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**Effect on honey, dusty off-
flavours and acetic acid in
sparkling wines made from
varying amounts of sour
rotten grapes**

**Belinda Kemp
2020 CCOVI Lecture Series**

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- “Honey off-flavour” compounds during grape ripening
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What is sour rot?



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**Photographs
courtesy of
Dr Wendy
McFadden-
Smith,
OMAFRA.**



Figure 1. Pictures of red and white grapes with sour rot infection

What is sour rot?



- Caused by combination of physical factors: wounding and berry splitting, microbiological factors including yeasts, bacteria & fungi, and spread by vinegar flies.
- Non-saccharomyces involved in the rotting process (*can differ in species depending on region*) (Barata et al. 2011)
- Sour rot = decrease in °Brix from conversion of sugar to acetic acid, gluconic acid, uronic acid, ethyl acetate & glycerol.
- Most important is oxidation of ethanol into acetic acid (vinegar) (Barata et al. 2011)

For further information about sour rot please refer to reports from OGWRI funded projects by Wendy McFadden-Smith (OMAFRA) on their website.

Ontario sour rot research reports

<https://ontariograpeandwinereseach.com/en/projects>

Detection thresholds for acetic acid and ethyl acetate



Detection Thresholds

Definition (*Cliff & Pickering 2006*)

The concentration at which there is 75% correct detection

➤ Acetic acid (*differs with wine style i.e. Icewine*)

0.8 g/L Maximum permitted volatile acidity concentration in still wine in Ontario is 1.3 g/L of acetic acid.

[Corison et al., (1979), Vintners Quality Alliance (1999)]

➤ Ethyl acetate (*differs with wine style i.e. Madeira*)

White still wine: 170 mg/L

Red still wine: 160 mg/L

(Corison et al., 1979)

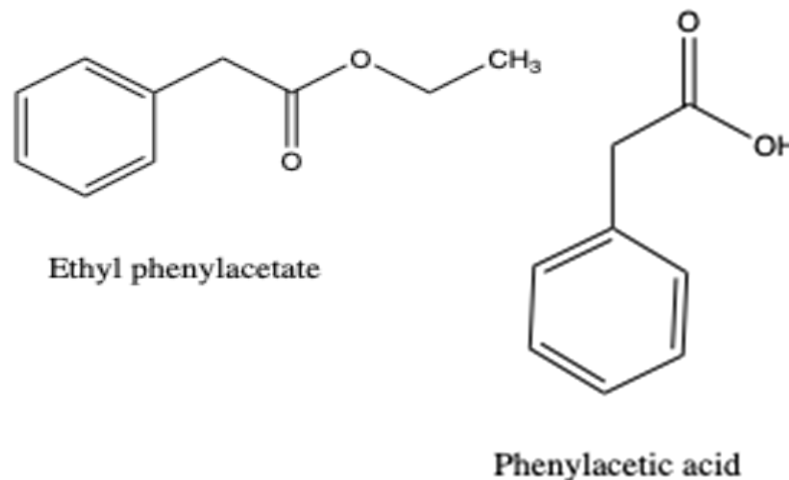
Phenylacetic acid (PhAA) & Ethylphenyl acetate (EPhA)

<https://pubchem.ncbi.nlm.nih.gov/compound/Phenylacetic-acid>



- **Phenylacetic acid (PhAA)** is a monocarboxylic acid.
- Role as a toxin, a plant metabolite, a *S. cerevisiae* metabolite, a plant growth retardant, an allergen and an auxin.
- PhAA is a direct degradation product of phenylalanine possibly from shikimic acid pathway. (*Tat et al. 2007*)
- Precursor of EPhA

- **Ethylphenyl acetate (EPhA)**
- Produced from PhAA by some yeast during fermentation & maturation.



Thresholds for Ethylphenyl acetate (EPhA) & Phenylacetic acid (PhAA) in wine



Detection thresholds

- Phenylacetic acid (PhAA) in red wine (*Aglianco*) **73ug/L**
(*Tat et al. 2007*)
- Ethylphenyl acetate (EPhA) in sparkling wines **263ug/L**
(*Kemp et al. 2019*)

Consumer Rejection thresholds (CRT) (*Campo et al. 2012*)

- Combination of EPhA & PhAA
- EPhA is **140ug/L** & PhAA **700ug/L** (red wine)

CRT not determined in sparkling wine
Reason: Lack of 75% agreement



Detection & rejection thresholds in wine of PhAA & EPhA



Some might not care!



