Cool Climate Oenology & Viticulture Institute
Brock University

Developing Ontario Appassimento Wines: The impact of drying method, yeast strain and botrytis on wine quality and consumer choice

CCOVI Lecture Series
March 16, 2016
Dr. Debra Inglis

Why Develop Appassimento Wines for Ontario

- **Can we further develop flavours in our grapes for use in high end wines despite cool, wet, less optimal fall months**
- **Adopt methods and technologies from other regions around the world that mitigate production risks, stabilize wine quality differences year-to-year and contribute to distinctive regional wine styles**
  - Ripen fruit post harvest off-the-vine, then ferment into wine (appassimento wines) – unique Ontario style

Things to watch for in Appassimento Grape Drying that may impact wine

- Increase in oxidation compounds during the drying process in the grapes that translate into oxidation faults in the wine (acetic acid, acetaldehyde and ethyl acetate)
- botrytis fungal development during the drying process from favourable humidity conditions (above 90% RH) that takes away from wine quality

The Appassimento Project -5 year

1. **Comparative study of 5 techniques used to dry the grapes using Cabernet franc**

2. **Yeast strain trial: comparison of a yeast isolate from local riesling grapes for use in appassimento wine production**

3. **Impact of *Botrytis cinerea* on chemical profile, sensory attributes and consumer acceptance of appassimento wines**

Project1 : Comparative study of 5 techniques used to dry the grapes using Cabernet franc

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Cabernet franc: 5 drying regimes compared


On-vine


Barn

Drying chamber


Kiln

Greenhouse

Project1 : Comparative study of 5 techniques used to dry the grapes using Cabernet franc

- **Elucidate for each method the environmental conditions present during the different stages of drying to enhance the understanding of the method and the potential impact of climate-related risks**
- **For 4 seasons**
  - Cabernet franc grapes, comparing drying regimes
  - First year was a trial year to work out methods, three complete years of data for 4 of 5 techniques
  - Fruit changes during drying monitored, fermentations completed each year, chemical and sensory analysis completed on the wines
  - Final year, we need to complete all volatile flavour analysis for past wines

Grapes and Drying Targets in Brix

- Cabernet franc were donated from Pillitteri Estates Winery each year
- Target Brix of fruit at harvest: 23ºBrix
- Target Brix for drying: 26ºBrix and 28ºBrix
- All wines fermented using the same protocol, in triplicate, using EC1118 yeast from Lallemand

On-Vine Drying: Temperature and Relative Humidity

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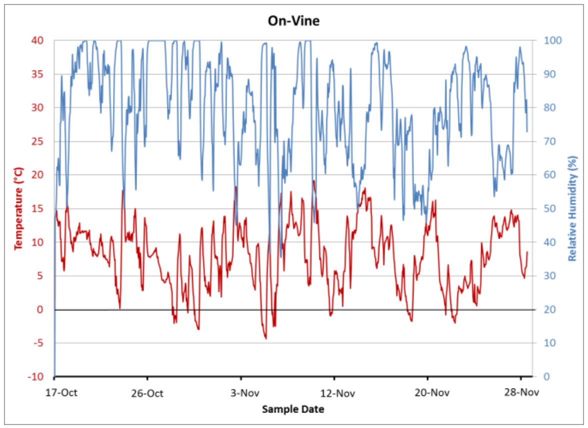
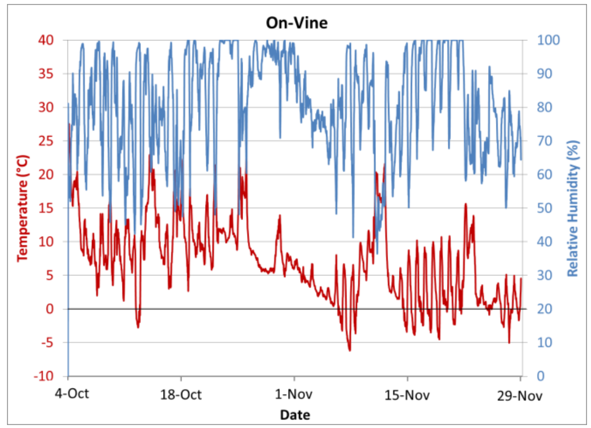
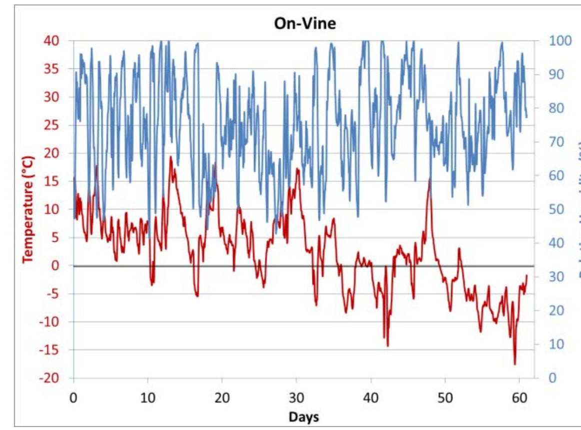
2011



2012



2013



Long Duration Treatment (2 plus months)

- Exposure to climate risks
- Rain, fog, dew, wind, freeze-thaw, wildlife
- Highly variable temperature and humidity

Barn Drying: Temperature and Relative Humidity

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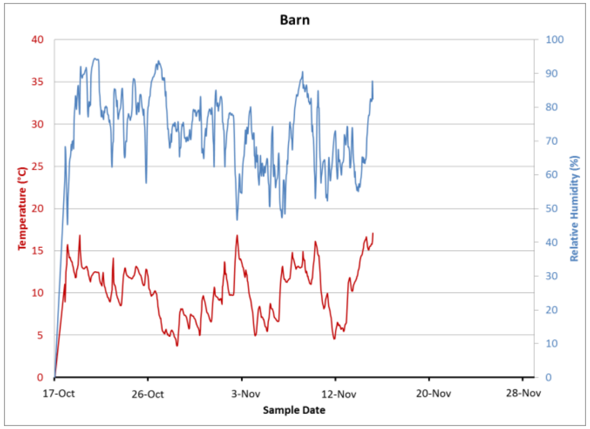
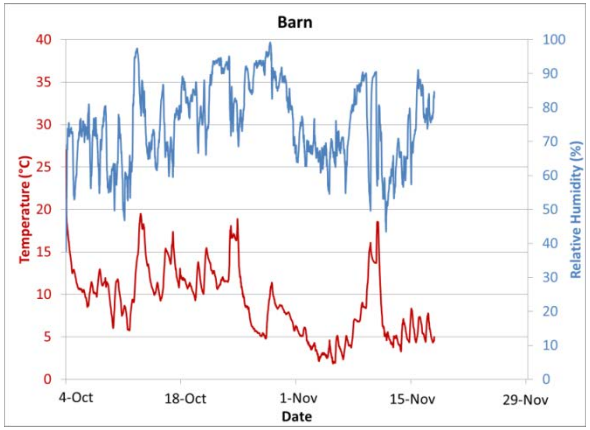
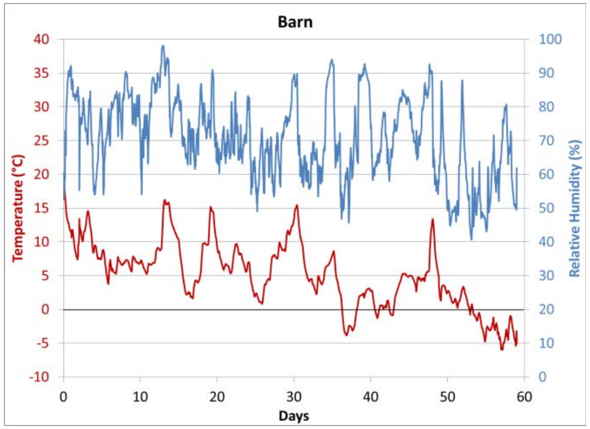
2011



2012



2013

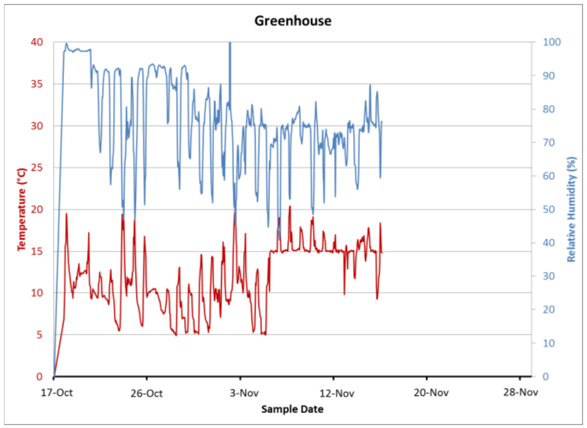


Mid to long term duration (1-2 months)

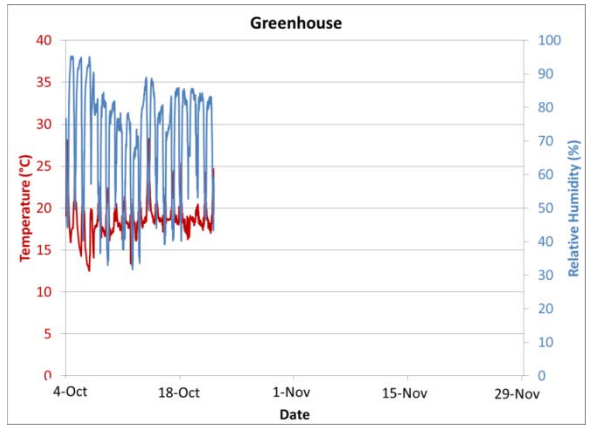
- Protected from rain, wildlife but impacted by external climatic conditions
- Temp and humidity correlated to external climate conditions (r = 0.836)
- Not as variable as on-vine

