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Trends in the research of malolactic fermentation

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Legal limits for total SO_2 in major winemaking nations (in mg/l)

Country	Wine type (RS)	Limit	Legal Reference/Description
USA	All	350	27 CFR 4.22(b)(1)
AUS	<35 g/l sugars	250	ANZFSC 4.5.1: Clause 5(5)(a)
	>35 g/l sugars	300	
NZ	<35 g/l sugars	250[b]	
	>35 g/l sugars	400[b]	
EU	white/rosé, <5 g/l	200	EC No 606/2009, Annex I B
	red, <5 g/l sugars	150	
	white/rosé, >5 g/l	250	
	red, >5 g/l sugars	200	
	specific wines	300	Eg.: Spätlese (can be dry), Bordeaux Sup., Côtes de Bordeaux, C. de Bergerac, Navarra, Penedès, several French VdP and Hungarian and some Greek sweets
	specific wines	350	E.g.: Auslese (can be dry), sweet wines from Romania, Czech Rep., Slovakia and Slovenia
	specific wines	400	E.g.: Beerenauslese, TBA, Eiswein, French sweet wines such Sauternes, Barsac, etc., sweet Greek with >45 g/l sugars, sweet Eastern European wines
CAN	All	350[b]	Canadian Food & Drug Reg, B.02.100
India	All	450	Prevention of Food Adulteration Act & Rules, Appendix C, Table 3
Japan	All (>1% abv)	350[b]	Japan's Specifications and Standards for Food Additives
RSA	white, <5 g/l sugars	160	Liquor Products Act 60 of 1989 Regulations Regulation 32 (Table 8)
	reds, <5 g/l sugars	150	
	All, >5 g/l sugars	200	
	specific wines	300	E.g.: noble late harvest and naturally dried

[a] unit is mg/kg
[b] Canada prescribes a maximum of 70 mg/l free or 350 mg/l combined SO_2

http://www.orwine.org/

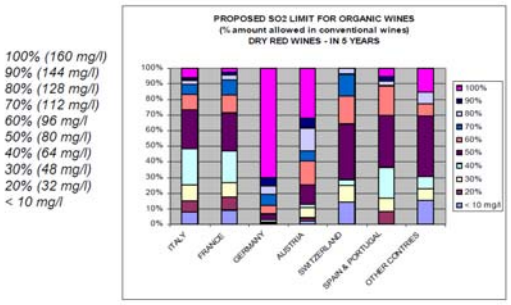

Figure 5: Proposed SO_2 limits for dry red wines

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Acetaldehyde a new enemy?

SPIEGEL ONLINE WISSENSCHAFT

Likörweine enthalten Krebsgift in hoher Konzentration

"Liquor-wines contain toxic carcinogen in high concentrations"

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Contains Acetaldehyde….??

SPIEGEL ONLINE WISSENSCHAFT

Acetaldehyd-Konzentration in alkoholischen Getränken

	Mittelwert	Spanne
Bier	9 mg/l	0 - 63 mg/l
Wein	34 mg/l	0 - 211 mg/l
Spirituosen	66 mg/l	0 - 1159 mg/l
Likörweine	118 mg/l	12 - 800 mg/l

Alle Angaben in Milligramm pro Liter (mg/l). Den höchsten Gehalt hatte mit 1159 mg/l ein deutscher Obstbrand. Werte um die 700 mg/l besaßen die höchstbelasteten Proben unter chinesischen Schnäpsen (721), mexikanischem Bacanora (696) und mexikanischem Tequila (670).

Als Molekül macht es nicht viel her. Gerade mal ein ordinäres Sauerstoff-, zwei Kohlenstoff- und vier Wasserstoffatome braucht es, schon hat man Acetaldehyd. Auch Mediziner und Biologen machen im Allgemeinen nicht viel Aufhebens um die Substanz mit dem sperrigen Namen. Sie ist ein normales, wenn auch rasch umgesetztes Zwischenprodukt des Kohlenhydratstoffwechsels von Pflanzen und Tieren. Und dennoch: Hinter dem biochemischen

http://www.spiegel.de/wissenschaft/mensch/0,1518,579783,00.html

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Acetaldehyde and cancer

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The role of acetaldehyde outside ethanol metabolism in the carcinogenicity of alcoholic beverages: Evidence from a large chemical survey