Some might hate it!

Honey, sweet, off-flavour

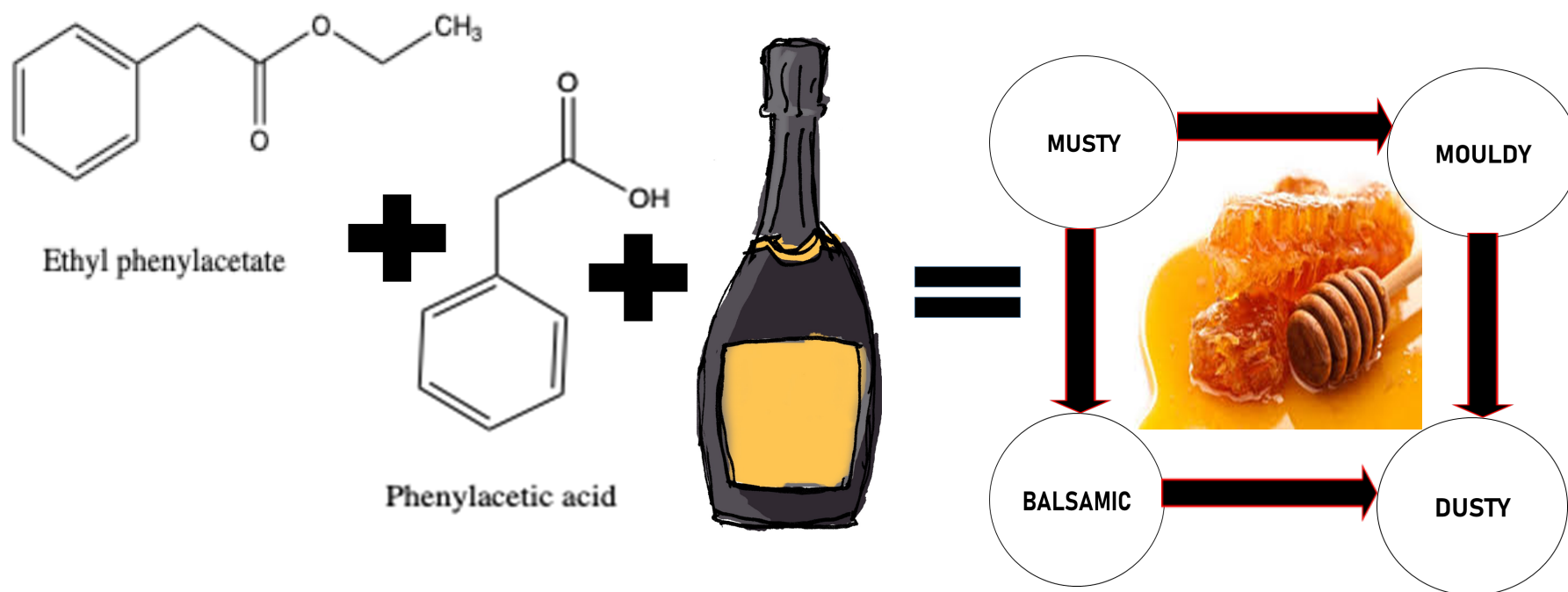


Figure 2. Diagram illustrating the influence of EPhA and PhAA on wine aroma.

Honey, dusty off-flavour in wine & honey



- EPhA produced from PhAA
- PhAA can increase during wine aging
(some yeasts produce more PhAA than others + N additions)

Other “honey” compounds in wine but not associated with sour rot

Is it a fault in wine?

- High concentrations that mask other compounds/aroma
- Unpleasant, musty odour reduces wine complexity & likeness
- Fault in red wines from Italy, Spain & Portugal

Concentrations of EPhAA & PhAA (combined) causes the fault!

Is it present in honey?

Determines the botanical origin of some specific honey types!