Greenhouse Drying: Temperature and Relative Humidity

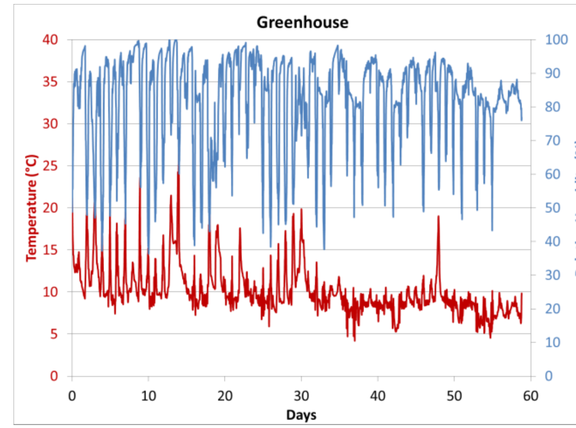
2011



2012



2013



Mid Duration Treatment (weeks)

- Protected from external climate (rain)
- More variability in humidity, can help control internal conditions with heat and air circulation

Kiln Drying: Temperature and Relative Humidity

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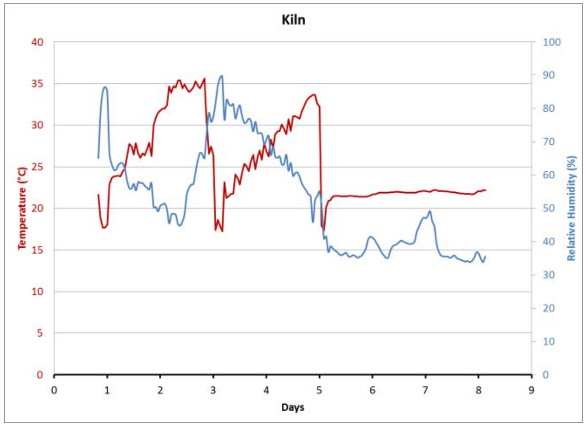
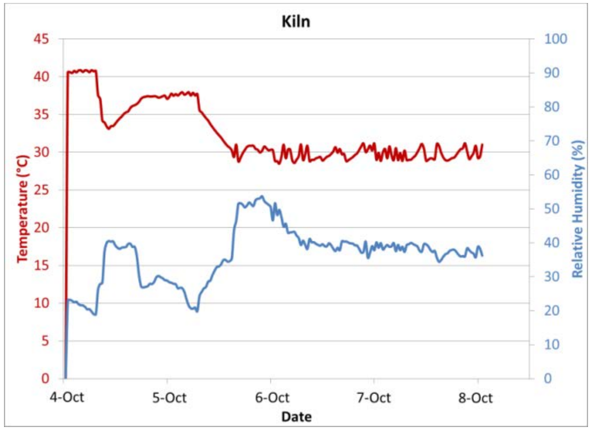
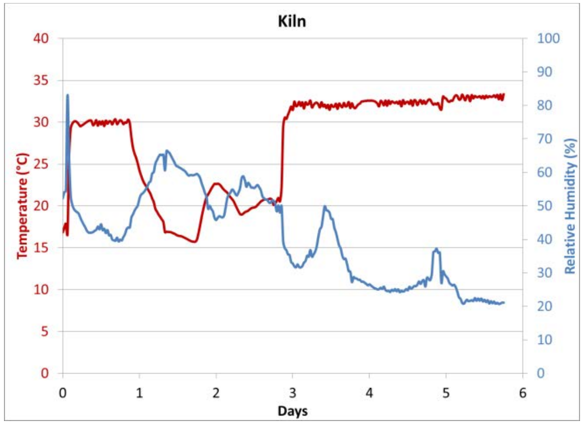
2011



2012



2013



Short duration (days)

- Protected from rain, wildlife
- Not correlated to external climate conditions
- High air flow
- Control temp, targeting approx. 30°C
- 2012, temp was increased on day 1 by mistake at winery

Drying Chamber: Temperature and Relative Humidity

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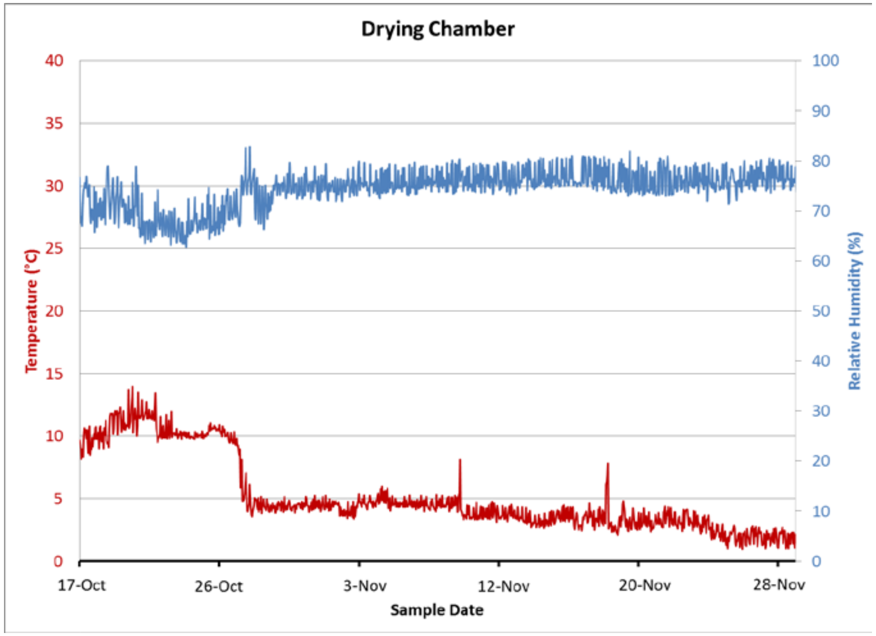
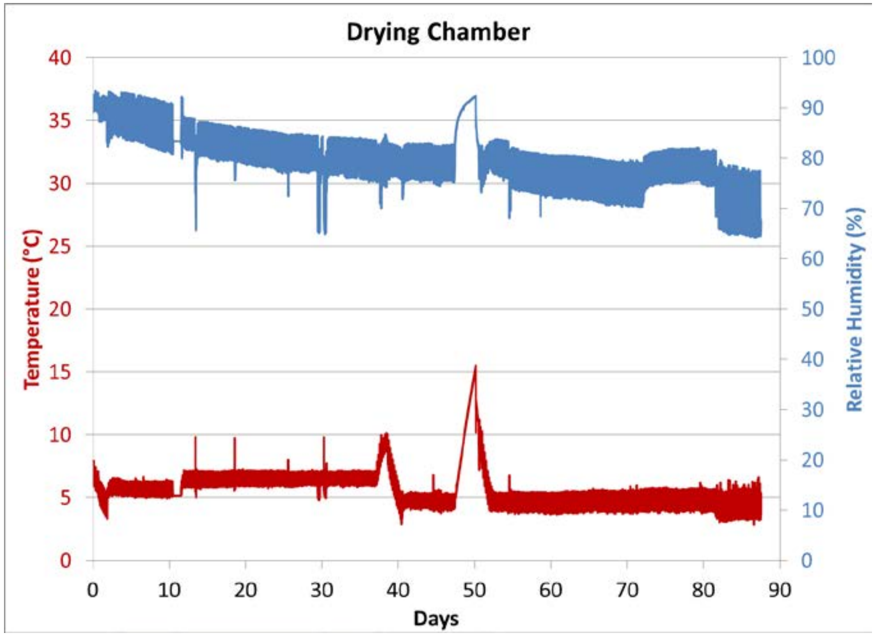
2011



2013



Longest duration

- Protected from rain, wildlife
- No external climate influence, temp and humidity controlled
- Temperature stays low, Humidity stays low
- Differences in conditions in 2011 vs 2013,
    - more botrytis in 2013, higher humidity at start in chamber

Drying treatments require different times to reach target Brix

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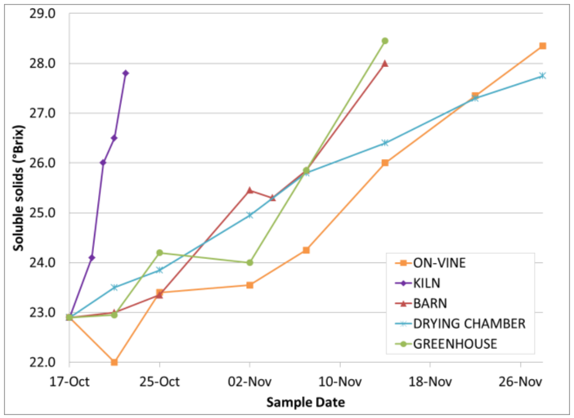
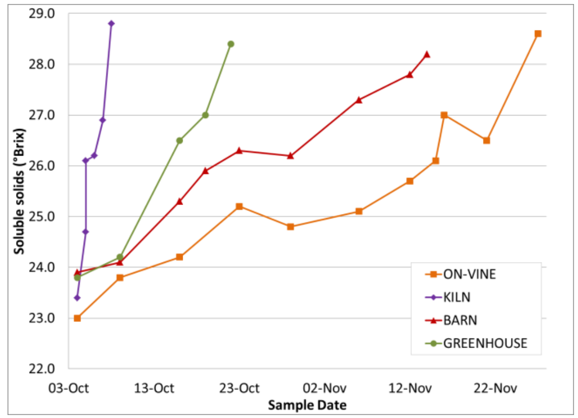
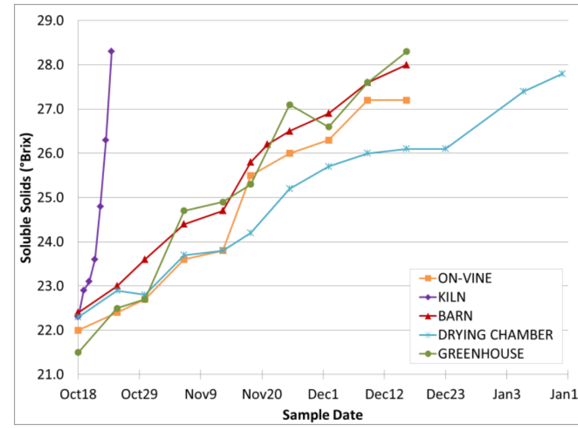
2011



2012



2013



Change in **soluble solids** for drying conditions.

