

Controlling Reductive Wine Aromas

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Wine Defects of Minor and Major importance

- Acetic acid (vinegar), acetaldehyde (leafy, cherry), acetate esters (solvent); butyric acid (rancid); ethyl acetate (nail polish remover); fermentation esters (banana), geranium (flowery); lactic (sauerkraut); leesy (baked bread); mousey (pungent); bottle shock (aromatically neutral); oxidation; lack of varietal character; filter pads; excess sulfite, horsey (barnyard); diacetyl (buttery); alcoholic; corky (musty, moldy)
- **Hydrogen sulfide (H_2S) – Rotten egg smell**
- **Mercaptan – burnt rubber, garlic smell**

Reduced Wines – The Most Common Problem in Winemaking

What causes it?

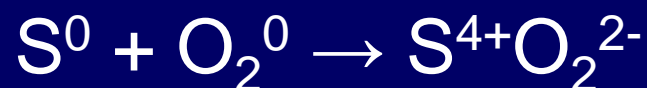
- Sulfur is reduced to hydrogen sulfide (H_2S)

What is Oxidation-Reduction?

- Oxidation is the process in which an atom loses electrons and undergoes an algebraic increase in oxidation number
- Reduction is the process in which an atom gains electrons and undergoes an algebraic decrease in oxidation number
- When there is an oxidation at one atom or molecule, then there is always a reduction in the other

Oxidation and Reduction

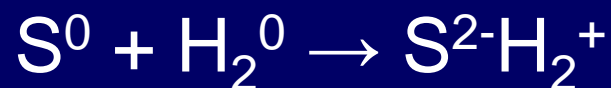
When Sulfur is oxidized:



ie. S^0 (sulfur) is oxidized to S^{4+}

O_2^0 (oxygen) is reduced to 2O^{2-}

When Sulfur is reduced:



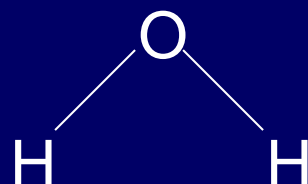
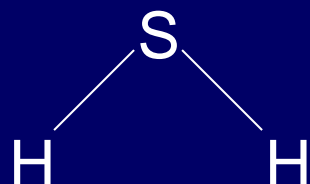
ie. S^0 (sulfur) is reduced to S^{2-}

H_2^0 (hydrogen) is oxidized to 2H^+

Oxidation and Reduction

However, in often simplified language, we generalize:

- Oxidation means the addition of oxygen and reduction is the addition of hydrogen
- The structure of H_2S is similar to the structure of water H_2O



Hydrogen Sulfide: H_2S

- Also called “sulfur hydride” or “sulfone”
- Common other names are “rotten egg gas”, “sewer gas”, “volcanic gas”, “sour gas” and “stink damp”
- H_2S is highly toxic and its toxicity is comparable to hydrogen cyanide (HCN)
- H_2S was responsible for the “great Permian Mass Extinction” according to one theory (versus the impact theory) 251.4 million years ago
- One naturally finds H_2S present in well water as well as in deep ocean volcanic vents

Recognition and detection thresholds for H₂S

- The LD₅₀ for H₂S in air is 800 ppm
- The recognition threshold in air is 4.7 ug/L or 4.7 ppb (= 4.7 X 10⁻⁶ g/L)
- The detection level in air for most people is 2.0 ug/L or 2.0 ppb (=2.0 X 10⁻⁶g/L)
- In Wine, the flavour threshold is reported as
 - 50-80 ppb (Wenzel et al, 1980)
 - 20-30 ppb (Dittrich and Staudenmayer, 1968)
- Today we use 30-50 ppb as the benchmark, but temperature, pH, alcohol, etc can affect the detection level

Reductions in Wine

1. Hydrogen sulfide: $S + H_2 \rightarrow H_2S$ (rotten egg)
 - M.P. = $-85.0\text{ }^{\circ}\text{C}$
 - B.P. = $-60.7\text{ }^{\circ}\text{C}$
2. Mercaptan: if H_2S in wine is not removed from the wine, it can (will) react with ethanol or acetaldehyde to form a new, even nastier compound called ethyl mercaptan or ethanethiol (burnt rubber, garlic, cabbage)
eg $3CH_3-CH_2OH + 3H_2S \rightarrow 3CH_3-CH_2-SH + 3H_2O$
 - M.P. = -148°C
 - B.P. = $+35^{\circ}\text{C}$

Mercaptan in wine is very persistent and hard to get rid of because of the higher B.P.

Sensory threshold in wine is 0.02-2.0 ppb

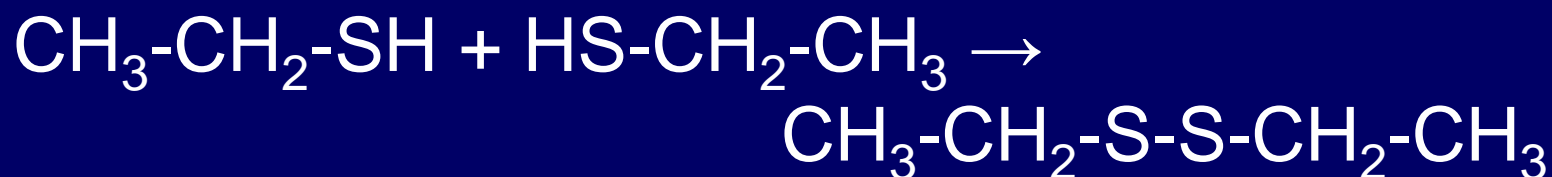
In 2000, the “Guinness Book of World Records” lists ethanethiol as the “smelliest substance in existence” (at 2.8 ppb)

Special Caution

- H_2S in a new wine where malolactic fermentation (MLF) and prolonged lees contact is intended and the wine will not be sulfited (free aldehydes) for weeks or months some of the H_2S will likely react with the ethanol and/or acetaldehyde to form ethyl mercaptan
- e.g. barrel fermented Chardonnay

Reductions in Wine continued

3. Diethyl disulfide: if ethyl mercaptan is not eliminated, then two molecules of mercaptan can react to form another molecule, even more nasty



M.P. = ?