- EPhA marker for authenticity of thyme honey made in Italy
(Piasenzotto et al. 2003)
- PhAA marker for authenticity of *Salvia officinalis* L. (sage) honey made in Croatia (Jerković et al. 2007)



FLORAL



FRUIT

FRESH:

pineapple, strawberry, melon, apple, pear, mango, peach, grape

BERRY:

blackcurrant, raspberry, blackberry, cherry, cranberry

DRIED:

prune, raisin, fig, apricot, cooked fruit, jammy, candied fruit

CITRUS:

citrus zest, lemon, orange, grapefruit, tangerine, candied peel

BURNED:

caramel, molasses, smoky, coffee, chocolate, burnt sugar, maple

CARAMEL:

butterscotch, toffee, brown sugar, beeswax

NUT:

toasted, chestnut, hazelnut, walnut, almond, peanut, pine nut

LACTIC:

milk, fresh butter, cooked butter, rancid butter, yogurt

CONFECTIONARY:

vanilla, white chocolate, cotton candy, marshmallow, fondant

WARM



FRESH

REFRESHING:

lemon verbena, peppermint, eucalyptus, anise, herbaceous, rain

CAMPHOROUS:

rosemary, lavender, thyme, menthol, balsamic



GREEN: fresh plants, grass clippings, raw vegetables, wet grass, wet hay, hops, artichoke, green bananas, kiwi, stems, bay leaf

DRY: hay, tea, herbal tea, straw

GAMEY:

sweat, manure, barnyard, stable, leather, cheese, gym bag, fresh fish, cat pee, daisy, dandelion

ANIMAL

VEGETAL

WOODY

DRY: wood, sandalwood, cedar, boxwood, shell, incense, oak

RESINOUS:

pine resin, propolis, turpentine

SPICY:

cinnamon, clove, nutmeg, pepper, ginger



MEDICINE: plastic, turpentine, cardboard, soap

PETRO-CHEMICAL:

metal, cabbage, sulfur, ash, tar, tobacco

CHEMICAL

YEAST:

fermentation, vinegar, beer, cider, malt, baked bread

EARTHY:

musty basement, moldy, mildew, rotting leaves, humus, peat moss, mushroom, truffle, chalk, pollen



SPOILED

Background to our study



- Observations in Ontario identified this prevailing honey/off-flavour aroma in some sparkling wines made from Pinot noir grapes
- Some Ontario grapes can suffer from sour rot

“less honey and sweetness is a path to complexity and style”

Anthony Gismondi (*wine writer*)

Project details

Sparkling and still red wine quality



1. Quantify EPhA, PhAA, ethyl acetate & acetic acid levels from clean & sour rot infected grapes during ripening (Biomarker/predictor of sour rot?)
2. Produce Pinot noir sparkling & still red wines with varying amounts of sour rot. Determine EPhA, PhAA, ethyl acetate & acetic acid concentrations.
3. Determine the potential of the Brock yeast (CN1) to reduce acetic acid, EPhA & PhAA in sparkling & still red wines.