Drying Condition	**26°Brix**			**28°Brix**		
	Drying Period (days)			Drying Period (days)		
	2011	**2012**	**2013**	**2011**	**2012**	**2013**
On-Vine	30	43	33	42	56	61
Kiln	3	1	5	5	4	6
Barn	22	15	34	29	41	59
Greenhouse	22	12	38	29	18	59
Drying Chamber	22	-	52	44	-	88

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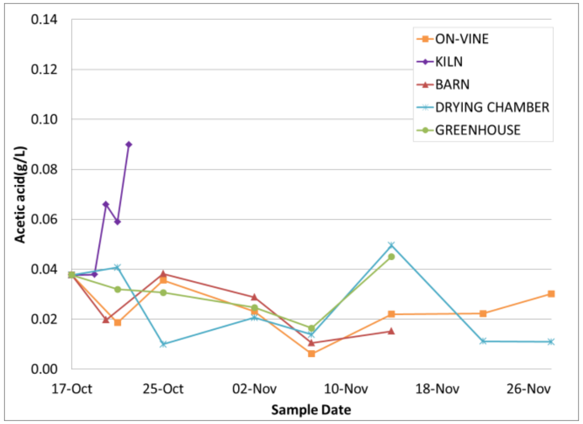
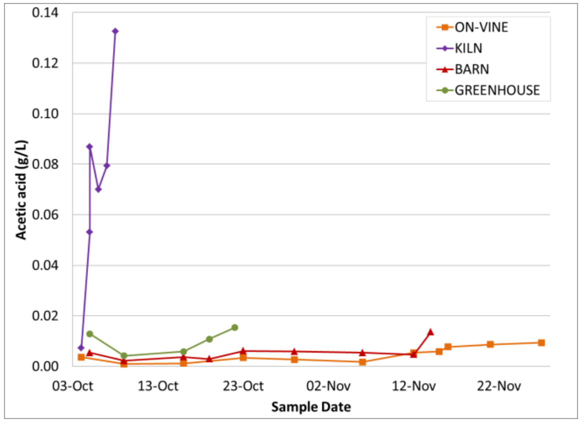
Things to watch for in Appassimento Grape Drying that may impact wine
- increase in oxidation compounds in the grapes like acetic acid, acetaldehyde and ethyl acetate during the drying process

Acetic Acid concentration increases with Kiln drying, something to watch

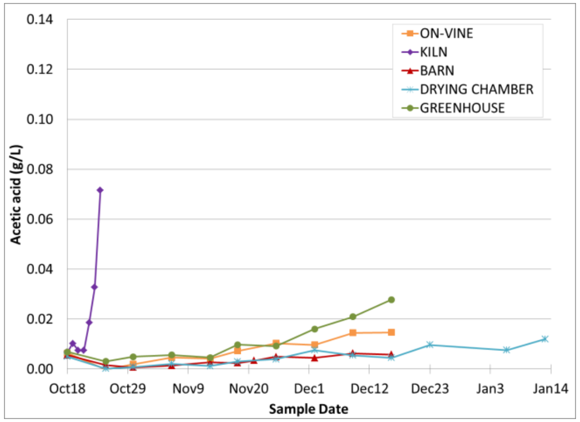
2011



2012



2013



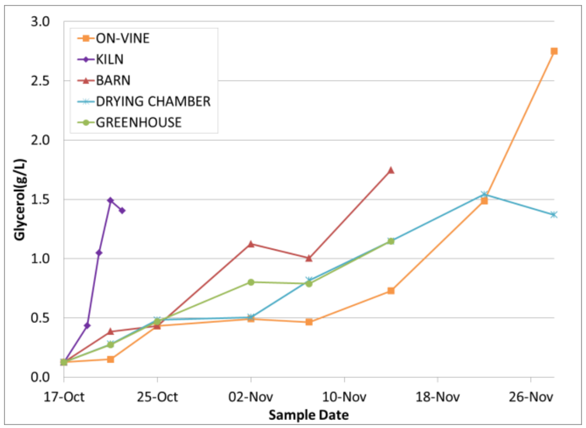
- All acetic acid values are quite low, even in the kiln
- Highest was 0.13 g/L acetic acid in 2012
- Higher acetic acid in kiln dried fruit NOT correlated to acetic acid bacteria on the fruit

Other compounds that varied through drying

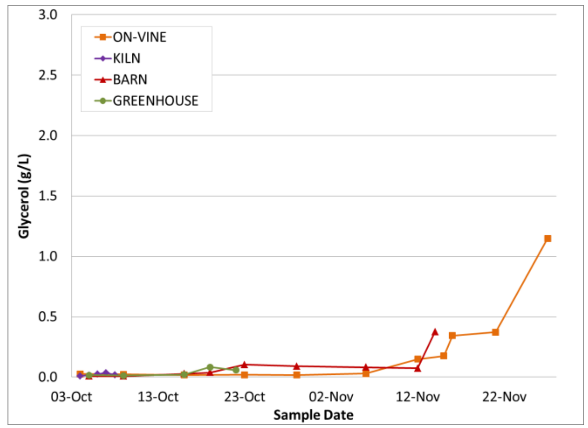
- **Acetaldehyde increases with all treatments**
  - Most pronounced with on-vine and kiln but still < 12 mg/L
- **Malic acid drops in all treatments**
  - Does not accumulate in berries with water loss
  - Usually between 2-2.5 g/L malic acid in the whithered fruit
- **Glycerol increases 10 to 20-fold across treatments in 2011, 2013**
  - little change in 2012 (free of botrytis)

Glycerol increases 10 to 20-fold across treatments in 2011, 2013, little change 2012 (free of botrytis)

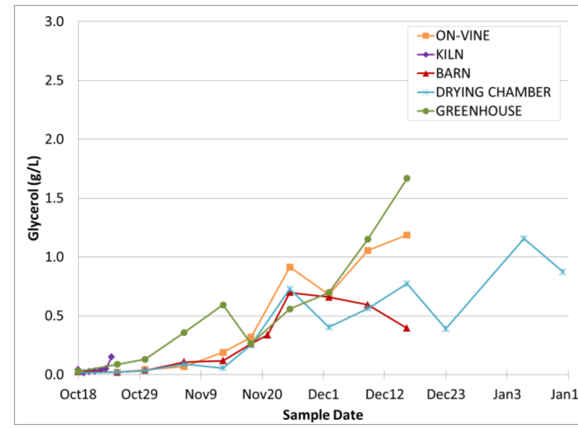
2011



2012



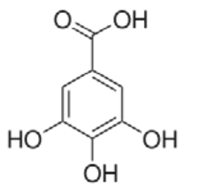
2013

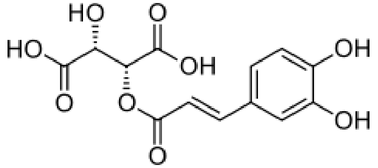


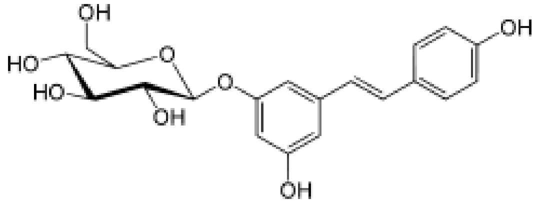
- Glycerol is a byproduct of botrytis

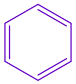
Polyphenolics 2011-2013 (V. DeLuca) Extraction and Identification of 25-30 different polyphenols

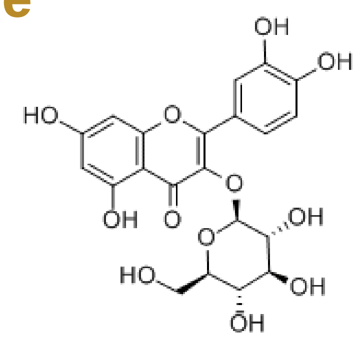
- **Simple phenols** (Gallic acid, Galloyl glucoside, Caftaric Acid)
- **Resveratrols** (transresveratrol, cis and trans-piceid)
- **Procyanidins** (Procyanidin, Procyanidin Dimers, Catechin, Epicatechin
- **Flavonoids** (Kaempferol, Kaempferol Glucosides, Quercetin, Quercetin glucoside, Quercetin glucuronide, Isorhamnetin glucoside, Myricetin, Myricetin glucoside, Myricetin galactoside, Myricetin rhamnoside
- **Anthocyanins** (Delphinidin-3-0-glucoside, Petunidin 3-O-glucoside, Malvidin-3-O-glucoside, Malvidin 3-O-acetylglucoside, Malvidin 3-O-coumaroylglucoside











Polyphenolic analysis summary (V. Deluca Laboratory)

- **Many polyphenols rise by 10 to 30 % in appasimento grapes compared to control grapes irrespective of the drying treatment used**
  - Simple phenols, Resveratrols, Procyanidins, Flavonoids & Anthocyanins
- **Resveratrol levels were higher in growing seasons when disease pressure was higher.**
- **Metabolite concentrations achieved were not specific to the drying method.**
- **Polyphenolic metabolite profiles have not yet been correlated to sensory attributes of the wines**
  - Is there impact on wine perception
- **Focus of the laboratory has now shifted to a search for transcript protein markers.**

Seed Analysis during Appassimento drying separated by drying technique (B. Kemp Laboratory)

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22.5 Brix	26 Brix	28 Brix
 Control – Oct 18, 2013	 On-Vine – Nov 20, 2013	 On-Vine – Dec 18, 2013
 Control – Oct 18, 2013	 Kiln – Oct 23, 2013	 Kiln – Oct 27, 2013

Seed Analysis during Appassimento drying separated by drying technique (B. Kemp laboratory)

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22.5 Brix	26 Brix	28 Brix
 Control – Oct 18, 2013	 Greenhouse – Nov 25, 2013	 Greenhouse – Dec 16, 2013
 Control – Oct 18, 2013	 Barn – Nov 21, 2013	 Barn – Dec 16, 2013

Seed Analysis during Appassimento drying separated by drying technique (B. Kemp Laboratory)

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22.5 Brix	26 Brix	28 Brix
 Control – Oct 18, 2013	 Drying Chamber – Dec 9, 2013	 Drying Chamber – Jan 14, 2014

Total Extractable seed tannin in appassimento grapes during drying process before fermentation (2013, B. Kemp Laboratory)