B.P. = +154°C (very non-volatile)

- This molecule is almost impossible to eliminate from wine (by normal means) and has a very cheese-like aroma

Historical measures to either avoid or eliminate H₂S and mercaptans in wine

1. During Winemaking, to avoid it
 - Put a late copper spray on the grapes (copper sulfate or copper oxychloride)
 - Add copper sulfate to the juice or fermenting wine (Australia)
 - Use copper alloy (bronze, brass) fittings in the cellar and in machinery
 - Bronze = copper + tin
 - Brass = copper + zinc
 - Install copper tubing (pipe) where the wine can be pumped through it in the cellars

Elimination strategies

2. Once you have it

- Aeration
- Sulfiting
- Copper sulfate fining and blue fining
- Copper citrate
- Silver chloride

Eliminating Hydrogen Sulfide

Aeration

- The simplest way sometimes is to aerate since H_2S has a low B.P. of only -60.7°C



- Aeration will also oxidize any present Cu^+ to Cu^{++}

Sulfiting

- Frequently a sulfiting eliminates H_2S



(Sulfur dioxide (sulfiting) added in aqueous solution is H_2SO_3)

- Most often aeration in combination with sulfiting gives the best results; and sulfiting also protects the wine simultaneously from oxygen causing other oxidation problems

Eliminating Hydrogen Sulfide

Copper Sulfate additions



- Copper sulfate can react with hydrogen sulfide and slowly with mercaptans (several days), but not diethyldisulfide
 - The wine must be “clean” with no yeast present before addition
- When copper sulfate is needed in excess of 2-3 mg/L (ie 0.5-0.75 mg/L as copper), then one has to “bluefine” with potassium ferrocyanide to remove any excess copper
- $\text{K}_4[\text{Fe}(\text{CN})_6] \cdot 3\text{H}_2\text{O}$ (yellow prussiate)
- Copper sulfate and Potassium ferrocyanide (blue fining) will barely work with diethyldisulfide because the sulfur in the disulfide linkage will not easily bind to the copper

Eliminating Hydrogen Sulfide

Other problems with potassium ferrocyanide

- If there is little or no iron (Fe) in the wine as we now have stainless steel equipment, the “blue fining” will hardly work well to remove the excess copper from the wine if one had to use in excess of 2-3 mg/L of copper sulfate for fining
- In order to get good flocculation of the “prussian blue” precipitate from the potassium ferrocyanide, one needs to add simultaneously during blue fining a gelatin/silica gel agent, which may also strip the wine
- To be certain no cyanide is left in the wine, the Hubach Test must be performed for cyanide residues

Eliminating Hydrogen Sulfide

Copper Citrate

- Copper(II)-citrate is a new compound
($\text{Cu}_2\text{C}_6\text{H}_6\text{O}_7 \cdot 2.5\text{H}_2\text{O}$)
- Recommended instead of copper sulfate since it is an “organic chelating agent” of copper meaning the copper does not totally go into the ionic form. Consequently, it does not leave as much residual copper in the wine
- The manufacturer claims only about 50% goes into wine
- **This is not legal in Canada yet**

Wine Treatment Agents

Agent	Formula	Composition	Notes
Copper Sulphate	$\text{CuSO}_4 \times 5 \text{ H}_2\text{O}$	25.5 % Copper	1 g = 255 mg copper
Copper Citrate	$\text{Cu}_2\text{C}_6\text{H}_4\text{O}_7 \times 2.5 \text{ H}_2\text{O}$	35 % Copper	1 g = 350 mg copper
Kupzit	$\text{Cu}_2\text{C}_6\text{H}_4\text{O}_7 \times 2.5 \text{ H}_2\text{O}$ in Bentonite	2 % Copper Citrate	50 g Kupzit = 1 g copper citrate = 350 mg copper

Eliminating Hydrogen Sulfide

Silver Chloride (AgCl)

- Works like a charm
- Silver preparations (“sulfidex”) were permitted in Europe, typically bound to diatomaceous earth and/or fixed onto filter pads
- It worked for H_2S and mercaptans and diethyldisulfide
- It left no silver in the wine and therefore, no additional blue fining was necessary
- It is no longer permitted in Europe because silver is considered a “heavy metal” compound but will it still be allowed in other countries???? We don’t know.

Wine Treatment Agents

Treatment	Impact on Wine
Copper Sulphate	<ul style="list-style-type: none">•Increased copper content•Bitter flavour•Possible cloudiness (casse)•Blue fining possibly required
Silver Chloride (bound to diatomaceous earth)	<ul style="list-style-type: none">•Increased silver content•Bitter flavour•Possible cloudiness•Blue fining possibly required
Copper Citrate (Cupric Citrate) Copper is chelated to citric acid which is bound to bentonite as a carrier	<ul style="list-style-type: none">•Low increase of copper content•Lower probability of blue fining•Can easily be filtered out

What is the cause of H₂S production (reduction of wine) -the relationship to nitrogen

- There is a link between low nitrogen values in juice and hydrogen sulfide production during fermentation—more to come shortly
- During fermentation, yeast metabolizes nitrogen, ie it needs nitrogen badly
- This needed nitrogen has to come from the medium, the grape juice
- It is present in the form of ammonia (NH₃) (small amounts) and is present in a bound form in the amino acids (there are about 20 amino acids)

Measuring Juice Nitrogen

- This nitrogen from ammonia and amino acids can be analyzed for and is expressed as Ammonia Nitrogen (mg N/L) and Free Amino Nitrogen (FAN) (mg N/L)
- The sum of both is expressed as Yeast Assimilable Nitrogen Concentration (YANC) (mg N/L)
- We should remember that the FAN is not as easy for the yeast to extract and use
- The yeast has to “work hard” to “pry off” the nitrogen from the amino acids

Yeast Nitrogen Usage

- The ammonia nitrogen is very easily metabolized by the yeast
- It is like “candy” for the yeast and the yeast prefers it since it can be immediately taken up and used
- The yeast needs nitrogen for the biosynthesis of its cell mass and to perform other numerous biosynthetic reactions

Amino Acids

- There are 20 amino acids
- Arginine, Proline and Glutamine are the most abundant amino acids in grape juice for most varieties and vintages
- Proline and Lysine are virtually not used by the yeast as a nitrogen source

Amino Acids in grapes during ripening

Table 3. Changes in the composition of the free amino acids in grape berries during ripening (grape variety Riesling, 1976; mg/L) (31).