Pinot noir during ripening 2019

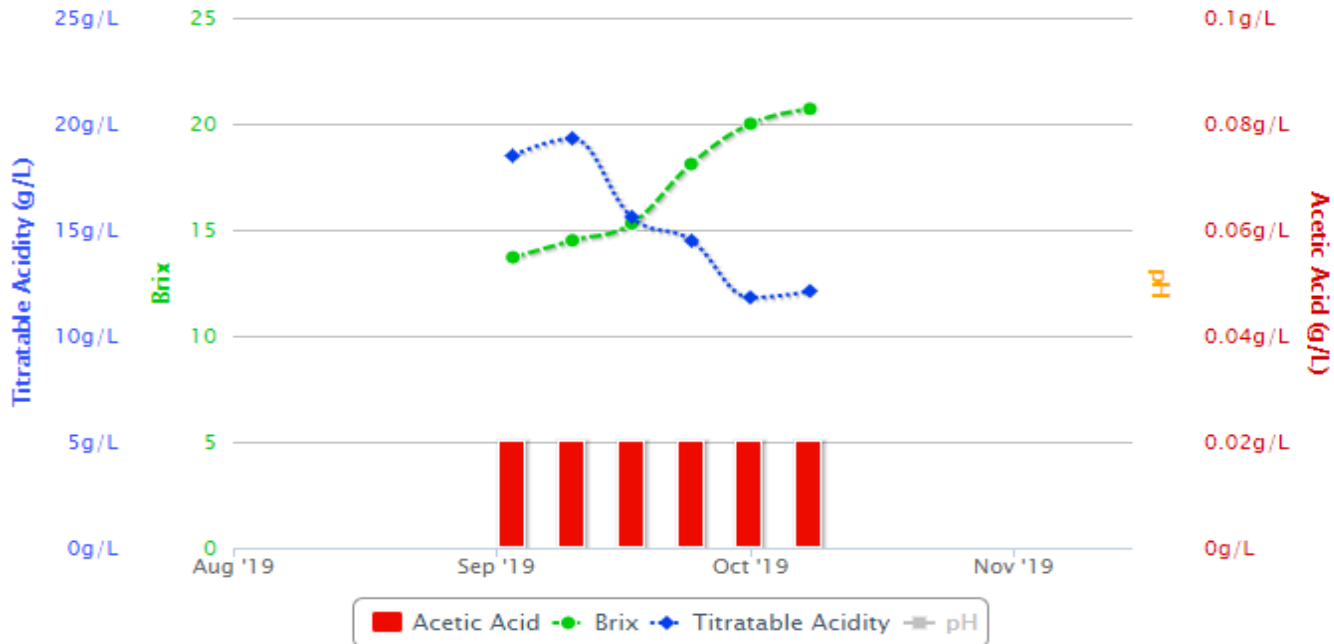


Figure 4. Acetic acid, °Brix and TA (g/L) in Pinot noir during grape ripening.

EPhA & PhAA during grape ripening 2019

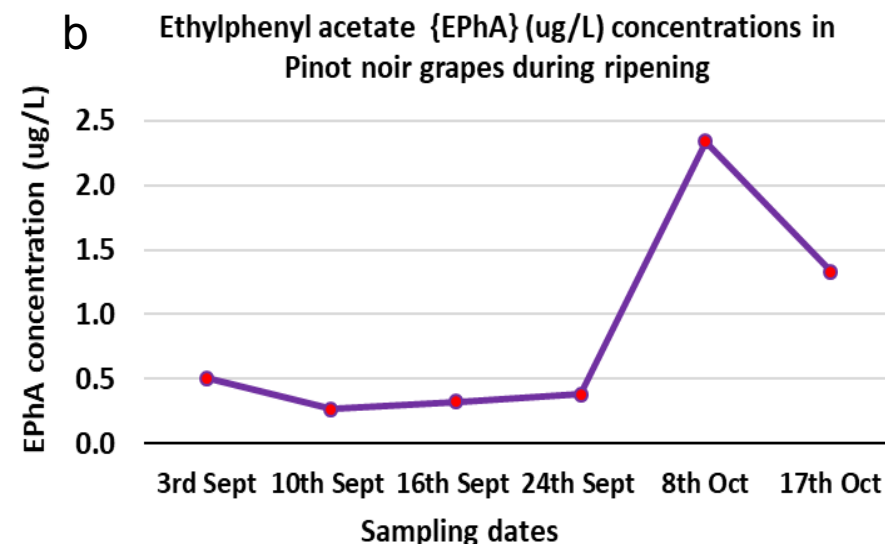
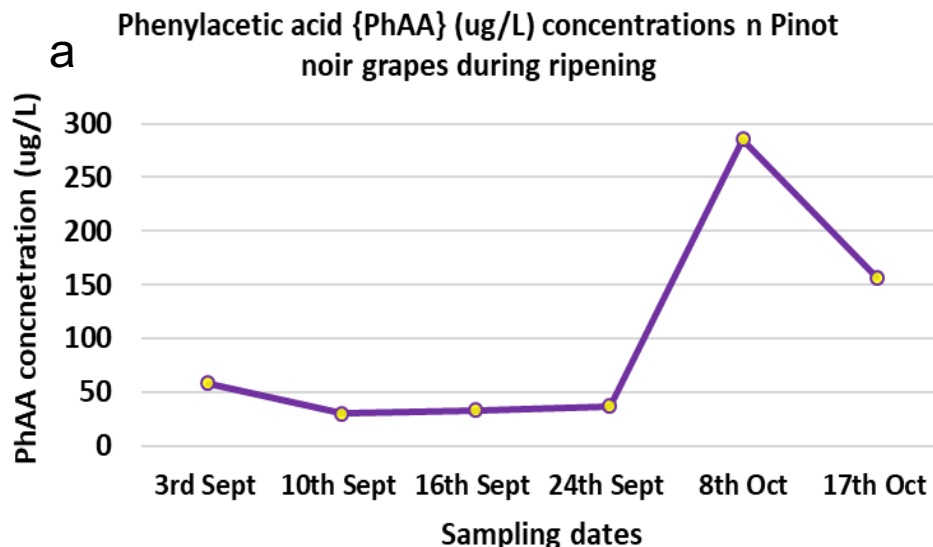


Figure 5 a & b. PhAA & EPhA concentrations during grape ripening in 2019.

