



# Effect of Yeast Inoculation Rate on Fermentation Kinetics, Chemical and Aroma Compounds in Cider Fermentations

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## Welcome

What is the WRE?

Promoting innovation through experimentation

How does it work?

Evaluating experiments through chemical and sensory analysis



Virginia Winemakers Research Exchange  
<https://winemakersresearchexchange.com>



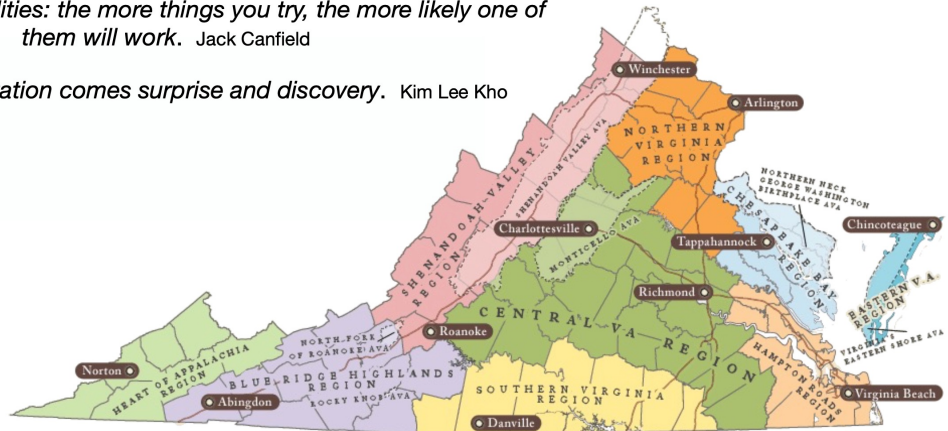
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# Why Do Experiments?

*No wise man tries every day what he has proved wrong the day before.*  
James Truslow Adams

*The Law of Probabilities: the more things you try, the more likely one of them will work.* Jack Canfield

*With experimentation comes surprise and discovery.* Kim Lee Kho



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## Comparing the effect of yeast dosing rates on fermentation kinetics, chemical and aroma compounds in Albemarle Pippin cider

*Potter's Craft Cider*

Andy Hannas

In 2022 Andy Hannas experimented with varying dosage rates of yeast inoculum to determine if there was a perceptible difference in the treatments chemically and sensorily. During the 2022 experiment, Treatment A received 15 g/HL yeast dose and Treatment B received 25 g/HL yeast dose. Treatments were monitored daily during fermentation and chemical analysis was taken at the end of fermentation. The results of this experiment showed that a higher inoculation dosage led to a faster onset of fermentation and completion of fermentation, while also producing 0.54% vol. more ethanol compared to the lower yeast inoculation dose. A sensory panel of 25 cider producers found the samples to be significantly different. Significant differences were found in scores for fruit intensity, tree fruit and volume, but no significant differences in scores for sulfur-like off odors.

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## Before we start

- Set up of each station
- QR codes  
Network: CiderCon  
Password: PetersonFarms
- Make sure numbers match
- Triangle test or Comparison:  
*Is there a difference?*
- “No” is an important answer
- If so, what is different?
- Try to answer every question
- When finished please wait quietly
- Discussion will follow
- Q & A at end of session

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## Taste 1<sup>st</sup> Flight: Triangle test

Network: CiderCon  
Password: PetersonFarms

Descriptors:

- Stone Fruit
- Tropical Fruit
- Fruit Intensity
- Sulfur-like off odor



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### 2023- Comparing the effect of yeast dosing rates on fermentation kinetics, chemical and aroma compounds in Albemarle Pippin cider

*Potter's Craft Cider*  
Andy Hannas



2 Different yeast dosing rates

Goals:

- No off aromas (H<sub>2</sub>S, VA,)
- Preserve or intensify aromatics
- Complete fermentation to dryness

**Building off work completed in 2022: Yeast dosing experiment on Winesap**

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## 2022- Experiment Results

Comparing the effect *Saccharomyces cerevisiae* dosing rates on fermentation kinetics, aroma compounds, and cider quality

	Low dose	High dose
Yeast Dosage Rate	15 g/HL	25g/HL
GoFerm Protect Evolution	18 g/HL	18 g/HL



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## 2022- Comparing the effect of yeast dosing rates on fermentation kinetics, chemical and aroma compounds in Winesap cider