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Treatment	22.5°Brix Epicatechin (ug/ml extract)	26°Brix Epicatechin (ug/ml extract)	28°Brix Epicatechin (ug/ml extract)
On-vine	1230 ±59 b	1360 ±52 a	1371±55 a
Kiln	1230 ±59 a	1257 ±24 a	1277 ±42 a
Greenhouse	1230 ±59 b	1330 ±50 a	1403 ±68 a
Barn	1230 ±59 c	1394 ±77 b	1572 ±59 a
Drying chamber	1230 ±59 b	1320 ±19 a	1366 ±24 a

Total Extractable skin tannin in appassimento grapes during drying process before fermentation (2013, B. Kemp Laboratory)

Treatment	22.5°Brix Epicatechin (ug/ml extract)	26°Brix Epicatechin (ug/ml extract)	28°Brix Epicatechin (ug/ml extract)
On-vine	60 ±13 a	49 ±9 a	24 ±13 b
Kiln	60 ±13 ab	41 ±14 b	68 ±17 a
Greenhouse	60 ±13 a	33 ±6 b	62 ±12 a
Barn	60 ±13 a	48 ±13 a	43 ±17 a
Drying chamber	60 ±13 b	60 ±20 ab	77 ±20 a

Appassimento Wines
High Ethanol Wines

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Drying Condition	26°Brix Ethanol (% v/v)			28°Brix Ethanol (% v/v)		
	2011	2012	2013	2011	2012	2013
On-Vine	12.6	15.0	14.8	13.7	16.4	15.6
Kiln	14.2	15.1	15.1	15.3	16.3	15.8
Barn	14.8	14.9	16.8	14.8	16.0	17.0
Greenhouse	13.3	15.3	14.8	15.5	16.7	16.4
Drying Chamber	14.4	-	16.0	15.5	-	17.0

Descriptive Analysis of Wines 2011, 2012, 2013 vintages (G. Pickering Laboratory)

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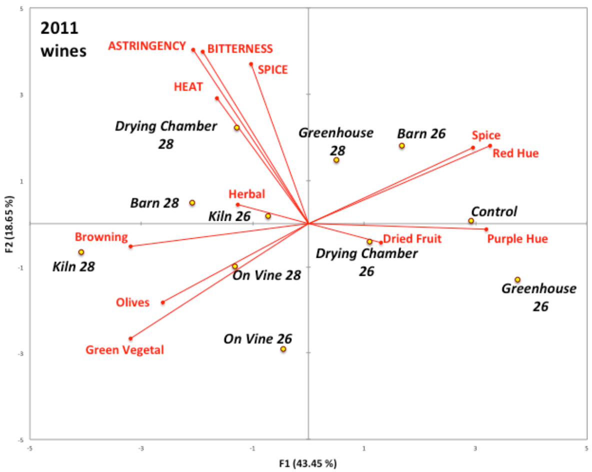
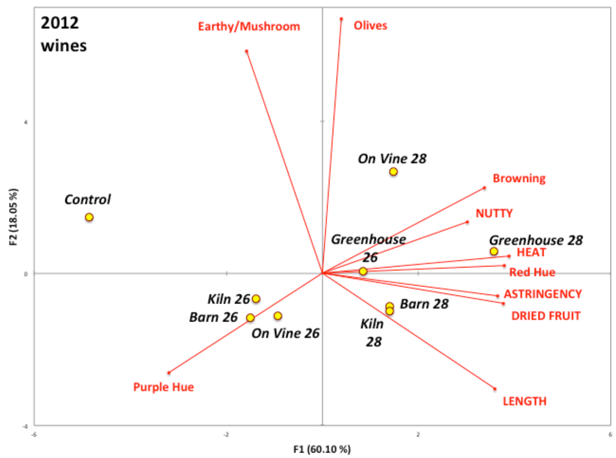
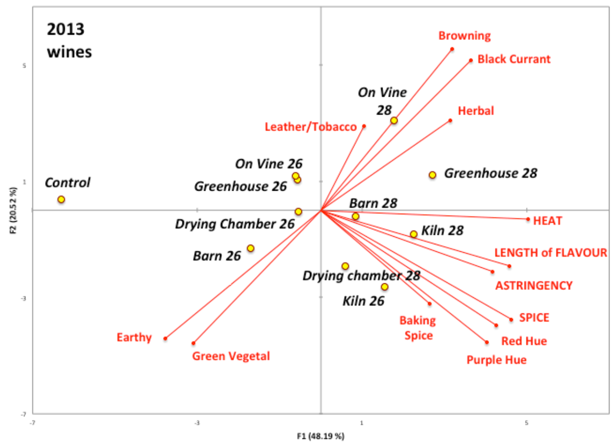






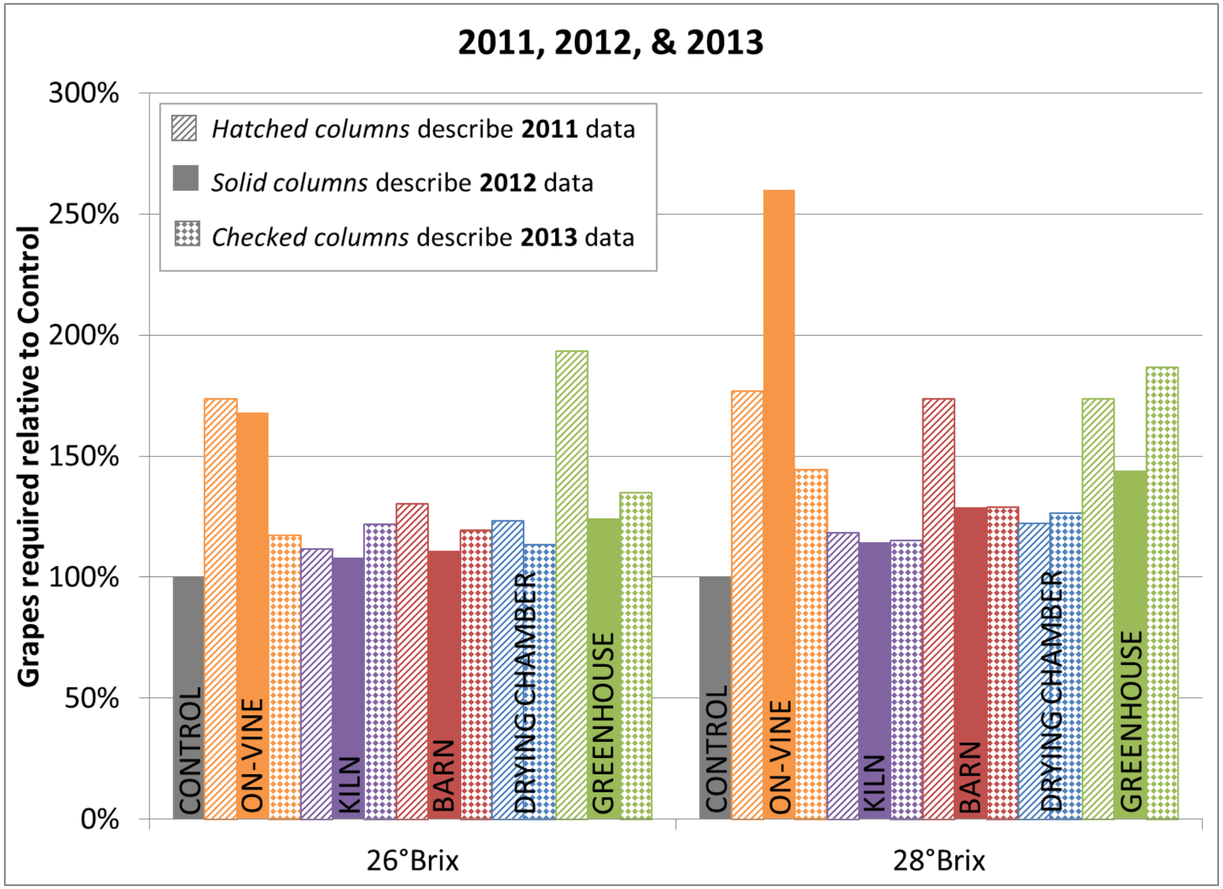
- Descriptive Analysis was performed for all wines for all 3 years using a trained sensory panel.
- Triplicate evaluations for up to 11 treatments each year!
  - CCOVI's custom sensory evaluation lab
  - 4-6 months after bottling
- Figures visualize the results of the Principal Component Analyses , which were performed on those descriptors that were significantly different between wines ($p(F)<0.05$).
- Labels in CAPITAL letters indicate flavor descriptors, those in lower case are aroma and colour descriptors.

Volatile compounds for flavour analysis

- GC-MS method now developed to analyze wines for volatile compounds
- Identification and quantification of volatile compounds in the wines, statistical analysis and interpretation of results
- Completed for all wines, data being analysed

Preliminary cost analysis 2011 vs 2012 vs 2013

- to generate the same must volume as the control for the various treatments, what % increase in grapes are required?