Date:	9 Aug.	23 Aug.	8 Sept.	22 Sept.
Amino acid				
Aspartic acid	60	48	75	74
Threonine	23	50	109	128
Serine	35	70	126	155
Asparagine	48	35	45	44
Glutamic acid	69	106	140	160
Glutamine	241	630	1000	1030
Proline	8	46	165	278
Glycine	7	9	10	17
Alanine	10	62	102	146
λ -ABA	18	14	22	22
Valine	9	7	18	57
Methionine	3	5	11	20
Isoleucine	8	13	18	71
Leucine	10	17	24	70
Tyrosine	12	12	24	28
Phenylalanine	17	14	21	72
λ -ABA	17	68	113	220
Ornithine	8	8	8	16
Lysine	5	3	5	10
Histidine	20	18	24	45
Arginine	150	257	497	660
Total	778	1492	2538	3322

Sponholz, Intl.
Symposium on
Nitrogen in
grapes and
wine, 1991

Ammonia and Amino Acid Usage Preference by Yeast

Most Preferred N sources

- Ammonia: is not very abundant in grape juice
- Glutamic Acid: is not so abundant in grape juice but is a preferred N source
- Glutamine: is quite abundant in grape juice and is a preferred N source

Ammonia and Amino Acid Usage Preference by Yeast

Next most preferred N Sources

- Since arginine is so abundant, the yeast will also readily use it, but it is a moderately preferred N source for yeast
- Excess of arginine in grape juice (over 1000 mg/L) is not desirable since it can release urea when yeast metabolize it, and the urea reacts with ethanol to form ethyl carbamate (carcinogenic compound)
- The federal limit of ethyl carbamate is 30 ppb but the LCBO allows only 20 ppb at the time of bottling

Wine Ethyl Carbamate levels pre-1980 vs post 1980

Table 16. Ethyl carbamate content ($\mu\text{g/L}$) in wines of the vintages 1951-1989.

Vintage	Samples	Minimum	Maximum	Average
1989	4	0	12.3	3.1
1988	17	0	16.9	2.2
1987	8	1.1	9.5	3.7
1986	30	1.9	5.2	3.7
1980-1984	5	3.1	10.1	5.2
1970-1979	41	5.7	35.8	18.9
1960-1969	13	12.2	37.8	20.4
1951-1959	9	10.9	48.6	27.3

Sponholz, Intl. Symposium on
Nitrogen in grapes and wine, 1991

Grape Juice Nitrogen in Niagara musts

Yeast Assimilable Nitrogen Content (YANC) in Table Wine and Icewine

Variety	2007 (Drought)			2008 (Rainy)		
	Ammonia (mgN/L)	Primary Amino Nitrogen (mgN/L)	Total YANC (mgN/L)	Ammonia (mgN/L)	Primary Amino Nitrogen (mgN/L)	Total YANC (mgN/L)
Table Wine						
Pinot Grigio	14	32	46	66	168	234
Pinot Grigio	61	92	153	95	233	328
Chardonnay	23	18	41	63	158	221
Chardonnay	48	47	95	86	263	349
Chardonnay	24	31	55	66	259	325
Riesling	46	44	90	77	128	205
Riesling	49	74	123	70	153	223
Viognier	15	81	96	102	247	349
Pinot Noir	35	74	109	105	208	313
Pinot Noir	14	178	192	127	295	422
Pinot Noir	32	56	88	90	209	299
Merlot	16	26	42	69	152	221
Merlot	37	48	85	86	178	264
Cabernet Franc	16	278	294	27	79	106
Cabernet Franc	1	45	46	29	92	121
Cabernet Franc	20	83	103	24	57	81
Cabernet Sauvignon	32	34	66	21	62	83
Cabernet Sauvignon	37	28	65	19	51	70
Shiraz	55	104	159	27	321	348
Shiraz	71	195	266	48	355	403
Icewine						
	2004*			2008		
Vidal	47.6	443.3	490.9	64	453	517
Riesling	84.2	375.8	460.0	107	288	395

* 2004 Icewine data was obtained from the lab of Dr. D. Inglis (Director, CCOVI) and are averages of 212 Vidal Icewine juice samples and 20 Riesling juice samples

All table wine data was obtained from single vineyard sites which are constant across the vintages

Table compiled by Marc Pistor, Inniskillin Wines

Nitrogen Values in NY Musts

Table 6. Amount of sugar (°Brix), pH, concentration (mg/L) of ammonia, some of the free amino acids (mg/L), total nitrogen (N), and total amino acids less proline (AA) in fresh New York grape juices of 15 varieties (11).

Grape variety	°Brix	pH	NH ₃	Arg	Cit	Gln	Thr	Pro	Asn	N	AA
Aurore	16.0	3.15	58	67	2	314	100	91	15	660	843
Catawba	16.6	3.04	2	91	4	131	76	69	6	380	571
Chardonnay	21.8	3.18	18	46	<1	63	90	628	6	310	1025
Concord	14.8	3.41	14	35	<1	60	38	20	3	330	361
Niagara	15.0	3.35	12	50	<1	92	45	22	4	220	376
Cayuga	16.8	3.02	na	20	1	37	25	42	4	na	221
Elvira	14.6	3.15	1	34	1	53	24	9	3	230	220
Ravat	21.2	3.20	2	136	1	72	39	260	7	550	724
Riesling	18.6	2.84	1	16	2	14	36	86	0	150	226
Seyval	18.4	2.93	2	26	1	72	19	101	4	300	337
Vidal	18.4	2.93	1	114	2	57	145	195	4	320	762
Baco noir	—	—	6	113	2	98	45	484	7	410	1056
Cabernet Sauvignon	20.8	2.96	-	32	2	13	48	489	-	—	656
Chancellor	16.2	3.23	1	116	1	61	35	39	4	440	392
Pinot noir	16.8	2.99	-	52	1	13	33	52	-	—	224

Varieties 1 - 5: high N fertilization (200 kg NH₄NO₃/ha), except Chardonnay (75 kg NH₄NO₃/ha). Varieties 6 - 15: no N fertilization and normal maturity. na = not analyzed. Results by courtesy of T. Henick-Kling, Geneva, NY (11).

