



Biogenic Amines

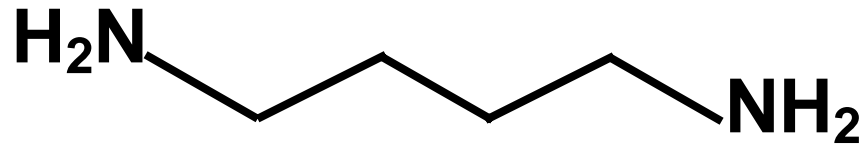
Relevance to the wine industry?

Napa Valley Wine Technical Group
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ETS Laboratories

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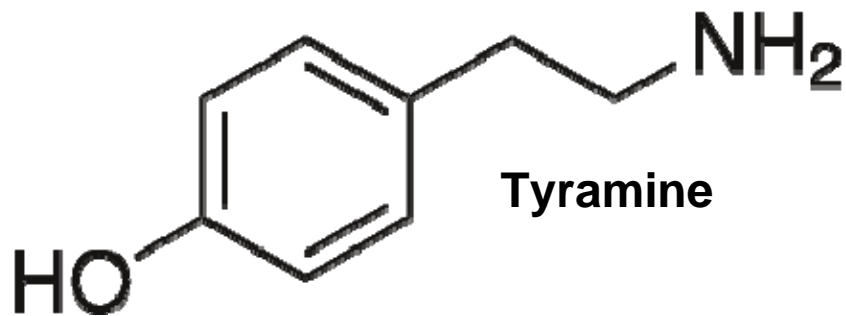
What are biogenic amines ?



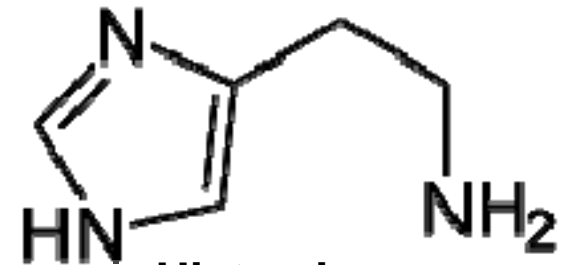
Putrescine



Cadaverine



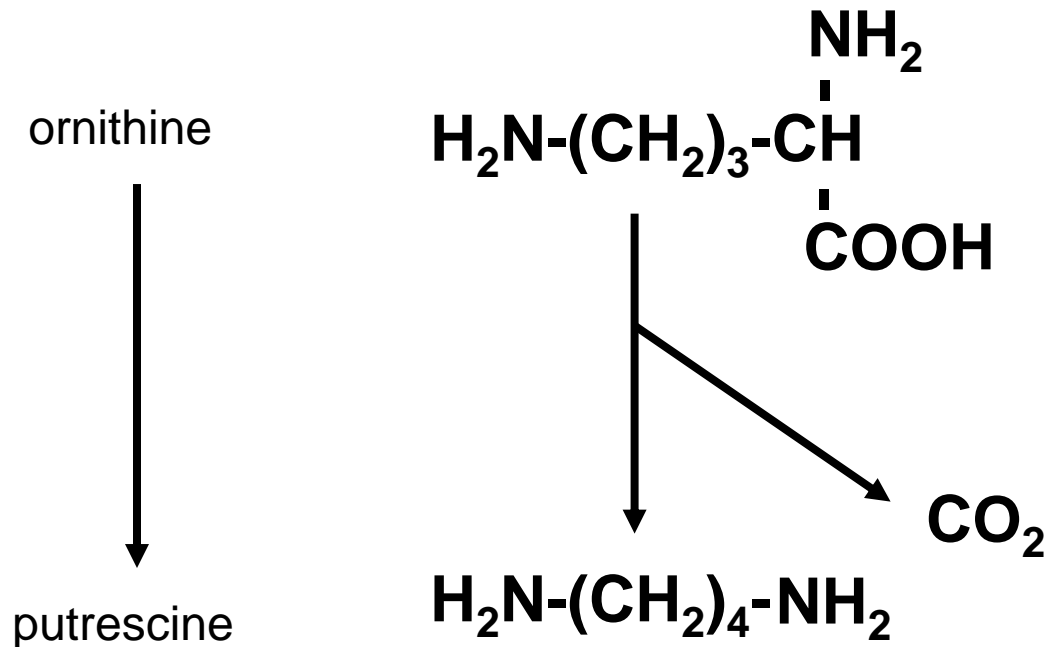
Tyramine



Histamine

How are biogenic amines formed ?