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Project 2: Can yeast choice overcome wine oxidation fault issues and assist in developing a unique Ontario style.
- new yeast isolate from local grapes that is a low producer of VA and ethyl acetate
–Jennifer Kelly, PhD student

A Novel Yeast for Regional Signature Wines

- An indigenous yeast with fermentative capacity was isolated from Riesling Icewine grapes
- Brock Isolate: *Saccharomyces bayanus*
  - Produces significantly lower concentrations of oxidation compounds (acetic acid, ethyl acetate and acetaldehyde) in finished wine vs. *Saccharomyces cerevisiae* EC1118 (Inglis and Heit, 2013)
  - Potential value in Appassimento wine
    - Grapes dried post-harvest may start with higher concentrations of oxidation compounds
    - Intent is to not further increase compounds in finished wine

Project Aims (Jennifer Kelly, PhD student)

Characterize *S. bayanus* Brock Isolate for Appassimento winemaking:

- **What are the upper sugar limits of juice that the yeast can ferment to dryness?**
- **How does it perform vs. *S. cerevisiae* EC1118?**
  - **Fermentation kinetics, oxidative compounds in finished wine, sensory profile of the wine**
- **Is there a consumer preference of appassimento wines fermented with the Brock yeast versus the commercially accepted EC1118 yeast?**

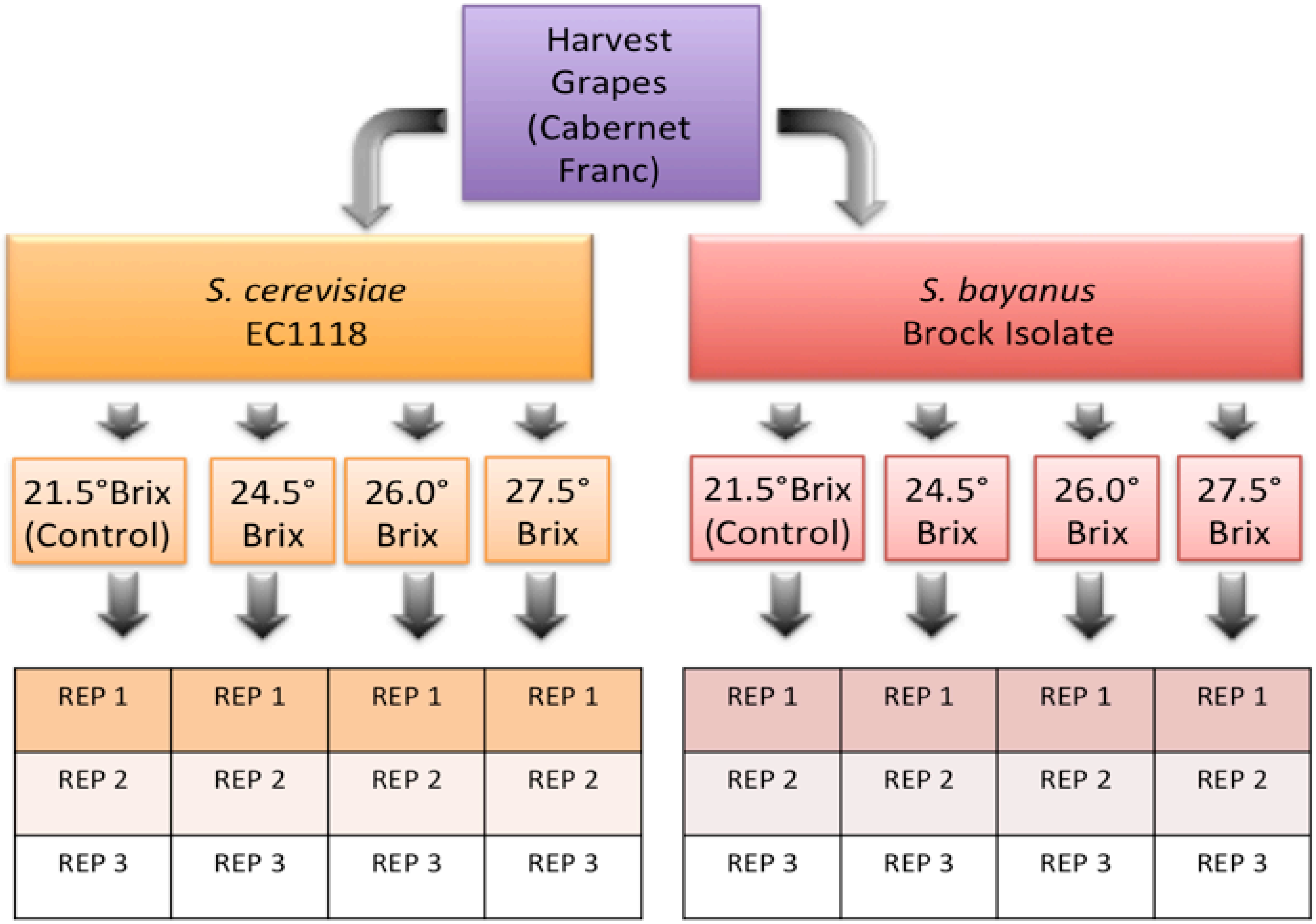
Winemaking Outline

Dried in barn

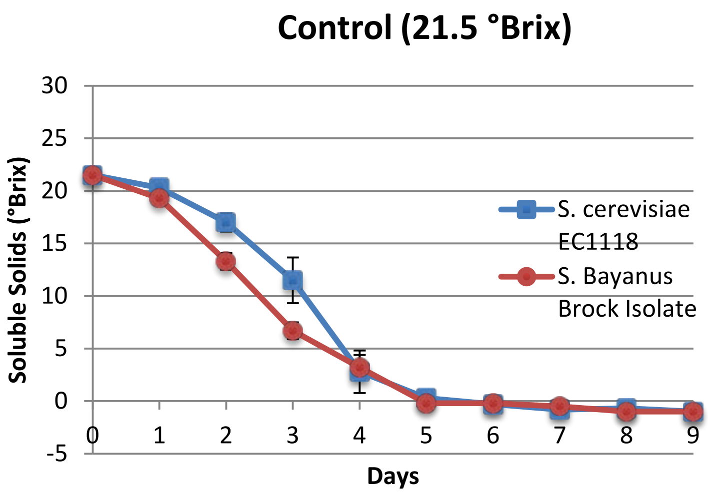
Inoculated using step-wise acclimatization at 5x10^6 cells/mL

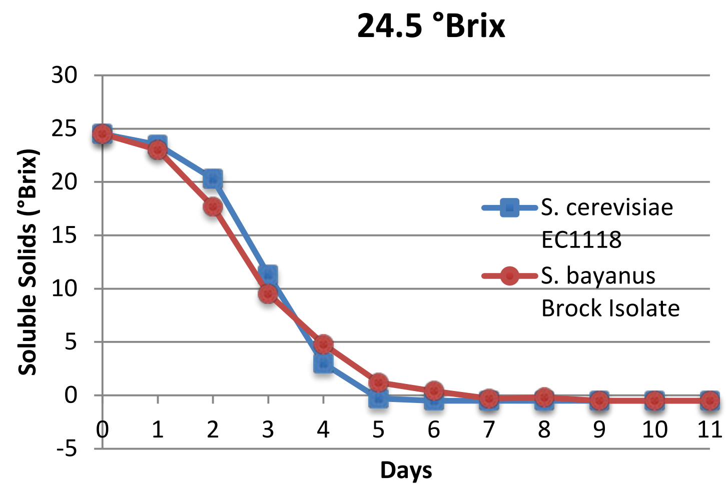
Daily Monitoring:

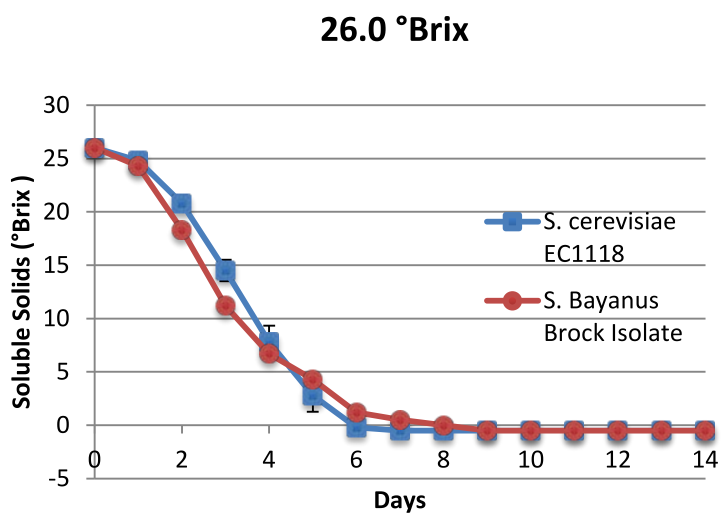
- °Brix
- Specific Gravity
- Temperature
- Cell Counting
- Sample collection for post-fermentation data

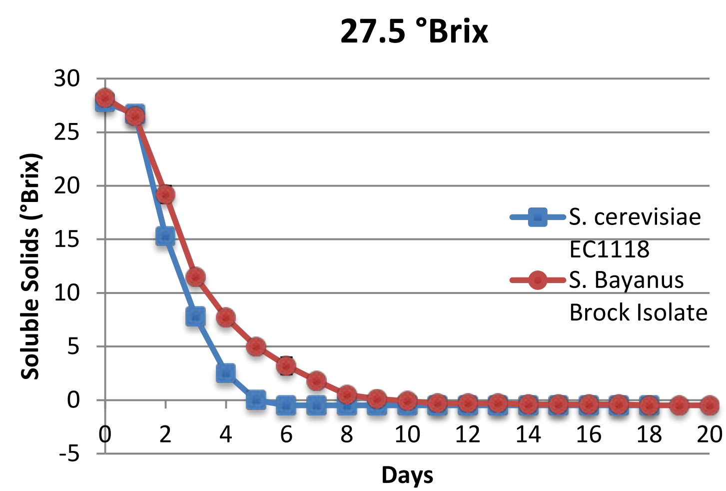


Fermentation Kinetics
How do the yeast species compare at each drying target?