Summary

- PhAA higher than EPhA during ripening
- Same trend for both
- Increase during ripening but with peaks for both compounds in 2019
- Acetic acid concentrations: below 0.02g/L throughout sampling

Sparkling wine experimental design



Hand picked sour rot bunches

Hand picked clean bunches

Whole bunch pressed sour rot grapes

Whole bunch pressed clean grapes

Settled with enzymes (24 hrs) Lallzyme Cuvee Blanc

*Sterile filtered juice prior to yeast build up

Treatments by % of sour rot juice (15L in triplicate)
0%, 10% (1.5L), 20% (3L), 30% (4.5L), 40% (6L) of *sour rot juice*
Fermentations with EC1118 & CN1 = 30 wines
YAN (mg N/L) Fermaid K added

Base wine → bottling & 2nd fermentation → Riddling, disgorging & dosage

**To ensure inoculation excluded all yeasts except EC1118 or CN1 yeasts*

Juice chemical composition



Table 1. Juice chemical analysis.

Treatment codes: Control (EC0). 10% sour rot juice (EC10), 20% sour rot juice (EC20), 30% sour rot juice (EC30) and 40% sour rot juice (EC40).

Analysis	EC0	EC10	EC20	EC30	EC40
°Brix	17.9 ±0.1	18.0 ±0.0	18.2 ±0.1	18.4 ±0.1	18.2 ±0.1
pH	3.1 ±0.0	3.1 ±0.0	3.1 ±0.0	3.1 ±0.0	3.1 ±0.0
*Titratable acidity (g/L)	12.6 ±0.1b	12.8 ±0.1b	13.2 ±0.1b	13.4 ±0.1a	13.2 ±0.1a
Ammonia (mg N/L)	29 ±2.5	28 ±3.6	28 ±2.9	27±1.7	26 ± 2.2
Amino acids (mg N/L)	78 ±5.2	84 ±16.0	83 ±8.9	77 ±5.7	80 ±6.5
YAN (mg N/L)	107±7.6	113 ±19.6	110 ±11.7	103 ±7.4	107 ±8.7

* Significance: $P = 0.05$

EPhA & PhAA concentrations in juice

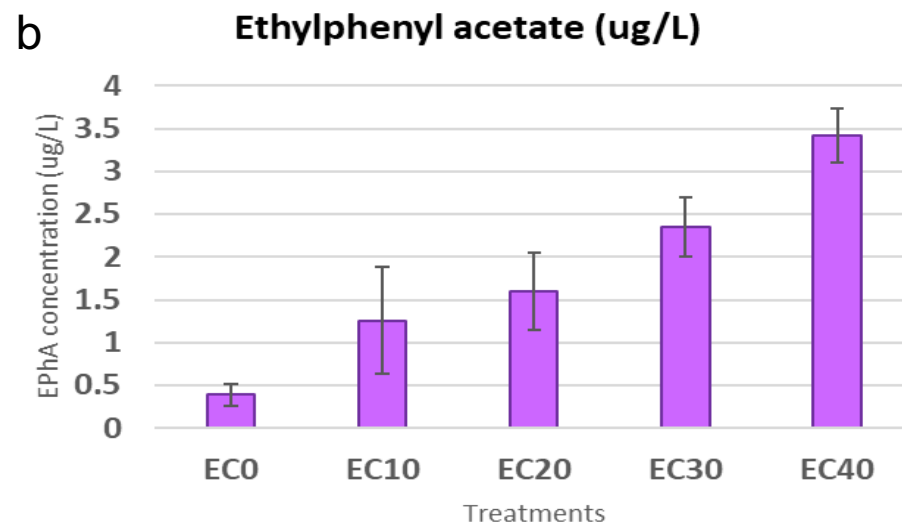
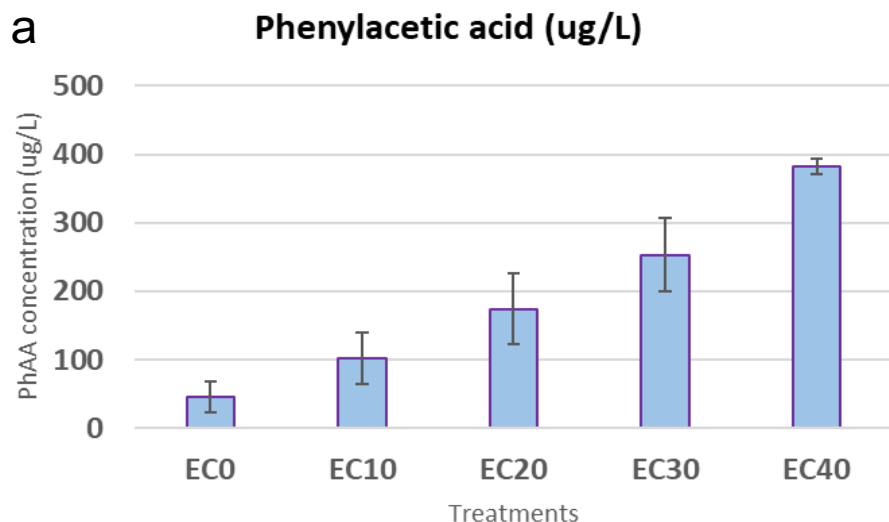