Amino Acids in must before and after Botrytis Infection

Table 8. The use of amino acids by *Botrytis cinerea* after infection of the grapes. Average amounts in 12 pairs of the 1972 vintage.

	Healthy grapes (mg/L)	Infected grapes (mg/L)	Difference	
			(mg/L)	(%)
Tryptophane	0.2	0.3	+0.1	+50
Lysine	45	37	-8	-18
Histidine	78	60	-18	-23
Arginine	959	574	-385	-40
Aspartate	57	31	-26	-46
Threonine	163	87	-76	-47
Serine + Amides	478	300	-178	-37
Glutamate	142	87	-55	-39
Proline	509	247	-262	-51
Citrulline	83	53	-30	-36
Glycine	15	14	-1	-7
Alanine	201	149	-52	-26
Valine	116	69	-47	-41
Methionine	37	15	-22	-59
Isoleucine	117	53	-64	-55
Leucine	160	63	-97	-61
Tyrosine	33	26	-7	-21
Phenylalanine	138	73	-65	-47
Ammonia	62	47	-15	-24
Total	3393	1985	-1408	-41

Sponholz, Intl.
Symposium on
Nitrogen in grapes
and wine, 1991

Influence of Nitrogen Fertilization on Amino Acid composition in Grapes

Table 5. The influence of N-fertilization on the amino acid content (mg/L) of grape berries (plants grown in Mitscherlich pots). Grape variety: Müller-Thurgau on Kober 5BB (33). P-, K-fertilization: 2 g/plant.

Amino acid	N (g/plant)				
	1	2	3	4	6
Aspartic acid	23	42	69	111	138
Threonine	46	165	256	229	284
Serine	41	152	304	260	330
Asparagine	14	50	93	108	171
Glutamic acid	132	223	330	355	380
Glutamine	125	767	2708	2045	4499
Proline	284	587	598	943	867
Alanine	25	107	198	194	275
Valine	20	72	108	121	112
Methionine	6	33	44	39	33
Isoleucine	17	58	75	86	76
Leucine	22	92	112	136	136
Histidine	36	143	196	209	197
Arginine	331	1360	2073	2233	2322
Total	1324	4349	7923	7926	10894

Sponholz, Intl. Symposium on Nitrogen in grapes and wine, 1991

Yeast Sulfur Metabolism

Sources of Sulfur compounds

- Both organic and inorganic sulfur compounds are present in grape juice and can be utilized by the yeast