*Potter's Craft Cider*  
Andy Hannas



- High dose (25g/HL)- faster fermentation rate and completion of fermentation (1 day earlier), and produced 0.54% alc. Compared to the Low dose (15 g/HL)
- A blind sensory panel of 25 Virginia cider producers found the samples to be significantly different
  - Fruit intensity higher in high dose

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**2023- Comparing the effect of yeast dosing rates on fermentation kinetics, chemical and aroma compounds in Albemarle Pippin cider**

*Potter's Craft Cider*  
Andy Hannas



2 Different yeast dosing rates

Goals:

- No off aromas (H<sub>2</sub>S, VA,)
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**Building off work completed in 2022: Yeast dosing experiment on Winesap**

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**2023- Comparing the effect of yeast dosing rates on fermentation kinetics, chemical and aroma compounds in Albemarle Pippin cider quality**



Treatment	Yeast dosing rate (g/HL)	Rehydration nutrient dosing rate (g/HL)	Est. Yeast Population- 24 hrs post-inoculation (cells/mL)
A	20	20	2.4 x 10 <sup>6</sup>
B	10	10	0.9 x 10 <sup>6</sup>

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<b>Flight 1</b>	Code	Treatment
<b>Group 1</b>	100	10 g/HL Yeast
	395	20g/HL Yeast
	944	20g/HL Yeast
<b>Group 2</b>	759	10 g/HL Yeast
	622	20g/HL Yeast
	412	10 g/HL Yeast
<b>Group 3</b>	588	20g/HL Yeast
	295	10 g/HL Yeast
	356	10 g/HL Yeast
<b>Group 4</b>	606	20g/HL Yeast
	550	20g/HL Yeast
	293	10 g/HL Yeast

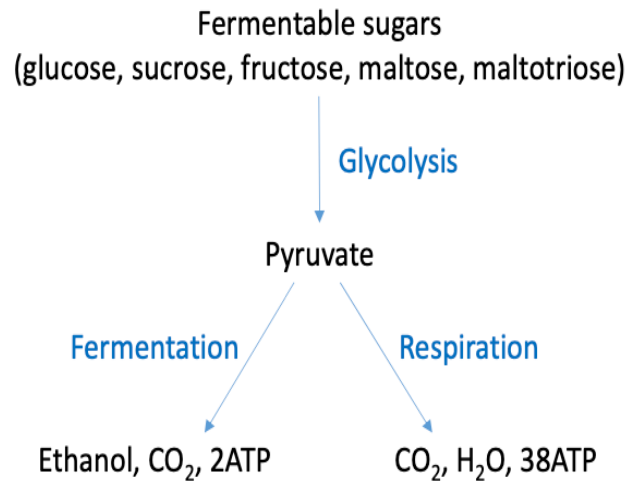
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## Factors that affect the type and concentration of aroma compounds?

- Cultivar or blend of apples used
- pH
- Yeast type
- Size of yeast inoculum
- Fermentation conditions
- Nutrition level

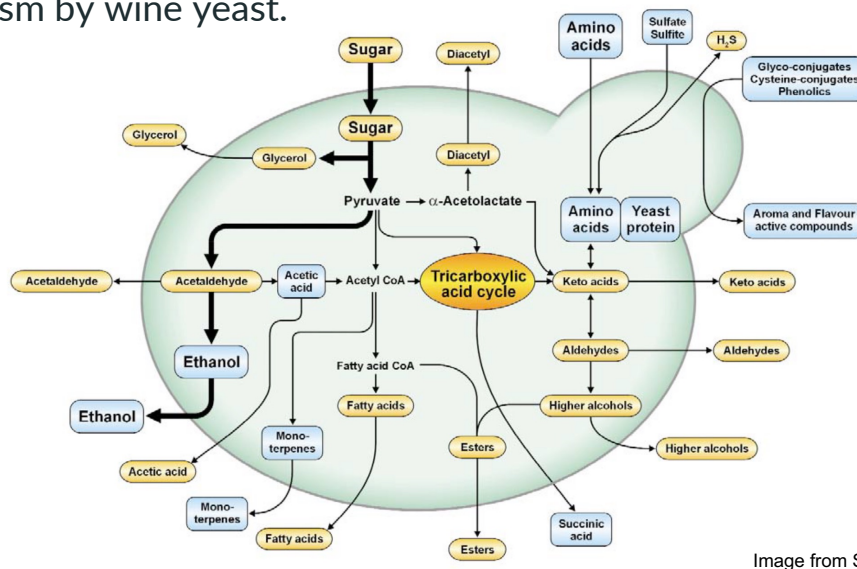
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## Sugar metabolism by *S. cerevisiae* yeast