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ABSTRACT

Acetaldehyde is a volatile compound naturally found in alcoholic beverages, and it is regarded as possibly being carcinogenic to humans (IARC Group 2B). Acetaldehyde formed during ethanol metabolism is generally considered as a source of carcinogenicity in alcoholic beverages. However, no systematic data is available about its occurrence in alcoholic beverages and the carcinogenic potential of human exposure

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Journal of Experimental & Clinical Cancer Research

RESEARCH Open Access

Short-term salivary acetaldehyde increase due to direct exposure to alcoholic beverages as an additional cancer risk factor beyond ethanol metabolism

Dirk W Lachenmeier[1*], Yulia B Monakhova[1,2]

Abstract

Background: An increasing body of evidence now implicates acetaldehyde as a major underlying factor for the carcinogenicity of alcoholic beverages and especially for oesophageal and oral cancer. Acetaldehyde associated with alcohol consumption is regarded as 'carcinogenic to humans' (IARC Group 1), with sufficient evidence available for the oesophagus, head and neck as sites of carcinogenicity. At present, research into the mechanistic aspects of acetaldehyde-related oral cancer has been focused on salivary acetaldehyde that is formed either from ethanol metabolism in the epithelia or from microbial oxidation of ethanol by the oral microflora. This study was conducted to evaluate the role of the acetaldehyde that is found as a component of alcoholic beverages as an additional factor in the aetiology of oral cancer.

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Addiction

REVIEW doi:10.1111/j.1360-0443.2009.02516.x

Carcinogenicity of acetaldehyde in alcoholic beverages: risk assessment outside ethanol metabolism

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ABSTRACT

Aims In addition to being produced in ethanol metabolism, acetaldehyde occurs naturally in alcoholic beverages. Limited epidemiological evidence points to acetaldehyde as an independent risk factor for cancer during alcohol consumption, in addition to the effects of ethanol. This study aims to estimate human exposure to acetaldehyde from alcoholic beverages and provide a quantitative risk assessment. **Methods** The human dietary intake of acetaldehyde via alcoholic beverages was estimated based on World Health Organization (WHO) consumption data and literature on

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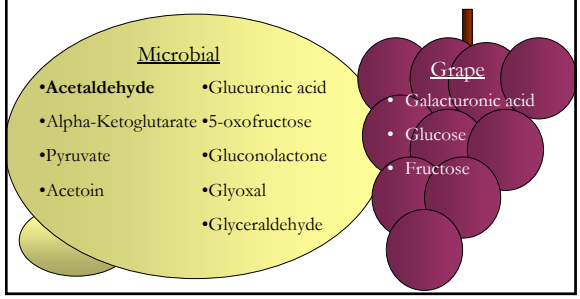
SO_2 Binding Compounds



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UHPLC Method

- 36 MPa (5,000 psi) System
- 2 µm particle size OCR phase
- Hydrazine-derivatisation (DNPH)
- Sample preparation
  - Control of oxidation by EDTA

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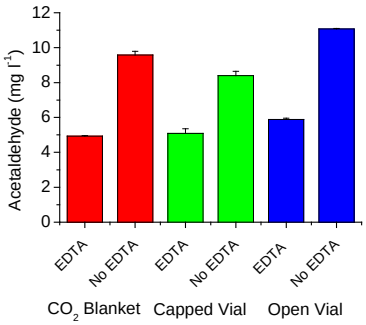
UHPLC Method



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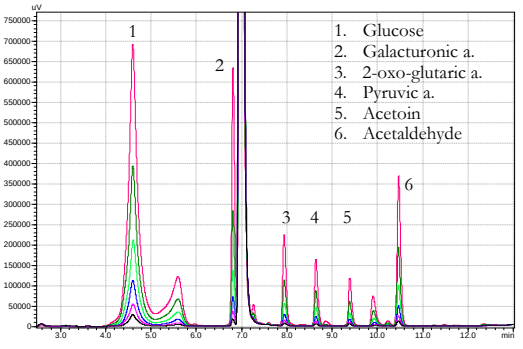
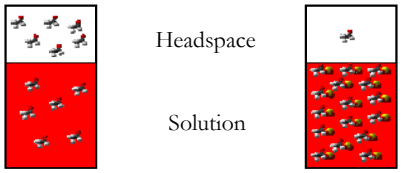


Acetaldehyde measurement

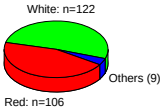
No SO_2 | SO_2 added

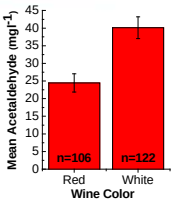


$$K_d = \frac{SO_2 + CH_3CHO}{HSO_3COHCH_3} = \sim 1 \times 10^{-6}$$

Analytical problems with GC (headspace or not)