- Enzymatic decarboxylation of amino acids
 - Living organisms can contain a number of decarboxylase enzymes
 - a carbon dioxide group is removed from the corresponding amino acid by the decarboxylase



How are biogenic amines formed ?

- In grapes
- In primary fermentation
 - formed by yeast
- Malolactic fermentation
 - formed by bacteria
- Post-malolactic fermentation
 - formed by bacteria

Where do the amino acids come from?

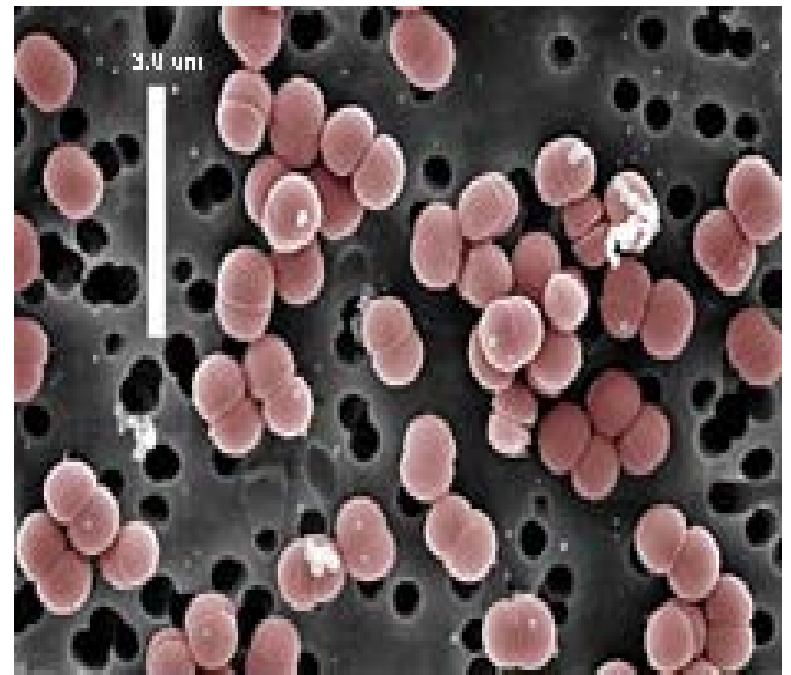
- From grape
 - free amino acids, vary by varietal and vintage
 - protein degradation
- From yeast
 - produced by yeast
 - break down grape proteins
 - excreted prior to or released during autolysis
- From bacteria
 - metabolize grape proteins release amino acids
 - produced by bacteria

Where do problem levels of biogenic amines form in the winemaking process?

- Most of the literature seems to agree that the highest levels of biogenic amines are produced during aging of the wine, or post malolactic fermentation
- Lactic acid bacteria are the primary producers of biogenic amines in wine
 - *Pediococcus*
 - *Lactobacillus*
 - *Oenococcus*

Pediococcus species

- *Pediococcus damnosus*
- *Pediococcus parvulus*
- Homofermentive species
- Prefers higher pH wines, 3.65 and higher greater risk



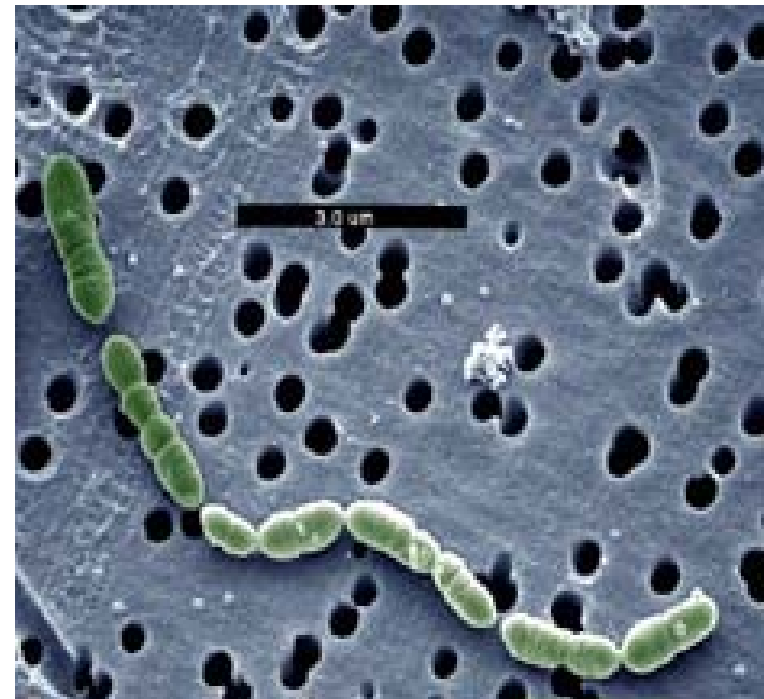
Lactobacillus species

- *L. hilgardii*, *L. brevis*, *L. plantarum*, *L. fermentum*
- Homofermentive and heterofermentive species
- Problem during sluggish/stuck ferments
- Prefer moderate to high pH, >3.5



Oenococcus oeni

- A heterofermentive lactic acid bacteria
- Grows at low pH >3.1
- The preferred bacteria for conducting malolactic fermentation



Do all Lactic Acid Bacteria Produce Biogenic Amines?