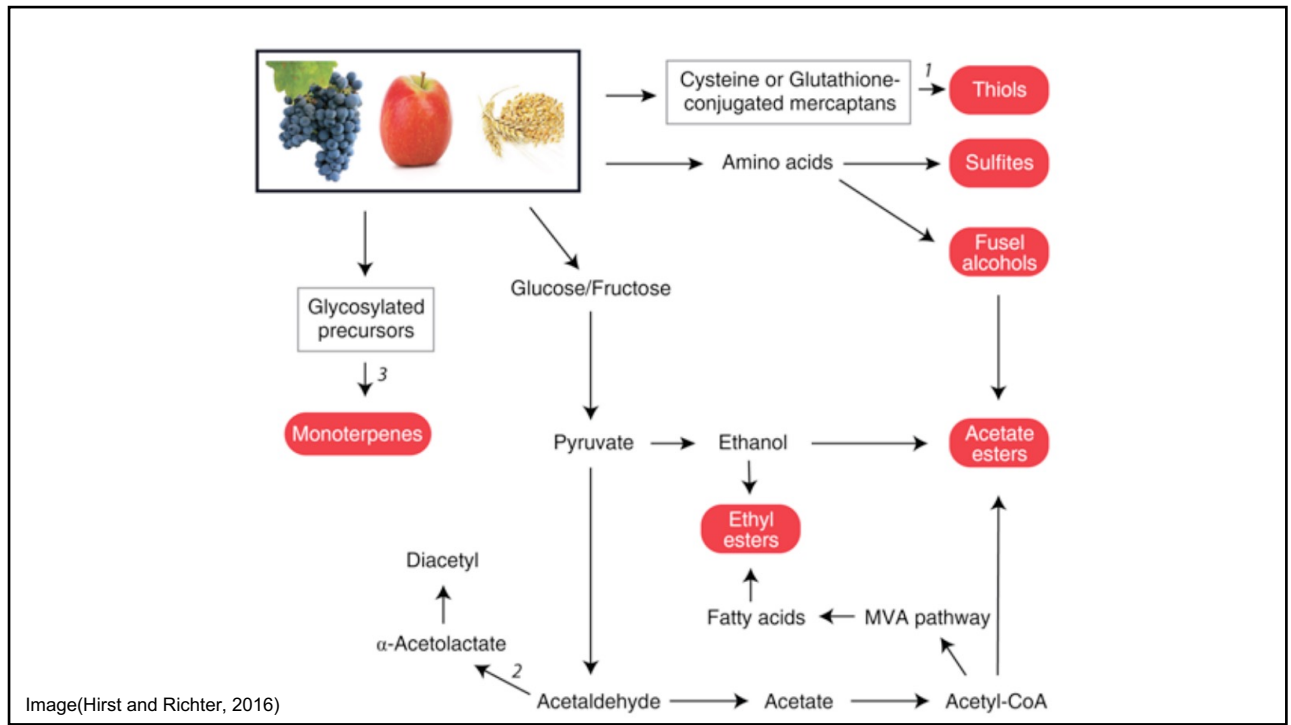


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Schematic representation of the derivation and synthesis of flavor-active compounds from sugar, amino acids and sulfur metabolism by wine yeast.



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## Sulfur, Sulfide, Sulfite

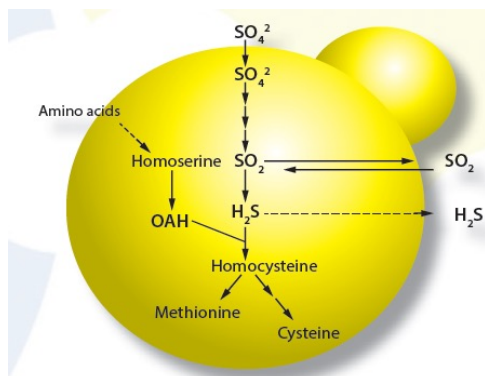


Figure 2: Wine yeast sulfate assimilation pathway

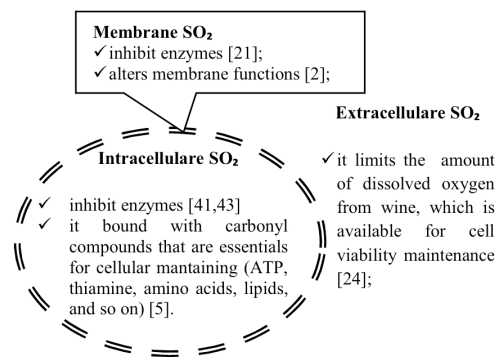


Fig 1. Different modes of action of SO<sub>2</sub> on microorganisms (adapted [8, 42])