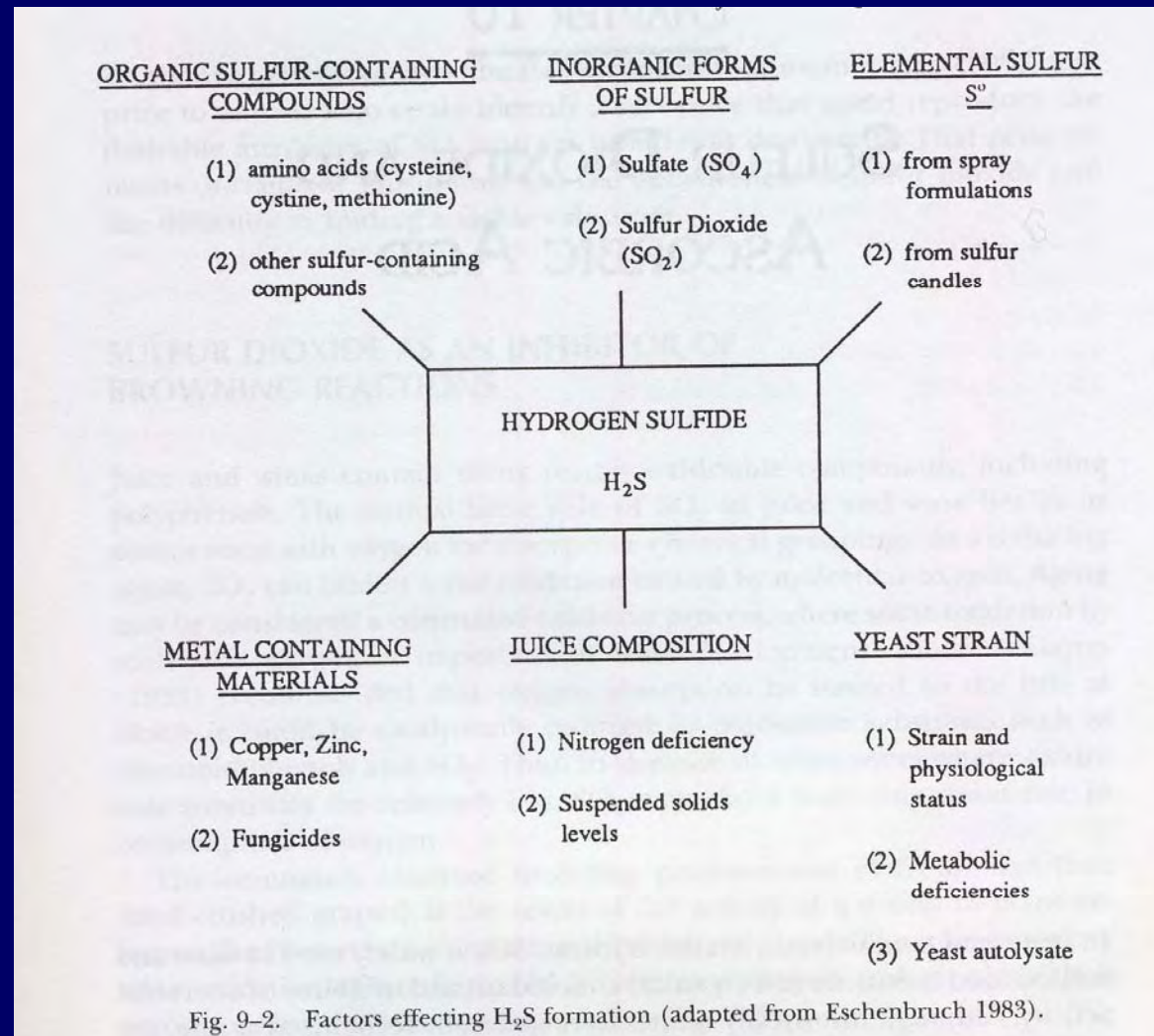
Inorganic Sulfur in grapes/juices

- Sulfate (SO_4^{2-})
- Sulfite (SO_3^{2-}) from addition of SO_2
- Elemental Sulfur (S^0) from spray residues
 - In Ontario, 21 day preharvest interval for S sprays

Organic Sulfur in grapes/juices

- Predominantly S- containing amino acids cysteine and methionine
- Less dominant are the vitamins thiamine and biotin

Factors Effecting H₂S Formation



Sulfate Reduction Sequence in making sulfur containing amino acids

- During fermentation, sulfur compounds can be reduced to hydrogen sulfide by the sulfate reduction sequence (SRS) if the yeast needs it to make cysteine and methionine
- In the absence of acceptor molecules (organic nitrogen compounds) for the sulfide when the SRS is triggered, the hydrogen sulfide is released into the wine from the yeast
- In order for the yeast to scavenge the hydrogen sulfide and incorporate it into cysteine and methionine, the yeast need plenty of nitrogen
- If the yeast does not need to make cysteine and methionine because it is available in the grape juice, then they do not trigger the SRS to make the sulfide

Sulfate Reduction Sequence: the path to make S-amino acids

Jiranek and Hallinan

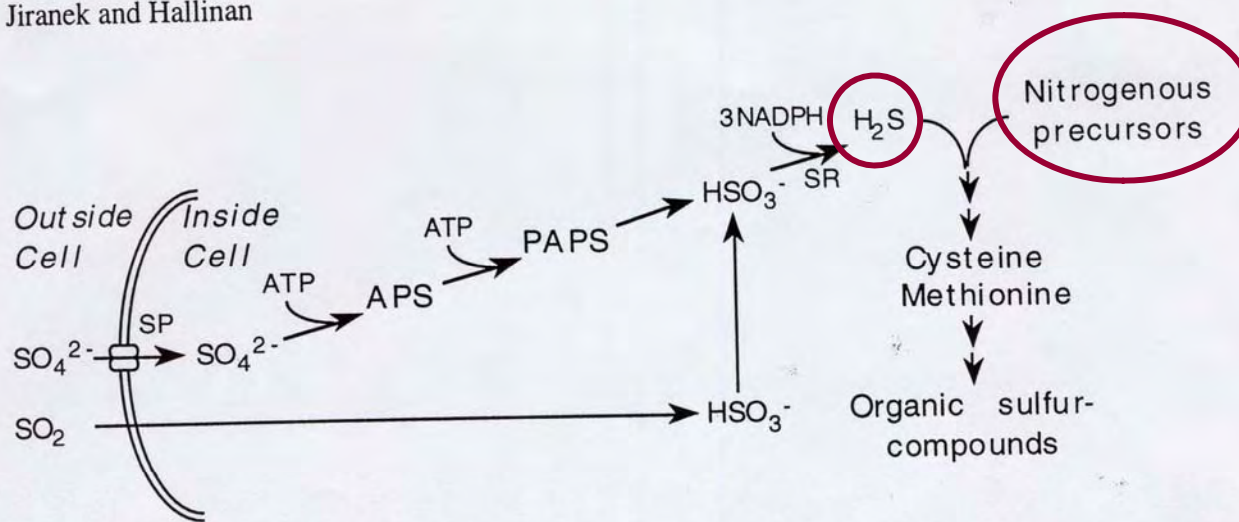
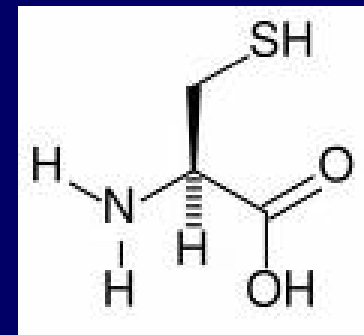
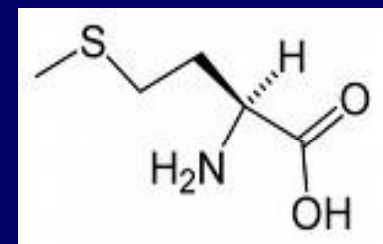


Figure 3. Sulfate reduction sequence and synthesis of organic sulfur compounds: APS, adenosyl 5'-phosphosulfate; PAPS, 3'-phosphoadenosyl 5'-phosphosulfate; SP, sulfate permease I and II; SR, sulfite reductase.