Figure 6 a & b. Concentrations of PhAA & EPhA (ug/L) in juice before primary fermentation. Treatment codes: Control (EC0). 10% sour rot juice (EC10), 20% sour rot juice (EC20), 30% sour rot juice (EC30) and 40% sour rot juice (EC40). *Error bars represent standard deviations.*

Summary points

- **Not** statistically significant (*ANOVA & Tukeys test*) between treatments despite increase in concentrations. Replicate variability within treatments.
- Concentration scales i.e. 3.4 (ug/L) in EC40 EPhA & 380 (ug/L) in EC40 PhAA

Acetic acid (g/L) concentrations in juice at harvest

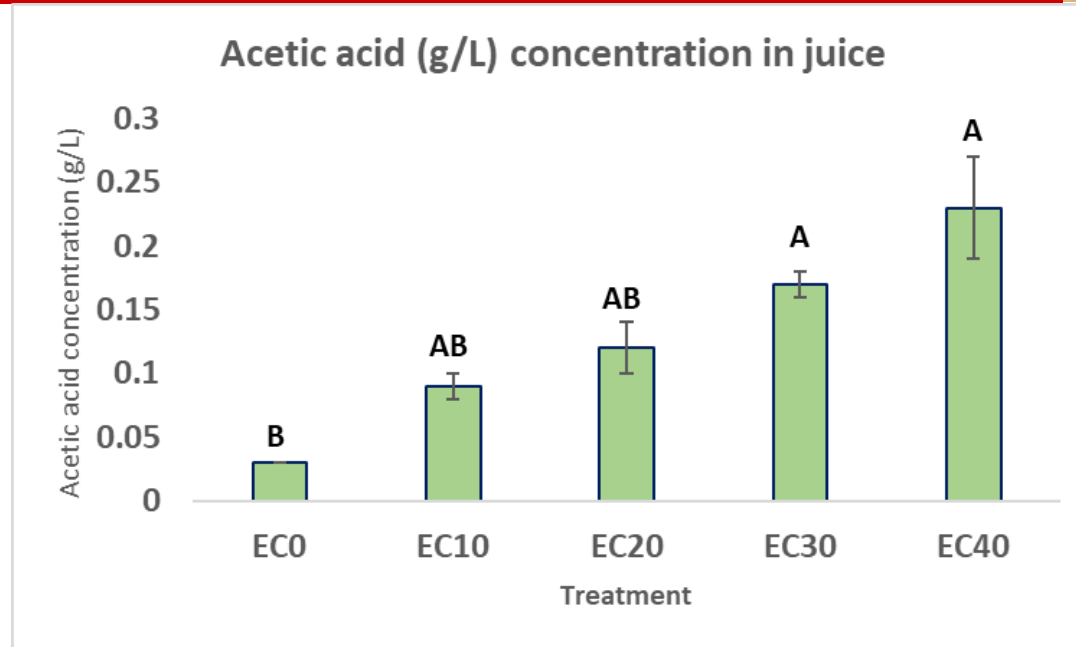


Figure 7. Acetic acid (g/L) concentrations in juice after treatment.

Treatment codes: Control (EC0). 10% sour rot juice (EC10), 20% sour rot juice (EC20), 30% sour rot juice (EC30) and 40% sour rot juice (EC40).