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## Sulfur compounds, including thiols, commonly found in wine. (Sweigers, et al. 2005)

Compound	Concentration in wine (µg/L)	Aroma threshold (µg/L)	Aroma descriptor
Hydrogen sulfide	Trace->80	10-80	rotten egg
Methanethiol (methyl mercaptan)	5.1, 2.1	0.3	cooked cabbage, onion, putrefaction, rubber
Ethanethiol (ethyl mercaptan)	1.9-18.7	1.1	onion, rubber, natural gas
Dimethyl sulfide	1.4-61.9	25	asparagus, corn, molasses
Diethyl sulfide	4.1-31.8	0.93	cooked vegetables, onion, garlic
Dimethyl disulfide	2	15, 29	cooked cabbage, intense onion
Diethyl disulfide	Trace-85	4.3	garlic, burnt rubber
3-(Methylthio)-1-propanol (methionol)	140-5000	500	cauliflower, cabbage, potato
Benzothiazole	11	50	rubber
Thiazole	0-34	38	popcorn, peanut
4-Methylthiazole	0-11	55	green hazelnut
2-Furanmethanethiol	0-350 ng/L	1 ng/L	roasted coffee, burnt rubber
Thiophene-2-thiol	0-11	0.8	burned, burned rubber, roasted coffee
4-Mercapto-4-methylpentan-2-one (4MMP)	0-30 ng/L	3 ng /L	cat urine, box tree/ blackcurrant, broom
3-Mercaptohexan-1-ol (3MH)	50-5000 ng/L	60 ng/L	passionfruit, grapefruit
3-Mercaptohexyl acetate (3MHA)	1-100 ng/L	4 ng /L	Riesling-type note, passionfruit, box tree

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## What the Current Research Tells Us: Beer

### How yeast pitch rate affects aroma and flavor compounds

- Increasing pitch rate does not increase net cell growth
- Increasing pitch rate increases:
  - Acetaldehyde production
  - Fermentation rate
  - Foam production
  - Potential for gas-stripping of aromatics

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## What the Current Research Tells Us: Beer

### Increasing pitch rate DECREASES:

- Cell division
- Nitrogen demand
- Drive aromatics toward solvent esters like Ethyl Acetate not fruity esters like isoamyl acetate
- Sulfur-based flavor compounds
- Higher alcohols

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## What the Current Research Tells Us:

### Underpitching leads to :


- Sluggish or stuck fermentation
- Increased residency times
- Increased overall pH
- Increased potential for off-flavors and aromas
- Opportunity for spoilage organisms to take hold

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## What the Current Research Tells Us: Wine World

 Desired aromas at  $10^5$  cells/mL

- Esters, lactones, mono-terpenes

 Undesirable aromas at  $10^5$  cells/mL

- Higher alcohols and medium chain fatty acids

YAN level affects are related to:

- Quantity of cells at initial stages of fermentation
- Rapid removal of nitrogen
- Net cell growth

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## Flight 1 Experiment: Methods and Results

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## 2023 Albemarle Pippin Juice

Juice Chemistry at Pressing (Imbibe Solutions data)