Cysteine



Methionine

Yeast Nitrogen Metabolism

- Nitrogen is the second most important nutrient for the yeast biosynthesis after carbon
- A great majority of the nitrogen comes from the degradation of amino acids
 - 60-90% comes from only 8 amino acids (Kliever, 1969)
- This is called FAN (free amino nitrogen)
- Ammonia is preferred but is less abundant in grape juice

YANC-Yeast Assimilable Nitrogen Content

- Not all grape varieties have the same YANC content, varies by location as well
- The weather (season, rain vs dry) in a particular year has a large impact on YANC (both Ammonia and FAN)
 - Previous table from Niagara showed in a dry year, the YANC values were much lower (2007) than in a wet year (2008)
 - Eg Chardonnay: Only 55 mg N/L in 2007 vs 325 mg N/L in 2008
 - Importance of measuring must nitrogen values

The Effect of Drought vs Rain on Must N

Yeast Assimilable Nitrogen Content (YANC) in Table Wine and Icewine

Variety	2007 (Drought)			2008 (Rainy)		
	Ammonia (mgN/L)	Primary Amino Nitrogen (mgN/L)	Total YANC (mgN/L)	Ammonia (mgN/L)	Primary Amino Nitrogen (mgN/L)	Total YANC (mgN/L)
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YANC-Yeast Assimilable Nitrogen Content

- Vineyard fertilization will also influence the FAN/YANC in the juice, both in terms of total abundance and in composition (previous table)
 - Remember the trouble when a lot of wines had defects because of nitrogen starvation in the vineyards (Atypical Aging, ATA)
 - 50-90 kg of N/Ha is accepted in CA, excess nitrogen will however cause excess vigor

YANC-Yeast Assimilable Nitrogen Content

- Cropping levels in the vineyard have a significant influence on the FAN/YANC content
 - High cropping will produce a shortage of YANC
- With an increase and adequate YANC levels, there is a great increase in ester formation (aroma) during fermentation (A. Rapp and Q. Versini, 1984/85)
- With a shortage of YANC, there is low ester formation (ie can lead to lack of varietal aromas and strange off aromas develop in the bottle)
 - ATA-atypical aging
 - UTA-untypical aging (Rapp and Versini)

YANC-Yeast Assimilable Nitrogen Content

- Bentonite fining of the juice with 100g/hL (1 g/L) may remove up to 30% of the amino acids
 - This consequently lowers the FAN which may result in a shortage of “YANC”
- The minimum required YANC to support yeast during fermentation accepted by researchers in model solutions is 150 mg N/L, but this also varies with yeast strain
- The preferred concentration of YANC to target is approx. **300 mg N/L**
- Most grape juices **do not** have this amount of YANC and some varieties are really short in certain years

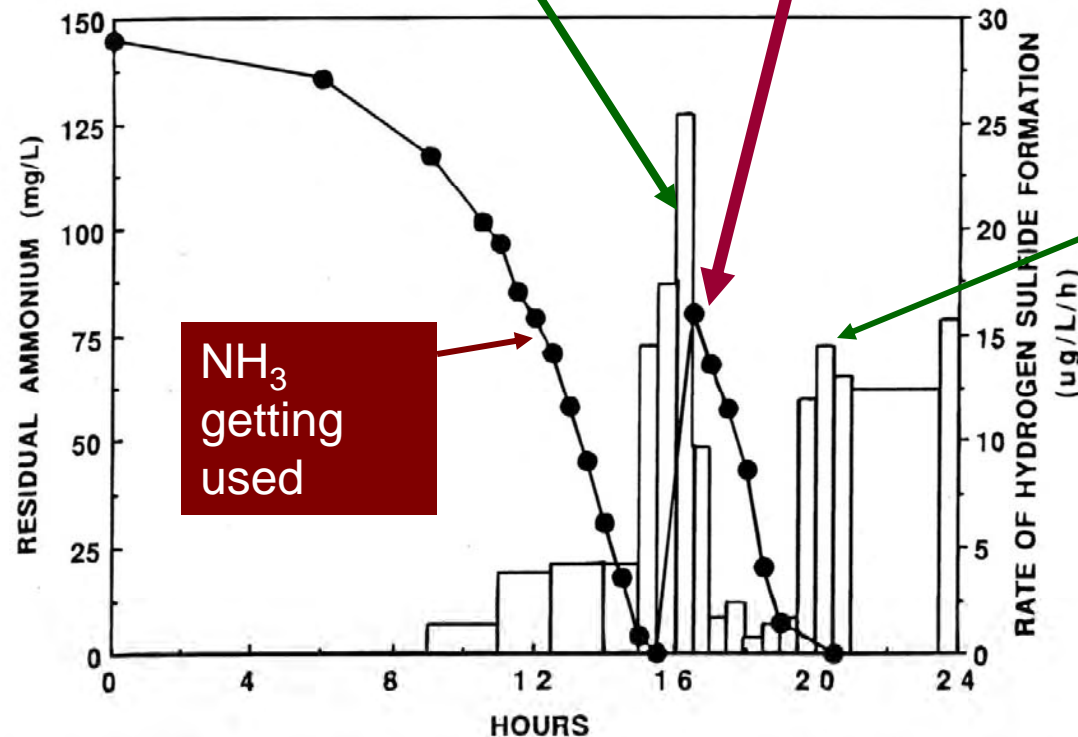
H₂S Production as a Consequence of YANC Shortage

- If there is insufficient nitrogen in the grape juice, the yeast turn on the SRS pathway to make cysteine and methionine, the S-containing amino acids
- The SRS pathway makes hydrogen sulfide from Sulfate
- In order for the yeast to scavenge the hydrogen sulfide and incorporate it into cysteine and methionine, the yeast need plenty of nitrogen
- If there is not enough organic nitrogen, the yeast release the hydrogen sulfide into the wine