SO_2 binders in New York wines





unit: g l^{-1}

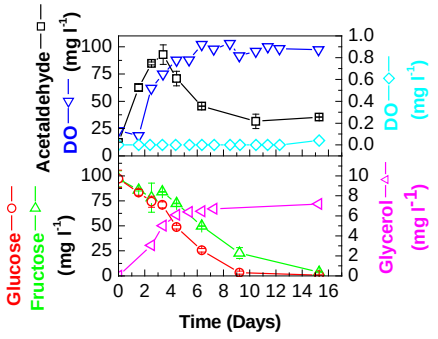
Wine Type	Glucose	Galacturonic Acid	Alpha-Ketoglutarate	Pyruvate	Acetoin	AcHO
White	4750	267	31	25	10	40
Red	1400	810	74	14	11	25
p-value	0.0033*	<0.0001*	<0.0001*	<0.0001*	0.7897	0.0002*

How much SO_2 is bound ?

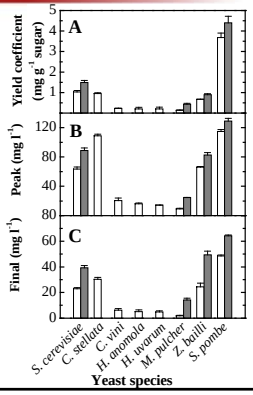
- Estimation of bound fraction using dissociation constants
  - 30 mg/l free SO_2 assumed

SO_2 binder	Percent of wine carbonyl bound to SO_2 [%]	
	Red	White
Acetaldehyde	55.4±4.3	71.5±4.3
Pyruvic acid	11.6±1.6	16.7±1.6
α-ketoglutaric acid	22.6±1.2	7.6±0.9
Galacturonic acid	10.4±0.6	2.7±0.2
Glucose	nil	1.5±0.3
Total	100	100
Bound SO_2	65.8 mg l^{-1}	81.2 mg l^{-1}

Acetaldehyde metabolism during AF



Acetaldehyde produced by different yeast genera and species



Factors for acetaldehyde formation during AF

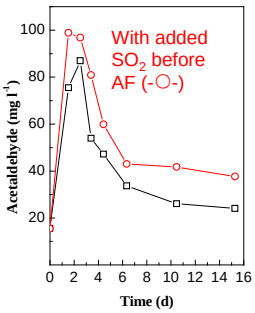
Factors:

- Yeast (EC1118, DV10)
- pH (normal and 3.6)
- SO_2 addition (0 and 30 ppm)
- Nutrients (none and 250 ppm)
- Temperatures (12 and 20 C)
- Variety (Gewürz and Pinot Blanc)

Significant Factors

- **SO_2 addition**
- Nutrient addition
- High pH values
- Low temperatures
- Yeast strain (*Sacch. cer.*)
- **Grape variety**

Acetaldehyde production during AF – SO_2



- 0.2-0.5 mg acetaldehyde per mg of SO_2 in model system

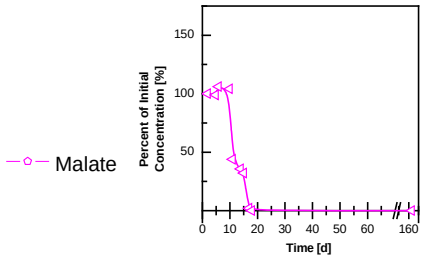
In wine fermentations

- Peak: 0.3 - 1 mg acetaldehyde per mg SO_2
- Final: 0.2 - 0.5 mg acetaldehyde per mg SO_2
- Result: 0.3 - 0.75 mg bound SO_2 per mg SO_2
- **50 mg SO_2 addition adds 15 - 37.5 mg bound SO_2**

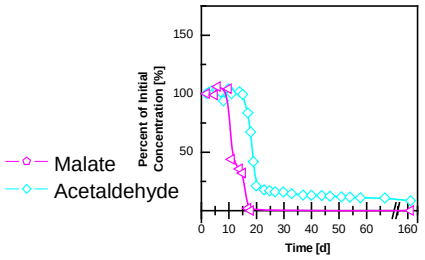
SO_2 Binders and MLF

- Strain effect
- Kinetics
- Inhibition

Metabolism of SO_2 Binders During MLF



Metabolism of SO_2 Binders During MLF



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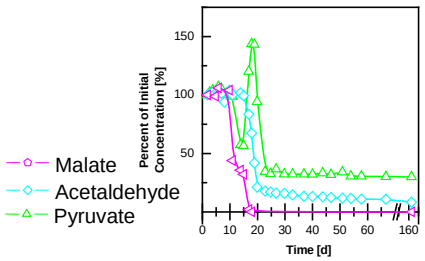