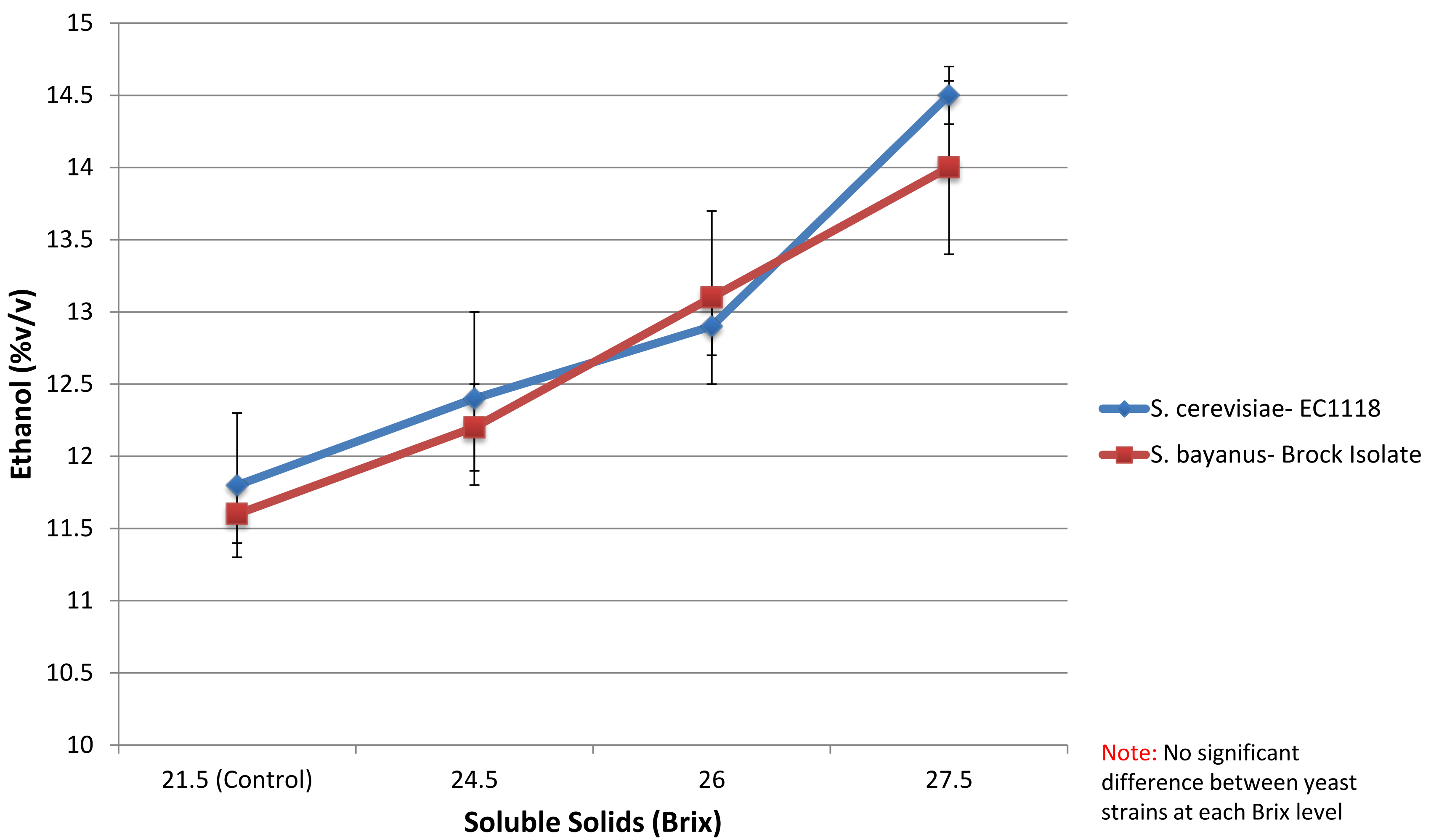






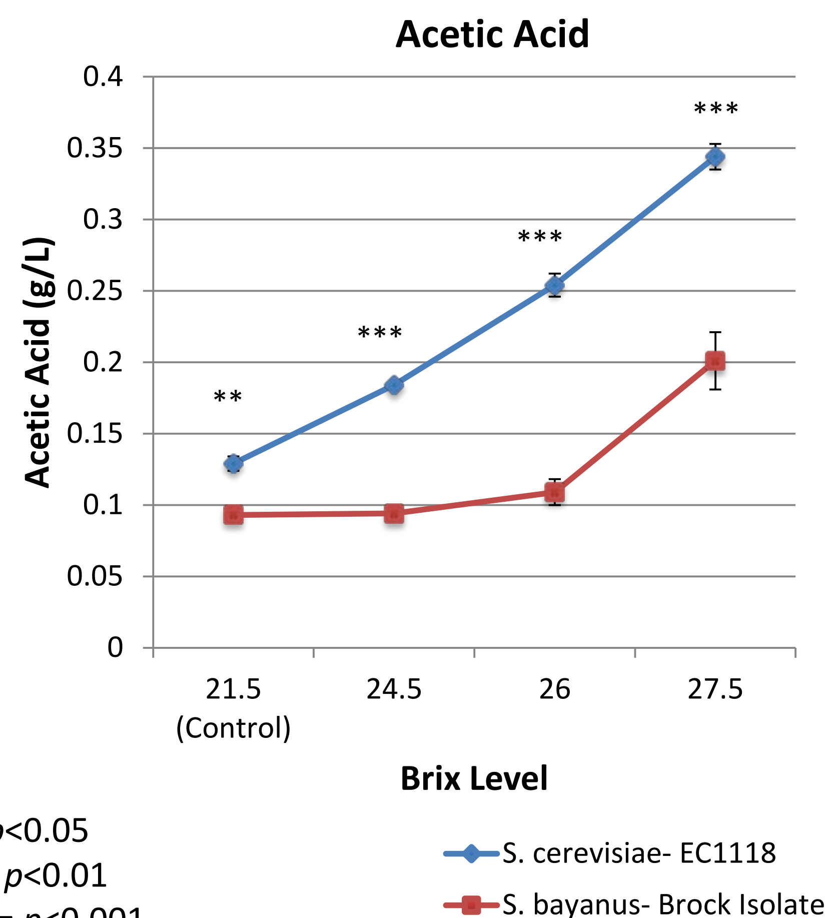


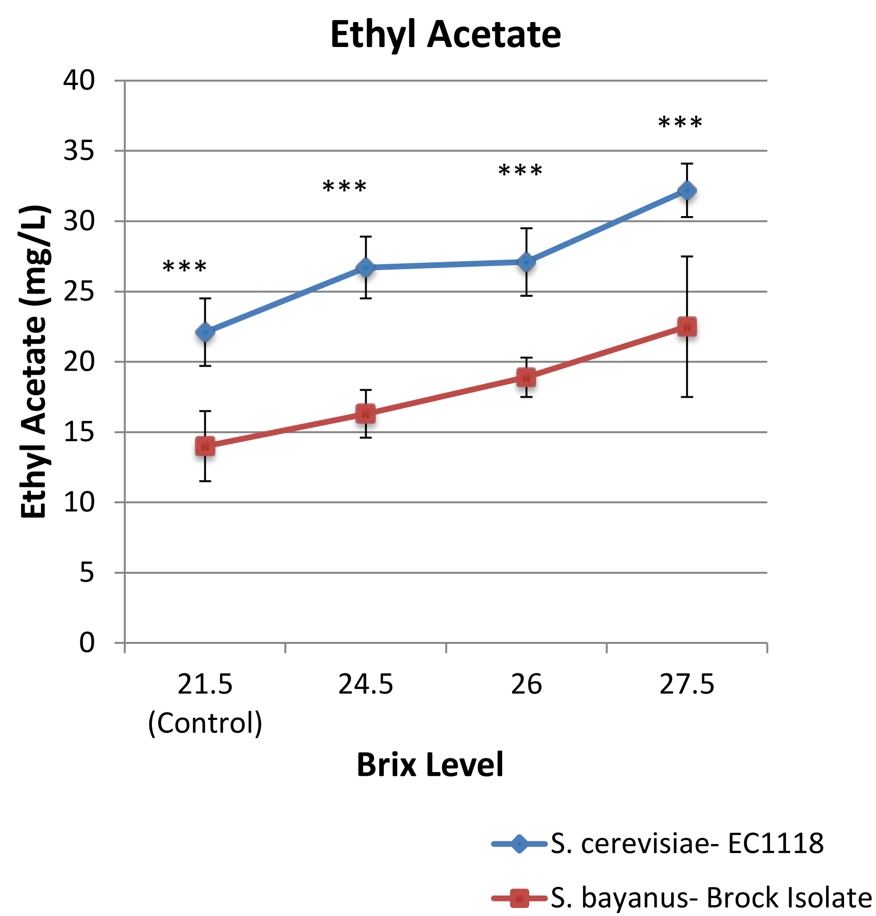
Can *S. bayanus* Brock Isolate produce similar ethanol levels to *S. cerevisiae* EC1118 in Appassimento wines?



Note: No significant difference between yeast strains at each Brix level

Can *S. bayanus* Brock Isolate reduce oxidation compounds in the wine?