Low Nitrogen leads to H₂S, ammonia supplementation reduces H₂S

Lots of H₂S when NH₃ gone

Ammonia addition, H₂S drops

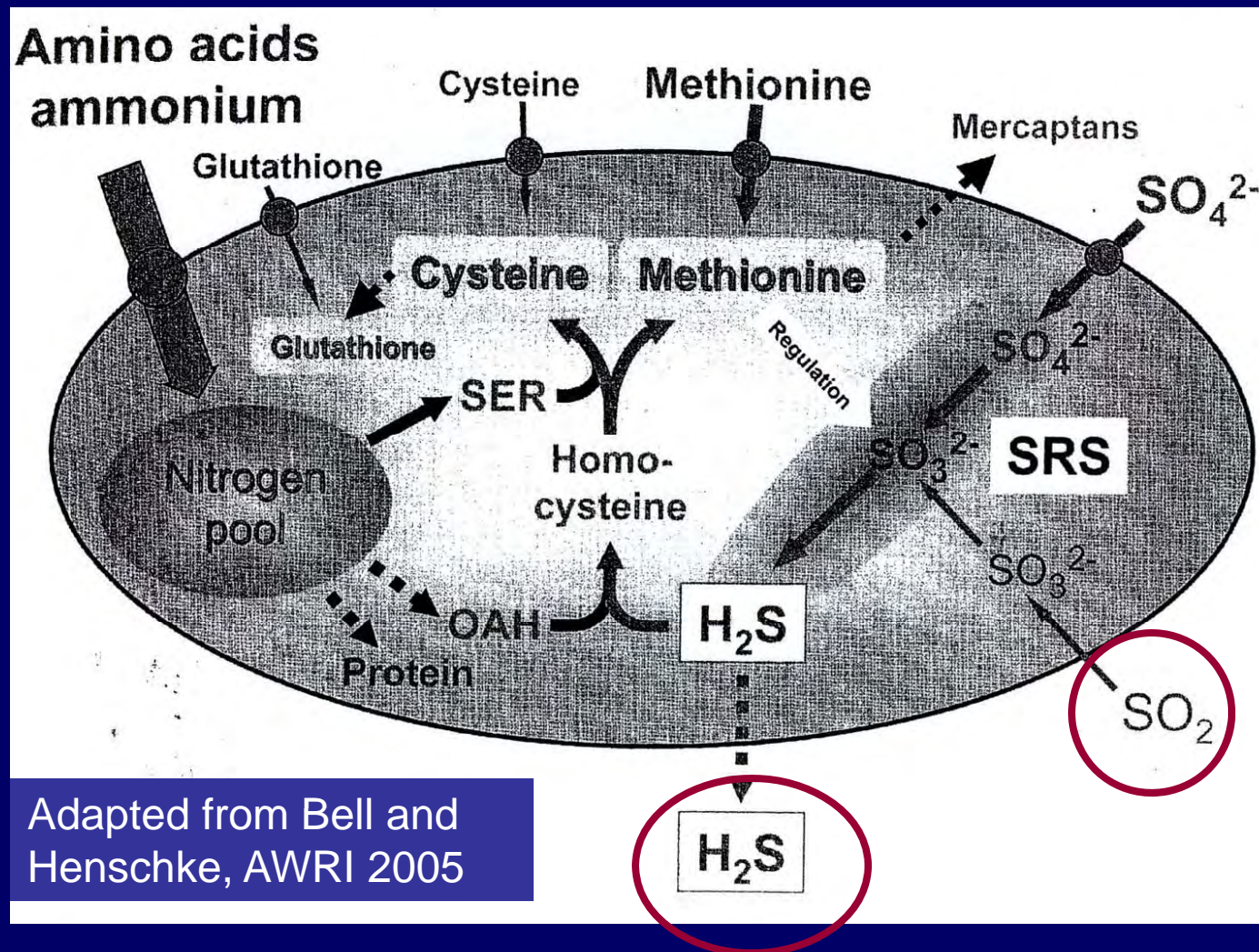


H₂S again
when NH₃
gone

Fig. 4. Affect of ammonium supplementation (solid symbol) on the rate of hydrogen sulfide evolution (histogram) in a synthetic grape juice medium inoculated with AWRI 72. Fermentation at 30°C was monitored continuously for hydrogen sulfide as described in the text.

Sulfate Reduction Sequence

if low Nitrogen, sulfite can form H_2S



The role of Sulfite in hydrogen sulfide production when nitrogen is limiting

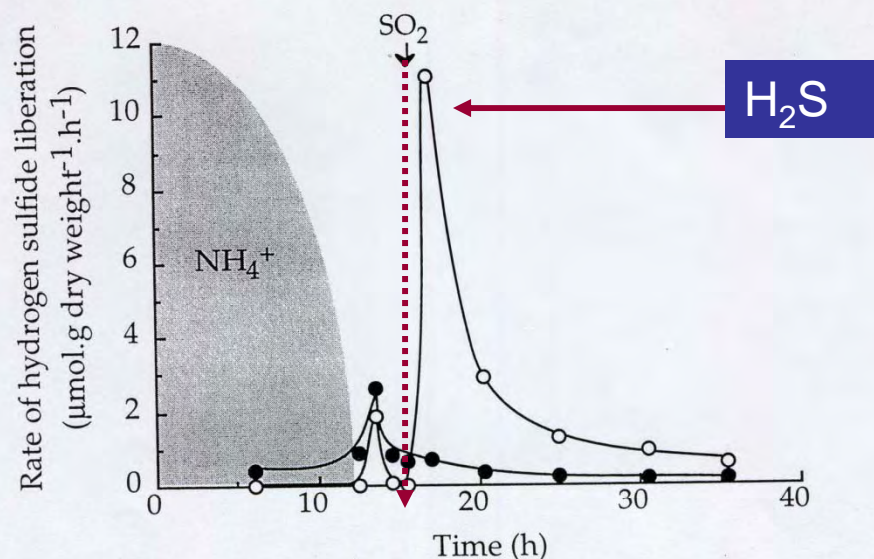


Figure 2. Total hydrogen sulfide liberation (O) and production from ³⁵S-sulfate (●) by strain AWRI 77. Fermentations were conducted in GJM containing 8.3 mM NH₄Cl and ³⁵S-sulfate (5 mM, 4 μCi/mmol specific activity). Sulfite was added to 260 μM at the time indicated (SO₂) following the point of ammonium depletion. Ammonium utilisation profile is shown schematically only (Redrawn from Jiranek *et al.*, 1995).