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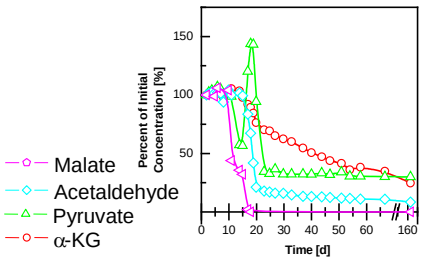
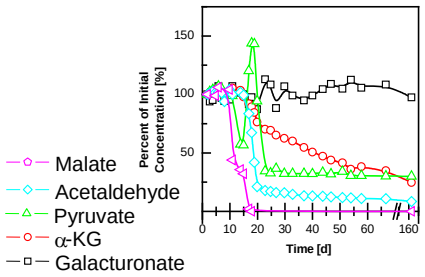


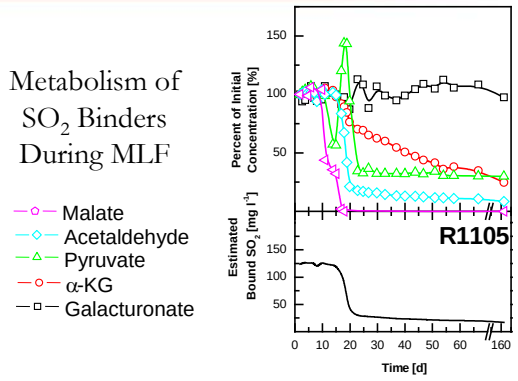
Metabolism of SO_2 Binders During MLF



Average degradation of SO_2 binders at 3 weeks post-ML (12 strains)

	ACHO	PYR	Alpha-KG	GA
Average (%)	87	85	48	0
Range (min)	83	68	28	-
Range (max)	92	100	72	-

Metabolism of SO_2 Binders During MLF



Considering the kinetics of bound-SO_2 reduction during MLF

Decrease before end of ML: 22%
In 1st week post-ML: 53%
In 2nd week post-ML: 6%
In 3rd week post-ML: 2%

On average, a 1 week delay after malic acid depletion allowed to reduce bound SO_2 levels by 75%

Scorecard for determining the ease of malolactic fermentation

	1 point each	2 points each	8 points each	10 points each
Alcohol (% vol)	<13	13 - 15	15 - 17	>17
pH	>3.4	3.1 - 3.4	2.9 - 3.1	<2.9
Free SO2 (mg/L)	<8	8 - 12	12 - 15	>15
Total SO2 (mg/L)	<30	30 - 40	40 - 60	>60
Temperature (°C)	18 - 22	14 - 18 or 18 - 24	10 - 14 or 24 - 29	<10 or >29
Yeast's nutritional needs	Low	Medium	High	Very high
Ease of Alcoholic Fermentation	No problems	Transient yeast stress	Sluggish / stuck AF	Prolonged yeast contact
Initial level of malic acid (g/L)	2 - 4	4 - 5 or 1 - 2	5 - 7 or 0.5 - 1	>7 or <0.5
Maximum AF rate (maximum loss of brix/day)	<2	2 - 4	4 - 6	>6
Note: Other, currently less well-known factors that are not considered in this scorecard may include the level of dissolved oxygen, polyphenolic content, lees compacting, pesticide residues, etc.				
Total score for the ease of malolactic fermentation:				

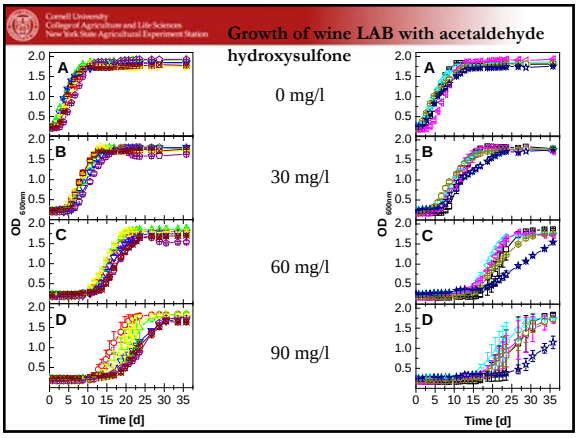
Score

Favorable (<13 points)
Not so favorable (13-22 points)
Difficult (23-40 points)
Extreme (>40 points)