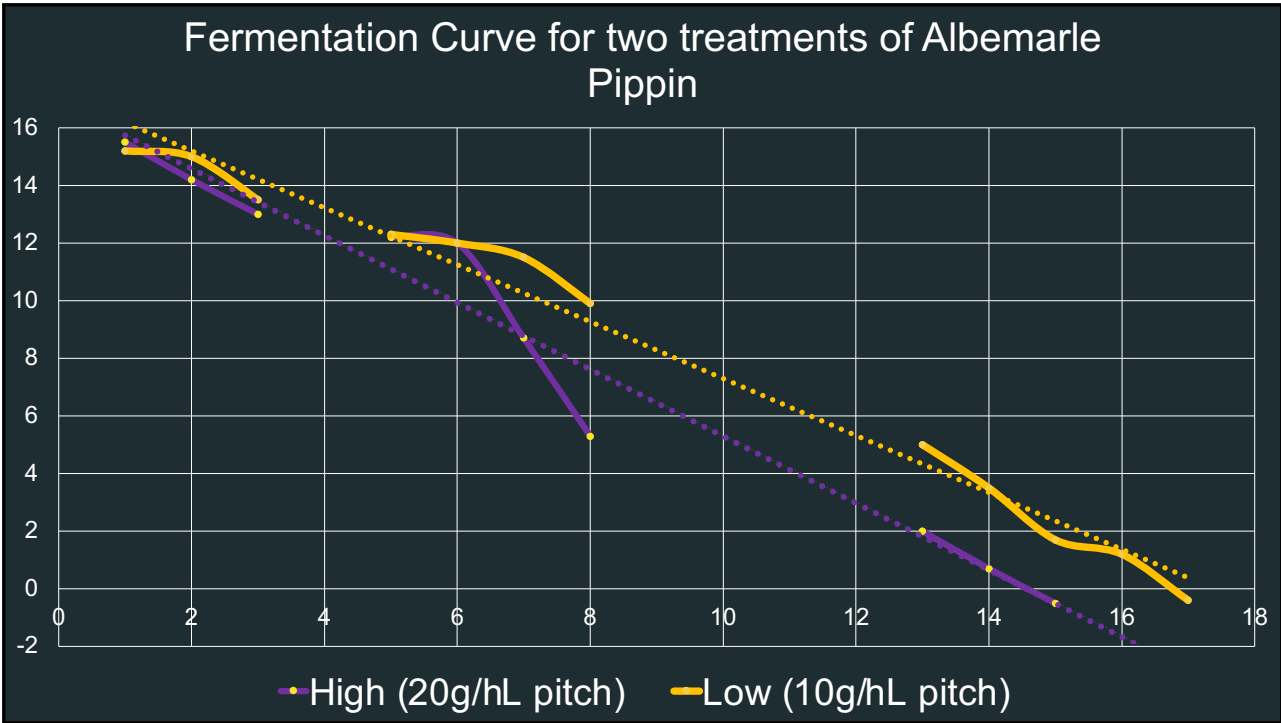
Brix	pH	Titrateable Acidity (g/L)	Malic Acid (g/L)	Lactic Acid (g/L)	Volatile Acidity (g/L)	YAN (mg N/L)
14.5	3.42	7	7.92	0.12	0.04	100

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## 2023 Experimental Design

	Treatment A- Manufacturer Recommendation	Treatment B-Low dose
Yeast Dosage Rate	20 g/HL	10g/HL
GoFerm Sterol Flash	20 g/HL	10 g/HL

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### Post Fermentation Chemical analysis (ETS Labs data)

Treatment	% Abv	R.S. (g/L)	pH	TA (g/L)	Malic (g/L)	Lactic (g/L)	VA (g/L)	Free SO2 (ppm)	Molecular SO2 (ppm)	Total SO2 (ppm)
A (20g/HL)	8.2	0.6	3.69	7.1	7.1	0.38	0.13	18	0.23	80
B (10g/HL)	7.91	4	3.68	7.0	7.18	0.37	0.08	14	0.19	77

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## Flight 1: Conclusions

### Cidermaker Conclusions and Considerations

- Microbial contamination
- Fermentation becoming stuck or sluggish

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## Taste 2nd Flight: Comparison

- Not a Triangle Test
- Each glass is a different treatment

### Descriptors:

- Stone Fruit
- Tropical Fruit
- Fruit Intensity
- Sulfur-like off odor



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## 2023- Comparing the effect of yeast dosing rates on fermentation kinetics, chemical and aroma compounds in cider

*Daring Wine Company*

Jocelyn Kuzelka



### 3 Different yeast dosing rates

Goals:

- No off aromas (H<sub>2</sub>S, VA,)
- Preserve or intensify aromatics
- Complete fermentation to dryness

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## 2023- Comparing the effect of yeast dosing rates on fermentation kinetics, chemical and aroma compounds in cider



	Treatment A	Treatment B	Treatment C
Yeast Dosage Rate	20 g/HL	10 g/HL	7g/HL
GoFerm Sterol Flash	20 g/HL	10 g/HL	10 g/HL