Error bars represent standard deviations. Significance $P = 0.001$.

Summary points

- Increase of acetic acid concentration with increase L of sour rot juice
- Ethyl acetate below limit of detection for the method

Base wine chemical composition



Table 2. Base wine chemical analysis.

Treatment codes: Control (EC0). 10% sour rot juice (EC10), 20% sour rot juice (EC20), 30% sour rot juice (EC30) and 40% sour rot juice (EC40).

Analysis	EC0	EC10	EC20	EC30	EC40
pH	3.1 ±0.0	3.1 ±0.0	3.1 ±0.0	3.1 ±0.0	3.1 ±0.0
Titrateable acidity (g/L)	10.9 ±0.1	10.9 ±0.1	11.2 ±0.1	11.5 ±1.0	11.5 ±0.1
*Malic acid (g/L)	5.3 ±0.0b	5.4 ±0.1b	5.6 ±0.2a	5.7 ±0.1a	5.6 ±0.1a
Residual sugar (g/L)	0.3 ±0.1 a	0.5 ±0.1b	0.5 ±0.1b	0.5 ±0.1b	0.5 ±0.1b
Alcohol (% v/v)	8.5 ±0.8	9.2 ±0.3	9.3 ±0.5	9.0 ±1.0	9.0 ±1.2

* Significance: $P = 0.001$

EPhA & PhAA concentrations in base wine

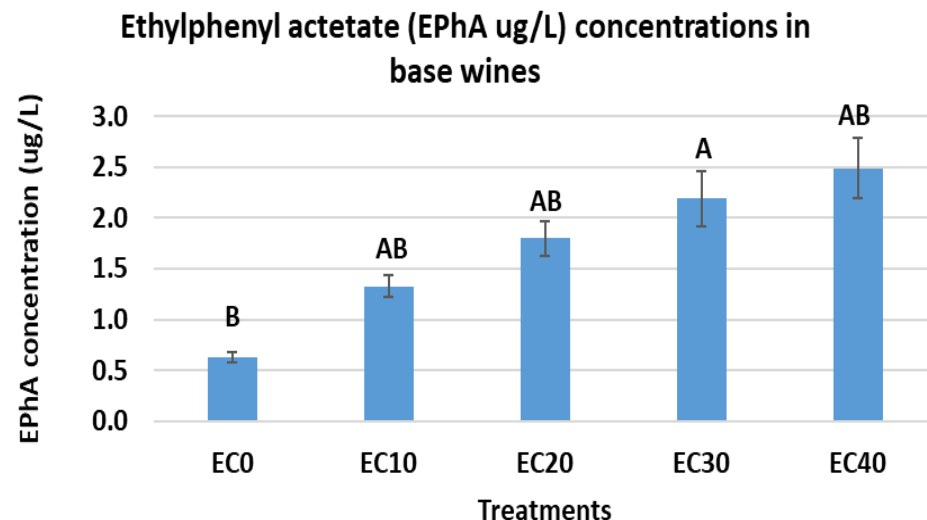
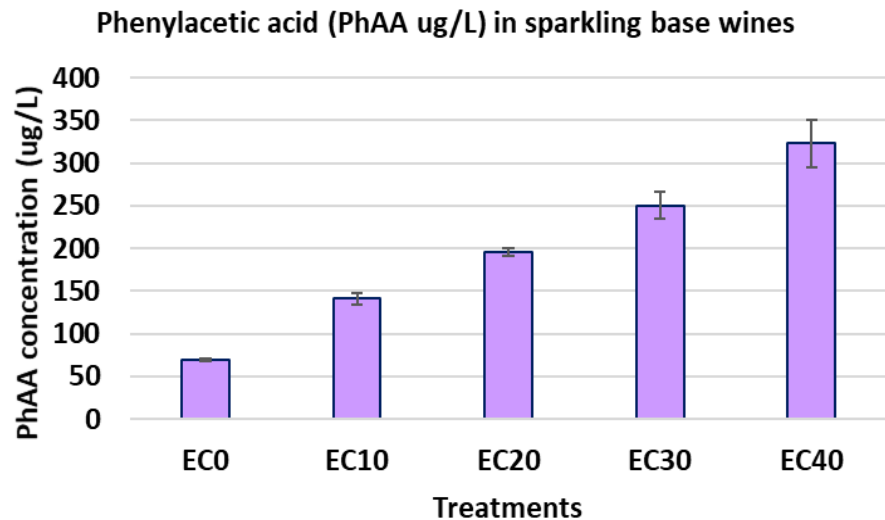


Figure 8. EPhA & PhAA concentrations in base wines.