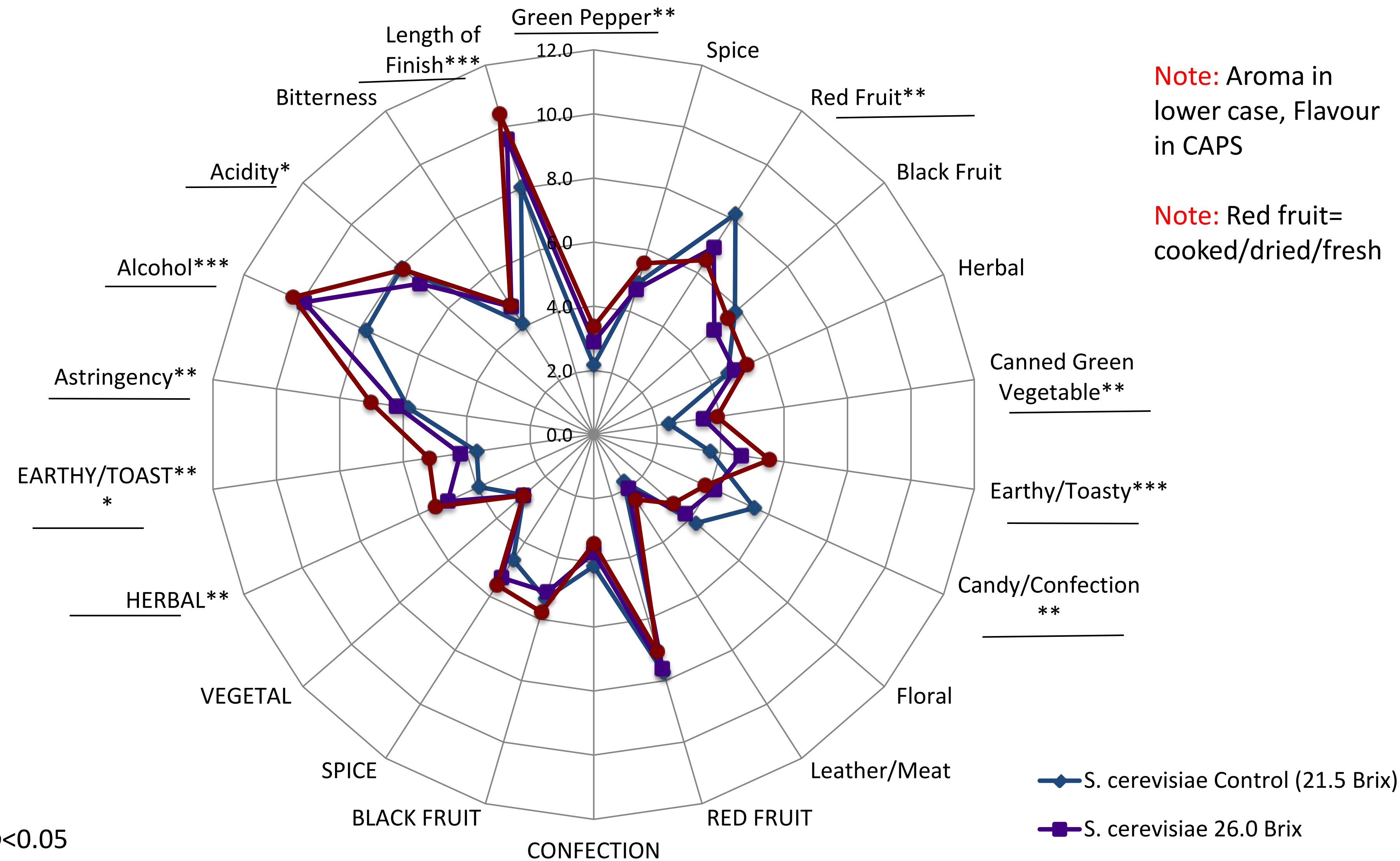


*= $p<0.05$
**= $p<0.01$
***= $p<0.001$

Sensory Evaluation

- **Descriptive analysis**
  - How does the profile differ from *S. cerevisiae* EC1118?
- **Panel = 11 discriminatory palates**
  - Attributes are identified and quantified using human subjects
  - Trained over 12 weeks
  - 15 cm line scale

Spider Plot: *S. cerevisiae* EC1118 Trends
Control (21.5 °Brix) vs. 26.0 °Brix vs. 27.5 °Brix
All Attributes

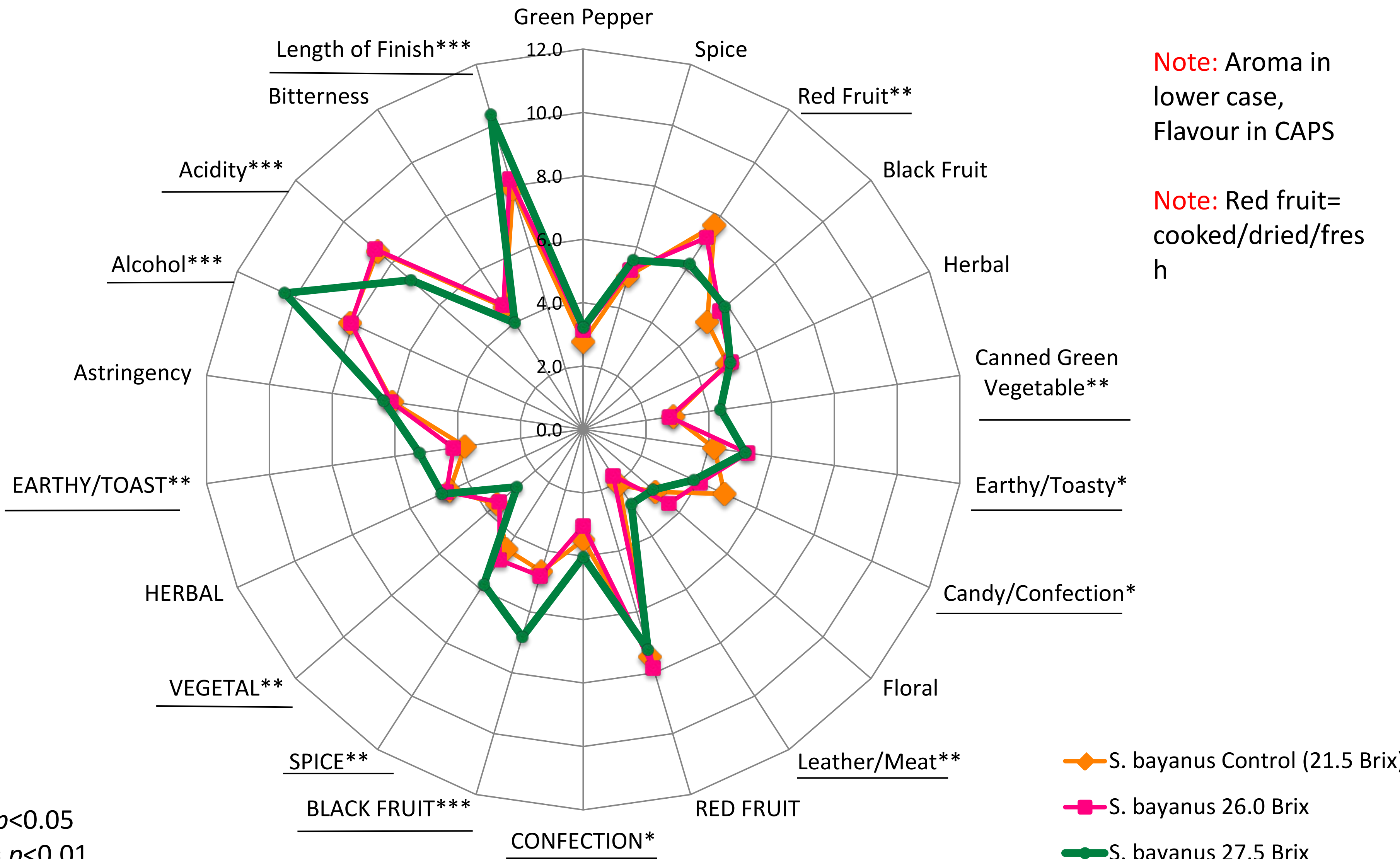


Note: Aroma in lower case, Flavour in CAPS

Note: Red fruit= cooked/dried/fresh

*= $p<0.05$
**= $p<0.01$
***= $p<0.001$

Spider Plot: *S. bayanus* Brock Isolate Trends Control (21.5 °Brix) vs. 26.0 °Brix vs. 27.5 °Brix All Attributes



Note: Aroma in lower case, Flavour in CAPS

Note: Red fruit= cooked/dried/fres h

*= $p<0.05$
**= $p<0.01$
***= $p<0.001$

PCA Chart
27.5 Brix
S. cerevisiae versus *S. bayanus*

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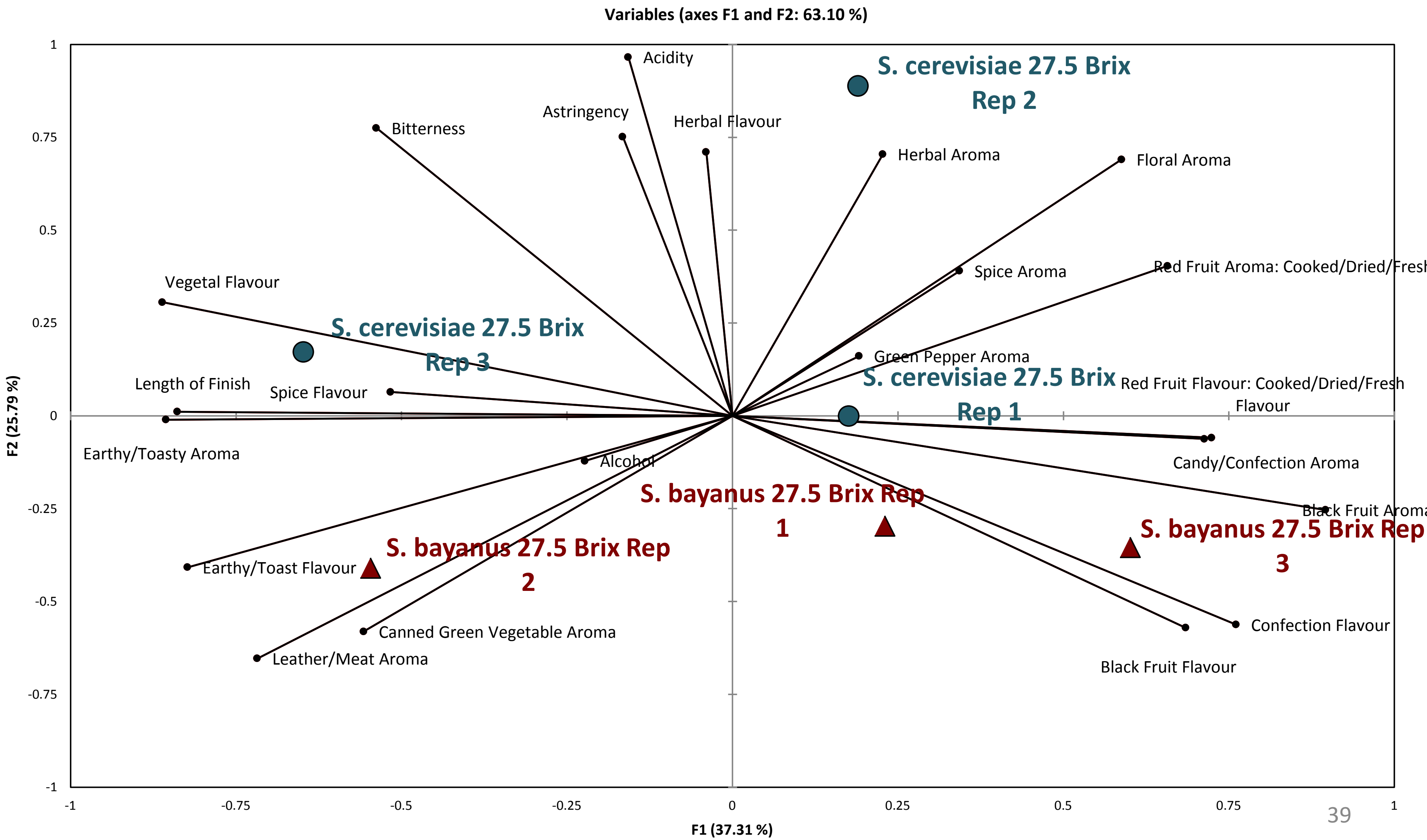
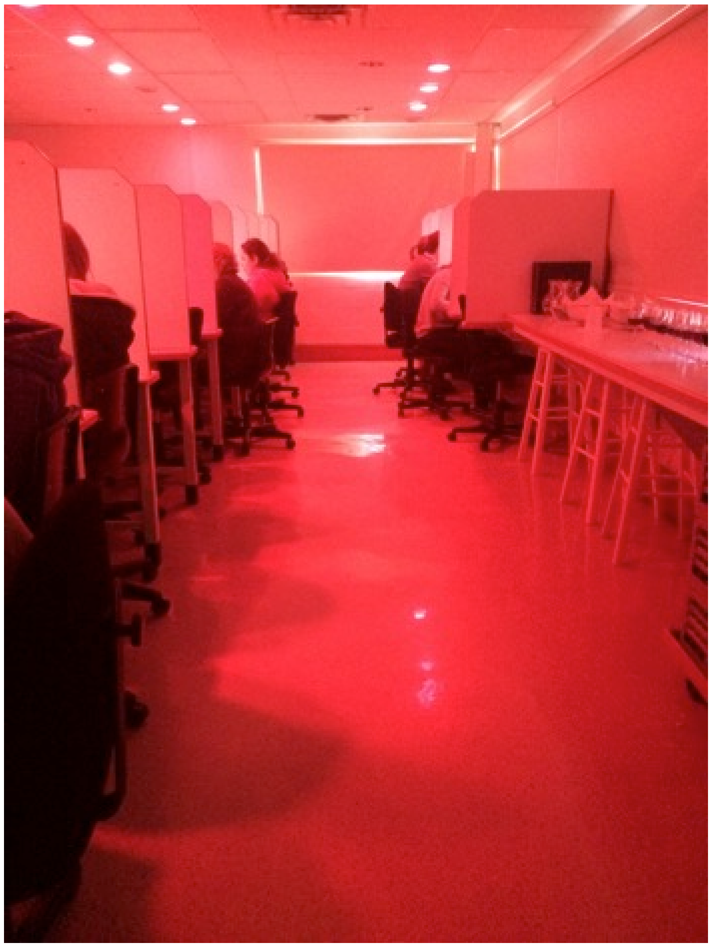