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Flight 2	Code	Treatment
Group 1	557	10 g/HL
	336	7 g/HL
	521	20g/HL
Group 2	242	20g/HL
	650	10 g/HL
	148	7 g/HL
Group 3	443	7 g/HL
	162	20g/HL
	721	10 g/HL
Group 4	256	10 g/HL
	439	20g/HL
	674	7 g/HL

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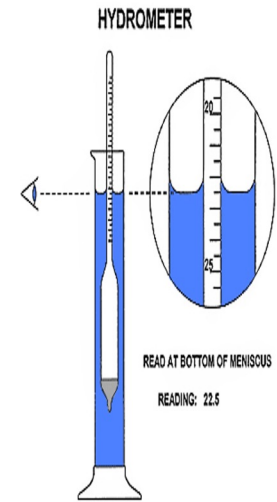
## Flight 2 Experiment: Methods and Results



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## 2023 Dessert Apple Blend- Juice Chemistry

°Brix	11.8
Titrateable Acidity (g/L)	3.4
pH	3.61
Malic Acid (g/L)	4.08
Lactic Acid (g/L)	0.01
YAN (mg/L as N)	13
Volatile Acidity (g acetic Acid/L)	0.01

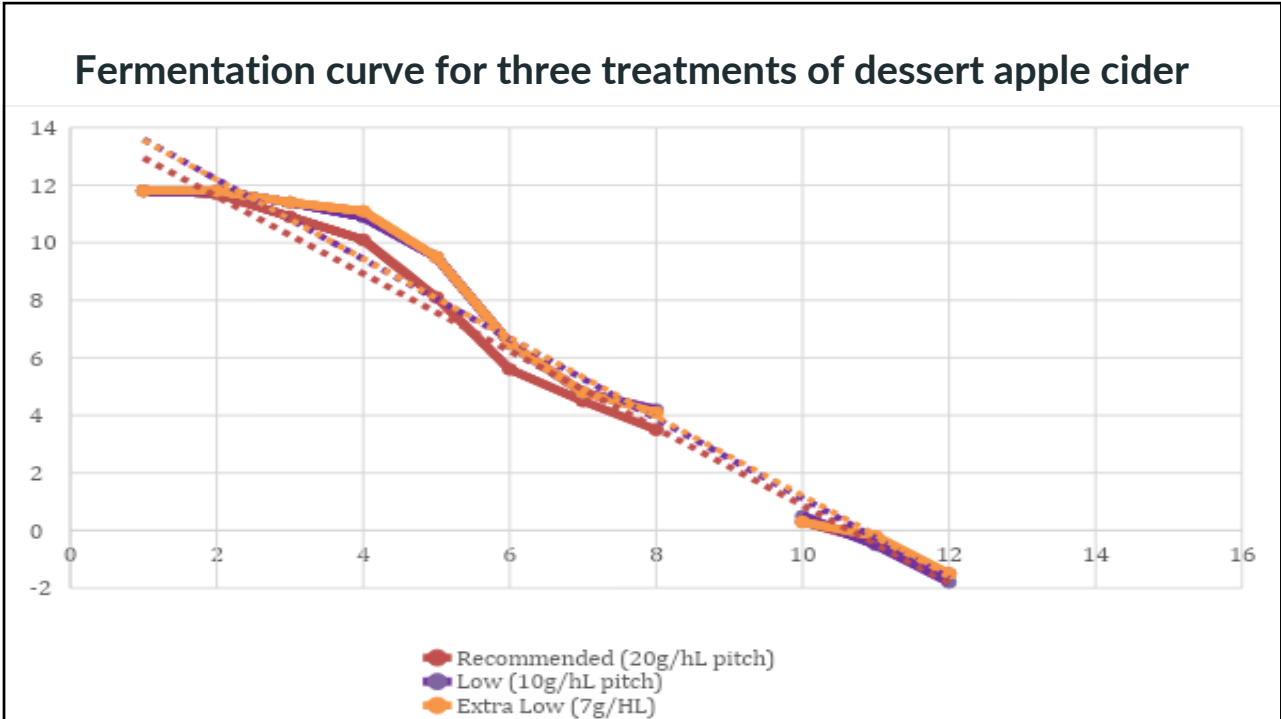


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## Yeast Cell Counts- 24 hours post-inoculation

Treatment	Yeast Inoculation Rate	Estimated Population	Viability
A	20 g/HL	17.6 x 10 <sup>6</sup> cells/mL	95 %
B	10 g/HL	10.2 x 10 <sup>6</sup> cells/mL	96 %
C	7 g/HL	7.2 x 10 <sup>6</sup> cells/mL	72 %