Diammonium Phosphate: **The Miracle compound to avoid H₂S in wine**

- Diammonium phosphate (DAP) $(\text{NH}_4)_2\text{HPO}_4$ is a plant fertilizer as well as a yeast nutrient
- 20% of the weight of DAP is nitrogen
ie 1.0 g/L DAP added to juice = 0.2 g/L of Nitrogen
(or 200 mg N/L from each 1 g/L of DAP)
- The Bureau of Alcohol, Tobacco, Firearms and Explosives (ATF) in the US allows 1.0 g/L DAP
- The Canadian Regulations stipulate now GMP (Good Manufacturing Practice) as the limiting condition for DAP addition

Diammonium Phosphate: **The Miracle compound to avoid H_2S in wine**

- DAP will supplement the missing YANC needed to avoid H_2S in wine
- Yes, it is an incredible tool to prevent reduction of a wine (H_2S and mercaptan formation)
 - It will supply, if added at the right time, the nitrogen needed to complete the necessary biosynthesis of organic S-compounds such as cysteine and methionine
 - This is necessary to “scavenge” the H_2S produced by the Sulfate Reduction Sequence

Diammonium Phosphate: **The Miracle compound to avoid H_2S in wine**

- If there is a shortage of Nitrogen to achieve the biosynthesis of cysteine and methionine, then the H_2S will be released from the yeast into the wine and the wine becomes **REDUCED**
- Even if there is already H_2S in the wine in the early stages of fermentation due to a nitrogen deficiency, the addition of DAP will eliminate the H_2S within a few hours from the wine

Recommended Application of DAP

Two Stages

- Stage One: to the juice at the start of fermentation, add 50% of the amount of DAP into the fermenting vat
- Stage Two: at around 5% ethanol, add the remaining 50% of the DAP

Single Stage

- There is no negative effect if one adds the total amount at the beginning of the fermentation
- Important to consider in barrel fermentations

Beware

- No DAP should be added once the alcohol reaches above 7% alcohol (the yeast can not take it up effectively, too late by then)

IMPORTANT MESSAGE

- Whenever the yeast runs out of nitrogen during the early stages of fermentation (i.e. when their biosynthetic pathways are most active), YOU WILL GET H_2S – A REDUCED WINE- SINCE THE SULFATE REDUCTION SEQUENCE IS TURNED ON
- However, when there is enough nitrogen available for the yeast, the H_2S , even though it may be made by the SRS, won't be released into the wine (it gets scavenged into S-amino acids).

Practical considerations to cure minor reductive wine aromas with $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$

We must keep in mind that the

- Federal law for copper in wine is 1.0 ppm (1 mg/L)
 - Changed in 2006
- The LCBO's limit (as well as most liquor jurisdictions) for copper in wine is 1.0 ppm (1 mg/L)
- Note: WHO limit for drinking water is 2 ppm and EPA limit is 1.3 ppm
- Danger of **COPPER CASSE**

Sometimes small amounts of copper (Cu^{2+}) added can eliminate minor off smells of reduced sulfur compounds (H_2S and mercaptan)

The reaction of copper with H_2S is fast, the reaction of Cu^{2+} with mercaptan takes time

Checking for H₂S or mercaptan in wine

- Any wine checked correctly for reduced aromas must be clear and properly filtered. **No yeast or colloids may be present for a valid test**

A. Simple “Penny Test”

- Not a quantitative but rather a qualitative test
- Sometime adding a few pennies to 50-100 mL of wine in a glass will show quickly if a wine is helped with copper when compared with another glass with no pennies – side by side
- US Pennies: were until 1982 made of 95% copper + 5% zinc, today they are 97.5% zinc and 2.5% copper plating
- Canadian Pennies: today they are 94% steel, 1.5% nickel, 4.5% copper plating

(since the copper plating is on the outside, it is exposed to the wine)

A. Simple “Penny Test”

- If only H_2S is present the wine will clean up quickly
- If mercaptan is present the wine may clean up if left in contact for longer
- It is wise to leave the pennies in the glass for a while (up to several hours) to see if mercaptans are present
- This is a quick and easy test for reduced aromas

B. Test with a standard copper solution

- With this method, a much more analytical and quantitative result can be attained
- With this procedure, you can evaluate how much copper needs to be added to eliminate the reduced aromas
- In both tests (the penny and the standard copper solution test) the wine may need time to clean up if mercaptan is present
- Remember mercaptan elimination is a slow process since the Cu^{2+} has to pry off the HS from the ethyl mercaptan molecule

Preparation of a Standard Copper Sulfate solution

- Distilled Water - 1L (1000 mL)
- Analytical grade $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ - 2.0 g
(note: $\text{CuSO}_4 \cdot 5\text{H}_2\text{O} = 25\% \text{Cu}^{2+}$)

ie

- 2.0 g $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ /1000 mL water
= 0.5 g Cu^{2+} /1000 mL
= 500 mg Cu^{2+} /1000mL
= 0.5 mg Cu^{2+} /1 mL
(1.0 g $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ /500 mL water will give same concentration)

Quick Test using copper solution

- 1 mL of this solution is 20 drops from an eyedropper, which is 0.5 mg of Cu^{2+}

Drops of solution	Copper in 100mL of wine	Cooper concentration
1 drop	0.025 mg Cu^{2+} /100 mL	0.25 mg Cu^{2+} /L
2 drops	0.05 mg Cu^{2+} /100mL	0.5 mg Cu^{2+} /L
3 drops	0.075 mg Cu^{2+} /100mL	0.75 mg Cu^{2+} /L
4 drops	0.1 mg Cu^{2+} /100mL	1.0 mg Cu^{2+} /L

- Leave the 100mL samples for several hours on the counter or in fridge, and then compare smell to control wine
- If you see it has not cleared up the wine in this time period, then you would prepare 500 mL bottles or 1L bottles with the equivalent concentrations (0.25, 0.5, 0.75 and 1.0 mg Cu^{2+} /L), keep in fridge for 1 week to see if the mercaptans can be removed

Some Last Words

- Research has shown that wine produced from clean juices have superior organoleptic properties
 - Approximately 0.1% solids
- It is most important to ensure that YANC levels in juice and must is adequate to avoid the SRS
 - 300 mg/L of YANC should be enough
 - Adding 1 g/L DAP will give 200 mg/L of added Ammonia
- Check for H_2S and mercaptan with the simple penny test if you have a suspicion
- In the case that you find reduced aromas carry on using the standard copper sulphate solution
- Approach the Federal Government to allow the use of cupric citrate
 - Has been legal in many countries since 2006
 - Austria, Switzerland, South Africa, most of eastern Europe, and Australia and New Zealand (if bound to bentonite)

THANK YOU

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