Treatment codes: Control (EC0). 10% sour rot juice (EC10), 20% sour rot juice (EC20), 30% sour rot juice (EC30) and 40% sour rot juice (EC40).

Error bars represent standard deviations (\pm). Significance $P = 0.03$.

Summary points

- EPhA concentration statistically significant
- Low concentrations of PhAA & EPhA – EC1118 yeast

Acetic acid (g/L) concentrations in base wine

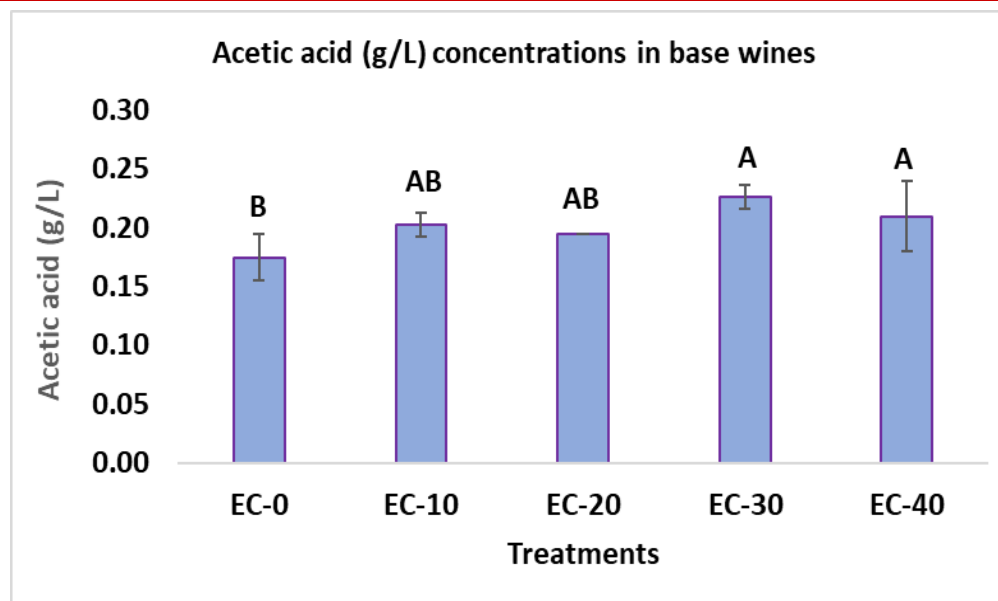


Figure 9. Concentration of acetic acid (g/L) in base wines.

Treatment codes: Control (EC0). 10% sour rot juice (EC10), 20% sour rot juice (EC20), 30% sour rot juice (EC30) and 40% sour rot juice (EC40).

Error bars represent standard deviations (\pm). Significance $P = 0.02$.

❖ **Ethyl acetate concentration range: 40 - 45 mg/L (*no statistical difference*)**

Summary of EPhA & PhAA results to date.....



Possible biomarker for sour rot during grape ripening?

- ✓ PhAA & EPhA increased during ripening
- ✓ Needs more years of data to confirm



Juice

- ✓ Increased PhAA & EPhA concentrations with increased sour rot %

Base wine

- Both PhAA & EPhA in base wines
- Juice & base wine concentrations similar
- Concentrations below threshold levels



Further research



- Bottling, 2nd fermentation, riddling disgorging & *dosage* with EC1118 yeast
- Comparison of data for EC1118 yeast with CN1
- Chemical analysis of 2019 finished sparkling wines
PhAA & EPhA of sparkling wines during aging

Do PhAA & EPhA concentrations fluctuate during aging on lees?

- Two more vintages 2020 & 2021
- Sensory analysis of 2019, 2020 & 2021

How can a winemaker transfer this knowledge to their winemaking?



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Canadian Grapevine Certification Network

CGCN-RCCV

Réseau Canadien de Certification de la Vigne

ANDREW PELLER

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In-kind partners

- Malcolm Lawrie owner of Lawrie Vineyard, NOTL, ON.

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WHO?

WHOM?

WHEN?

WHOSE?

QUESTIONS

HOW?

WHAT?

WHY?

WHERE?

WHICH?