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### Flight 2: Post-Fermentation Analysis

	% ABV	R.S. (g/L)	pH	TA (g/L)	Malic (g/L)	Lactic (g/L)	VA (g/L)	Free SO <sub>2</sub> (ppm)	M. SO <sub>2</sub> (ppm)	Total SO <sub>2</sub> (ppm)
A (20g/HL)	6.59	0.1	3.82	3.6	2.33	0.86	0.17	22	0.52	56
B (10g/HL)	6.65	0.3	3.88	3.2	1.81	1.24	0.16	30	0.5	59
C (7g/HL)	6.57	0.3	3.98	2.3	0.1	2.87	0.16	25	0.54	58

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## Flight 2: Conclusions

### Cidermaker Conclusions and Considerations

- Microbial contamination- native LAB
- Fermentation becoming stuck or sluggish
- Perhaps not for every fermentation

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## Sensory: what did you think?

- Could you tell a difference sensorily between the samples?
- What differences could you detect?
- Would you consider using lower yeast dosing rates based on what you tasted and learned today?

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thank you



Funding: The Virginia Wine Board

Andy Hannas, Potter's Craft Cider

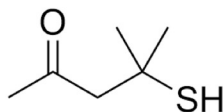
CiderCon24, the ACA, and CINA

For questions/comments: [jocelyn@panaceawine.com](mailto:jocelyn@panaceawine.com)

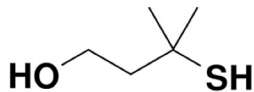
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### 3 main compounds to know

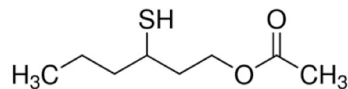
4-mercapto-4-methylpentan-2-one (4MMP)(0.8 ng/L) - boxwood, broom, cat pee



3-mercaptohexan-1-ol (3MH)(60 ng/l) - citrus (lime, grapefruit, orange)



3-mercaptohexyl acetate (A3MH)(4.2 ng/L) - passionfruit, gooseberry, guava,



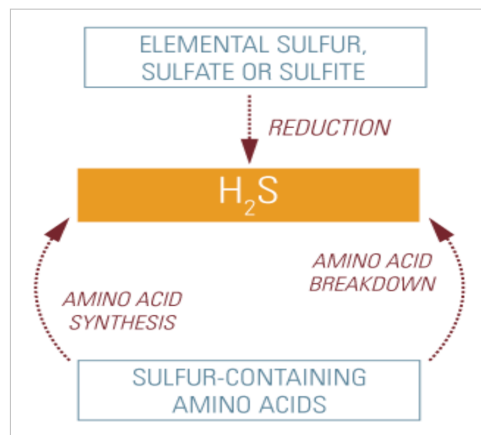
**There are other thiols: cooked leeks, mercaptans  
Enhance perception of green (IBMP)**

Slide courtesy of Joy Ting

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# Rotten Egg- H<sub>2</sub>S

- Threshold is 1 ppb
- CAUSES:
  1. Most often caused by yeast fermentation
  2. Use of a high H<sub>2</sub>S strain (Montrachet)
  3. Extended contact with heavy or dirty (primary) lees
  4. Variety of apple



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# Rotten Egg- H<sub>2</sub>S

- PREVENTION:
  - Nitrogen based supplements
  - Temperature control
  - Select appropriate yeast for environmental conditions present and variety
  - Rack off heavy lees within 2 wks post-AF
- CURE:
  - Copper Sulfate addition

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# Struck match-(Ethyl Mercaptan)

- Threshold 1.1 µg/L
- CAUSES:
  - Break down of sulfur-containing amino acids
  - When H<sub>2</sub>S is not removed quickly from cider
  - When cider left in contact with lees in reductive environment
- PREVENTION/CURE:
  - DO NOT AERATE
  - Copper addition



Adapted from: <https://www.etslabs.com/library/31>

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# Garlic/Onion- Dimethyl Disulfide

- Threshold 4.3 µg/L
- CAUSES:
  - Break down of sulfur-containing amino acids
  - When H<sub>2</sub>S is not removed quickly from cider
  - When cider left in contact with lees in reductive environment
- PREVENTION/CURE:
  - DO NOT AERATE
  - Same as for mercaptans

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