Need to have active decarboxylase enzymes

- Ornithine decarboxylase – ODC
- Tyrosine decarboxylase – TDC
- Histidine decarboxylase – HDC
- Lysine decarboxylase – LDC
- Arginine decarboxylase - ADC

The Perfect Storm: Biogenic amines

- Presence of excess amino acids in the wine
- Conditions conducive to growth of lactic acid bacteria
- Presence of lactic acid bacteria containing genes for decarboxylases corresponding to the amino acids present in the wine

Why should wine producers be concerned about biogenic amines ?

- Indication/perception there may be sanitation problems
- Negative impact on wine sensory traits
 - overt
 - covert
- Regulatory issues for wine
 - current
 - potential



How can biogenic amines affect wine ?

- Biogenic amines are small, positively charged molecules
 - Indirect – potentially can interact with aroma compounds found in wine based on charge interactions
 - Direct – some biogenic amines have associated aromas

How can biogenic amines affect wine ?

Overt spoilage:

- high levels result in off notes attributed to the smell of the biogenic amine, described as metallic, fermented meat, or sauerkraut
- Sensory descriptors; “moldy tuna”

• Covert spoilage:

- elevated levels may cause a loss in aroma
- Sensory descriptors
 - Wines have lost their “art”
 - Wines have lost their terroir
 - Wines have lost varietal character

Regulatory concerns

- Until recently, Switzerland had a regulatory limit that does not allow import of wines that exceed 10 mg/L or 10 ppm of histamine
- Many wine importers in the European Union are requiring analysis of biogenic amines on import documents.
- At this time, importers consider presence of biogenic amines an indicator of poor sanitation
- Proposed regulations will include biogenic amines under the classification of allergens



Regulatory concerns

Suggested Regulatory Guidelines in the Literature

- Switzerland – 10 ppm
- France – 8 ppm
- Belgium – 6 ppm
- Finland – 5 ppm
- Holland – 3.5 ppm
- Germany – 2 ppm



Survey of Biogenic Amine Levels in Wines

Surveyed 284 wines from California, Oregon and Washington

- Putrescine ranged from <1 ppm to 296 ppm, 86 wines >10
- Cadaverine ranged from <1 ppm to 4 ppm
- Histamine ranged from <1 ppm to 72 ppm, 49 wines >10
- Tyramine ranged from <1 ppm to 20 ppm, 11 wines >10
- Highest total biogenic amines in a single wine was 360 ppm



Survey of Biogenic Amine Levels in Wines

- 135 out of 284 samples had total biogenic amine >10 ppm
- 205 out of 284 samples had total biogenic amines >2 ppm
- 232 out of 284 samples had total biogenic amines >1 ppm



Survey of Biogenic Amine Levels in Wines

- Observed two facilities that had very high biogenic amine levels in the majority of their products
 - n = 8, average of 174 ppm
 - n = 18, average of 69 ppm
- One thing they have in common is sanitation issues



How do you prevent biogenic amine formation in wine ?

- Need to eliminate “uncontrolled” bacterial growth
- Use commercial strains of *Oenococcus* for conducting malolactic fermentation
- Some yeast strains appear to leave lower levels of available amino acids at the end of AF, leaving less for subsequent conversion by bacteria



Things that can contribute to formation/accumulation of biogenic amines

- Presence of amino acid precursors in grape, must or wine
- DAP
- Time of contact with skins, extended maceration (>10 days)
 - arginine in skins
- Time of contact with yeast lees
 - excretion (AA)
 - autolysis (AA and BA)
- Presence of microbial nutrients, micronutrients
- Wine pH
- Molecular SO₂



