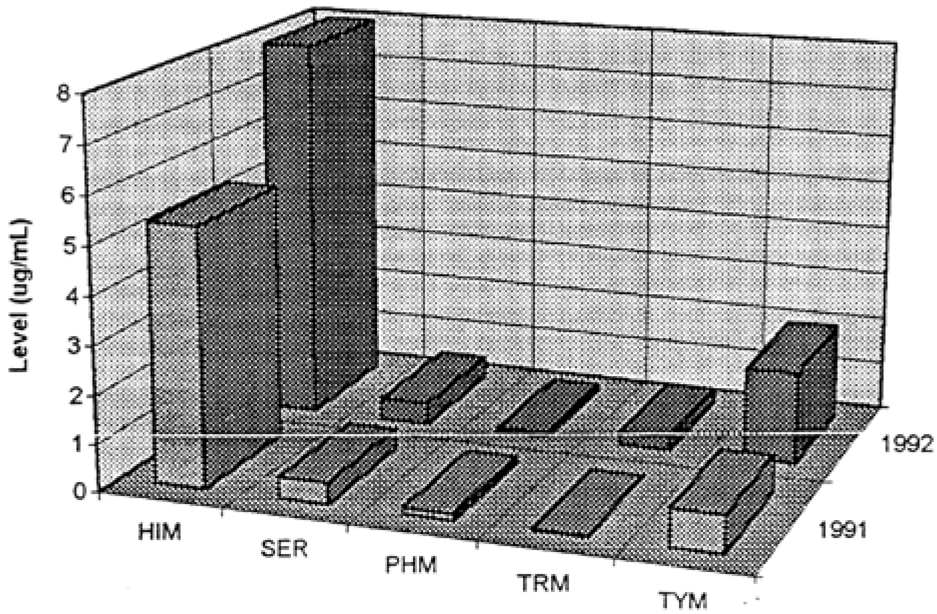
Sample	HIM	SER	PHM	TRM	TYM
Pinot Noir					
% positive samples	97	47	34	16	47
range (g/L)	nd-23.98	nd-2.23	nd-0.89	nd-5.51	nd-8.31
mean $\pm$ sd (g/L)	7.01 $\pm$ 6.05	0.44 $\pm$ 0.60	0.11 $\pm$ 0.21	0.19 $\pm$ 0.90	1.54 $\pm$ 2.43
Cabernet Sauvignon					
% positive samples	100	86	43	0	29
range (g/L)	0.16-2.85	nd-0.99	nd-0.14	nd	nd-0.93
mean $\pm$ sd (g/L)	1.31 $\pm$ 1.09	0.41 $\pm$ 0.32	0.06 $\pm$ 0.07	nd	0.14 $\pm$ 0.29

Sample	PUT	CAD	SPM	SPD	AGM
Pinot Noir					
% positive samples	100	47	47	68	47
range (g/L)	2.43-203.12	nd-2.07	nd-8.31	nd-2.35	nd-8.37
mean $\pm$ sd (g/L)	26.67 $\pm$ 39.01	0.34 $\pm$ 0.50	0.49 $\pm$ 0.83	0.57 $\pm$ 0.57	0.54 $\pm$ 1.41
Cabernet Sauvignon					
% positive samples	100	100	50	79	86
range (g/L)	4.54-19.81	0.06-1.51	0.23-1.17	0.34-1.68	0.17-2.40
mean $\pm$ sd (g/L)	9.40 $\pm$ 4.46	0.85 $\pm$ 0.40	0.27 $\pm$ 0.35	0.88 $\pm$ 0.65	0.60 $\pm$ 0.69

Table 2

	histamine
Malolactic Culture	
Leuconostoc oenos DSM 7008	+
Leuconostoc oenos EY-02D	+
Leuconostoc oenos EY-02D*	+
Leuconostoc oenos Erla	-
Leuconostoc oenos MT 01	+
MCW Malolactic culture	+
Bitec D	-
Bitec D*	+
Lavin EQ-54	+
Wine Isolates	
009B	+
12	+
18	+
26	-
29	+
47	-
49	+
51	-
53	-
62	-
66	+
67	+

Amines in Pinot Noir wines



Amines in Cabernet Sauvignon wines