Dr. Sibylle Krieger, Lallemand, Germany

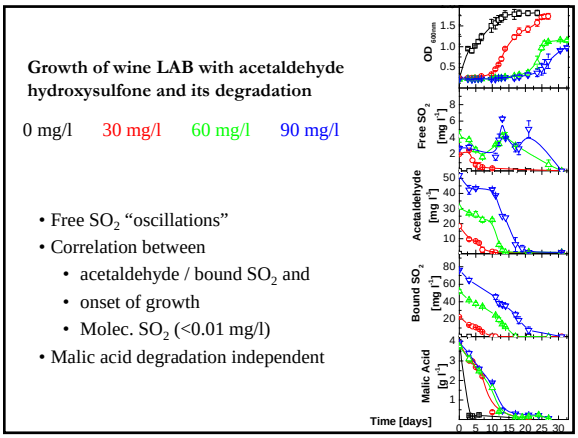
Testing the effect of pure acetaldehyde hydroxysulfonate on growth and metabolism of wine LAB

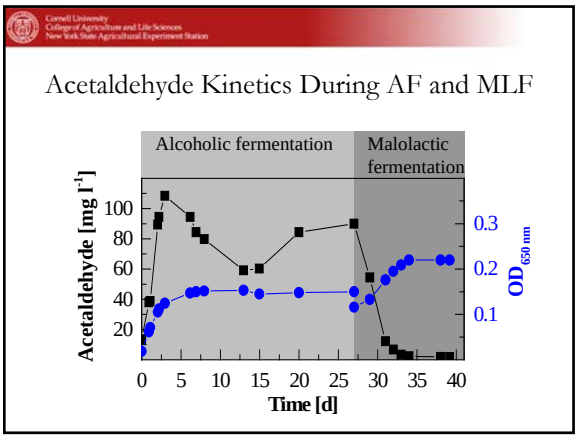
- Production of pure acetaldehyde hydroxysulfonate by
  - reaction of acetaldehyde with a potassium metabisulfite solution
  - liquid-liquid extraction
- Medium
  - MRS with malic acid
  - pH 4.5

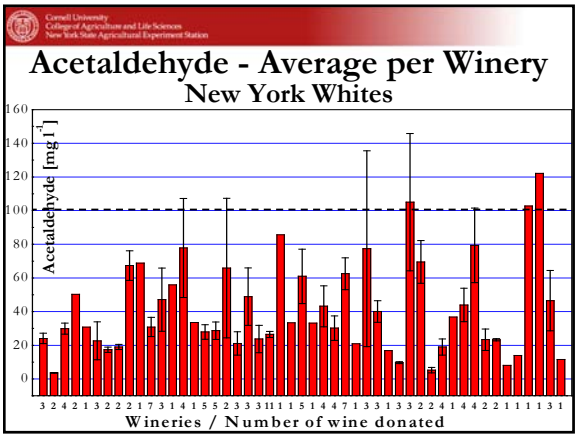


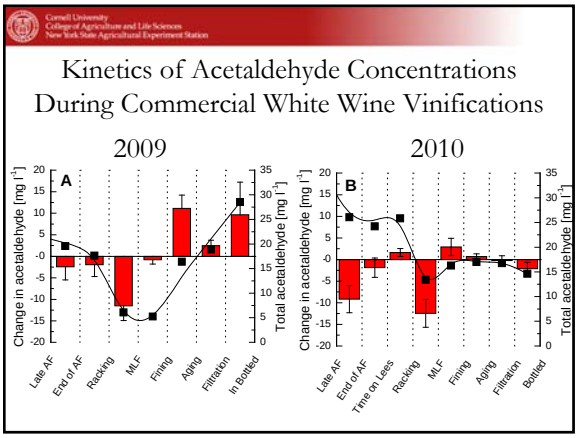
Effect of bound SO_2 on growth of wine LAB

Bound SO_2 (mg l^{-1})	Lag phase (d)	µMax (h^{-1})	Ymax (OD_{600nm})
0	3.55[a]	0.0172[a]	1.854[a]
30	7.66[b]	0.0124[b]	1.793[ab]
60	16.00[c]	0.0102[b]	1.765[bc]
90	20.38[d]	0.0077[c]	1.650[c]









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