Molecular SO₂ Revisited

pH	% Molecular SO ₂	% Bisulfite HSO ₃ ⁻	% Sulfite SO ₃ ⁼	Minimum ppm of Free SO ₂	
				0.8 molecular	0.5 molecular
3.00	6.1	93.9	0.012	13	8
3.05	5.3			15	9
3.10	4.9	95.1	0.015	16	10
3.15	4.3			19	12
3.20	3.9	96.1	0.019	21	13
3.25	3.4			23	15
3.30	3.1	96.8	0.024	26	16
3.35	2.7			29	18
3.40	2.5	97.5	0.030	32	20
3.45	2.2			37	23
3.50	2	98	0.038	40	25
3.55	1.8			46	29
3.60	1.6	98.4	0.048	50	31
3.65	1.4			57	36
3.70	1.3	98.7	0.061	63	39
3.75	1.1			72	45
3.80	1	98.9	0.077	79	49
3.85	0.9			91	57
3.90	0.8	99.1	0.097	99	62
3.95	0.7			114	71
4.00	0.7	99.2	0.122	125	78



Potential Control Processes

- Prevent growth of wild strains of lactic acid bacteria
- Inoculation with commercial strains of *Oenococcus*
- Sanitation
- Adequate molecular SO_2
- Nitrogen desert
- Micronutrient desert

Remediation ??

- Bentonite, research done in Germany on wines with relatively low levels of biogenic amines
- Observed better reduction of biogenic amines by fining treatments in must than in wine
- In wine, bentonite interacted primarily with histamine and to some extent putrescine

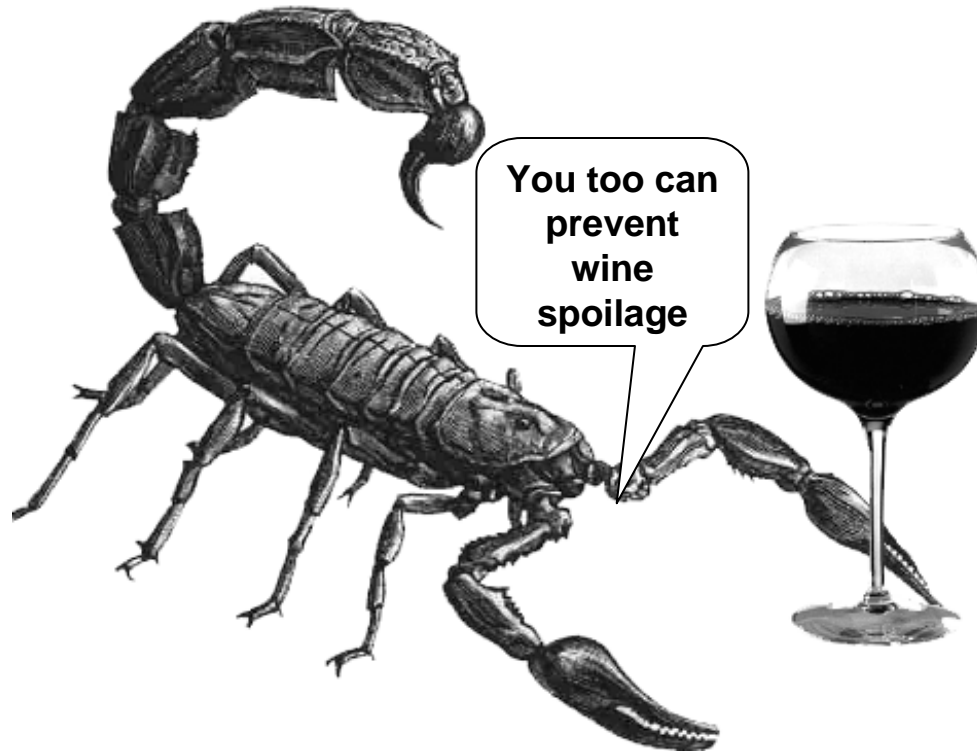
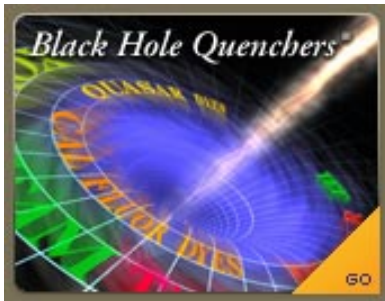
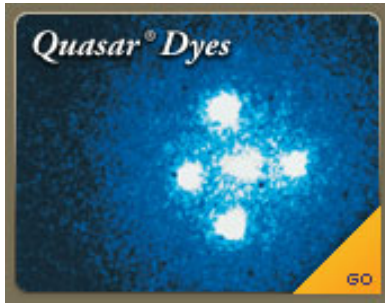


Subliminal Take Home Message

- pH
- Molecular SO₂
- Sanitation



Thank You



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