Sensory analysis conclusions



S. bayanus Brock Isolate in Appassimento Wine

- Shifted the sensory profile of the wine towards **increased black fruit flavour and aroma**
- **Reduced sourness and astringency** vs. *S. cerevisiae* EC1118 commercial yeast
- Has demonstrated its feasibility for industry use
- Consumer Preference????

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Project 3: Role of Botrytis in adding complexity to wines

-should we always discard botrytis infected fruit or can we first assess the impact of botrytis on wine profile?

-recent research points to a role of some botrytis infected fruit to add complexity in appassimento wines (noble rot form)

-Investigated 10% botrytis infection in the grapes

Botrytis infected Cabernet franc

Three Categories based on colour and physical appearance

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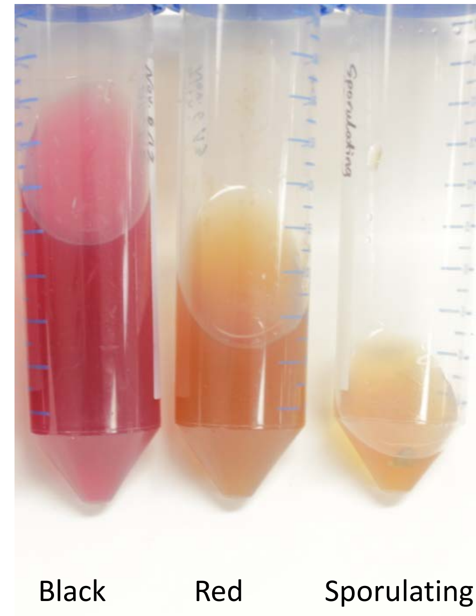
Red Black Sporulating

Separated fruit into 3 categories incubated in humid chamber to confirm botrytis

Day 0



Day 8

Berries from each category were plated out, botrytis confirmed in sporulating positive control and red berries

Chemical Comparisons of three fruit categories to confirm botrytis

	Black Berries (healthy berries)	Red Berries (botrytis berries)	Sporulating Berries (botrytis berries)
Brix	27.9	31.3	34.3
Glycerol (g/L)	0.1	9.3	11.1
Gluconic Acid (g/L)	0.1	1.5	1.4

Project 3: Impact of *Botrytis cinerea* on appassimento fruit – Sorting Team

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Project 3: Impact of *Botrytis cinerea* on appassimento fruit

Black, uninfected berries

Red, infected berries
but not sporulating

Fermentation set up with 0 and 10% by weight of botrytis infected berries

Project 3: Juice Analysis prior to fermentation with EC 1118

Juice Metabolite	Control	10% Botrytis
Brix	27.6 ± 0.2	28.1 ± 0.1
pH	3.65 ± 0.02	3.66 ± 0.01
TA (g/L Tartaric Acid)	4.8 ± 0.0	4.7 ± 0.0
Acetic Acid (g/L)	<0.02	<0.02
Glucose (g/L)	132 ± 5	128 ± 4
Fructose (g/L)	145 ± 10	142 ± 3
Glycerol (g/L)	0.04 ± 0.0	1.2 ± 0.1
Gluconic Acid (g/L)	0.14 ± 0.1	0.29 ± .02
Ammonia (mg N/L)	7 ± 1	7 ± 0
Amino acid (mg N/L)	91 ± 3	85 ± 0

Project 3: Fermentation Kinetics

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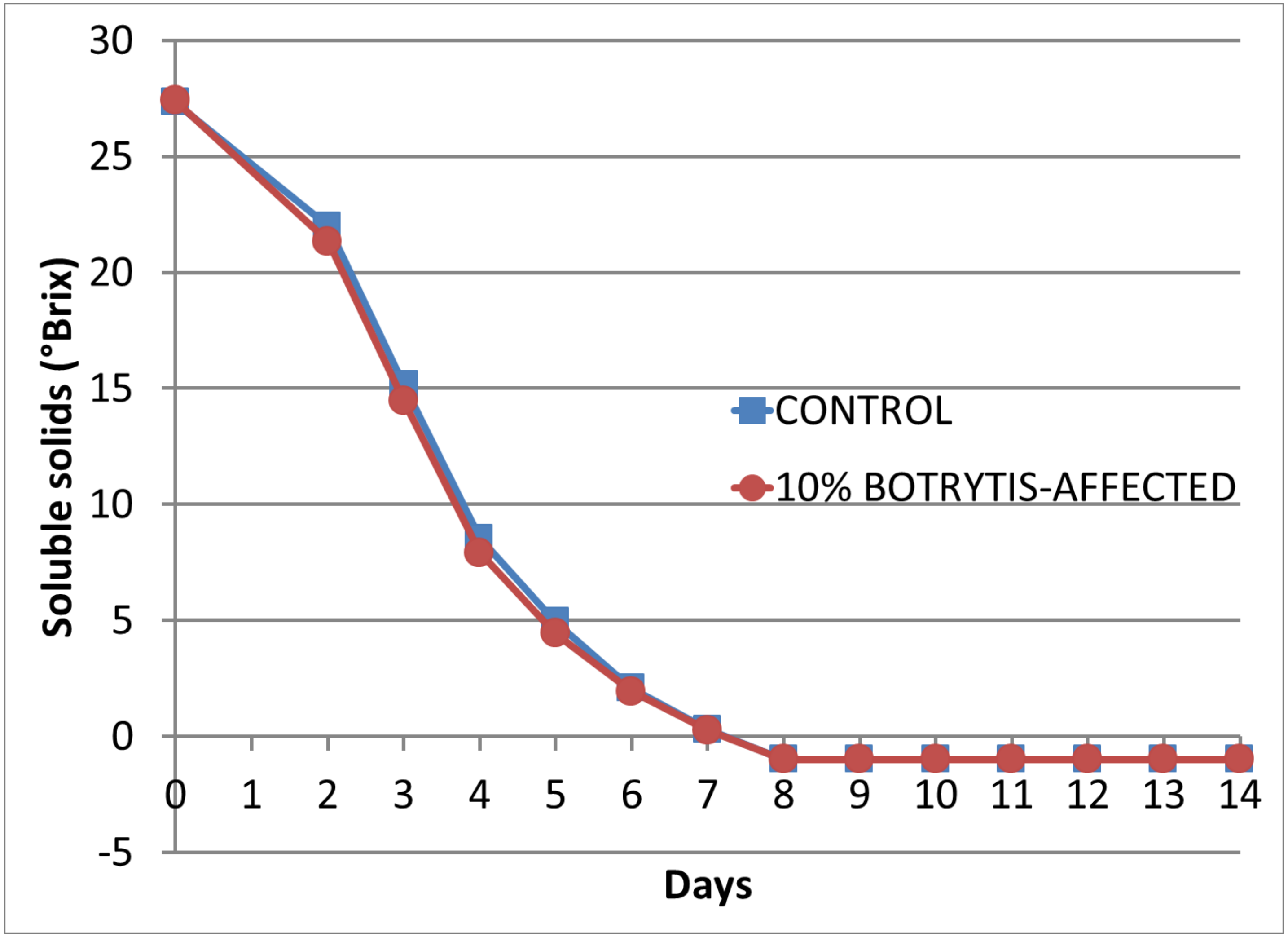

Project 3: Control vs 10% Botrytis Wine Analysis

Wine Metabolite	Control	10% Botrytis
pH	3.97 ± 0.03	4.01 ± 0.02
TA (g/L Tartaric Acid)	6.7 ± 0.0	6.4 ± 0
Ethanol (% v/v)	16.4 ± 0.2	16.4 ± 0.2
Residual Sugar (g/L)	0.17 ± 0.01	0.24 ± 0.02
Acetic Acid (g/L)	0.28 ± 0.00	0.35 ± 0.00
Acetaldehyde (mg/L)	108 ± 10	113 ± 8
Glycerol (g/L)	11.5 ± 0.4	12.7 ± 0.4
Gluconic Acid (g/L)	0.23 ± 0.02	0.34 ± 0.01

Project 3: 0 vs 10% Botrytis wines Sensory Analysis

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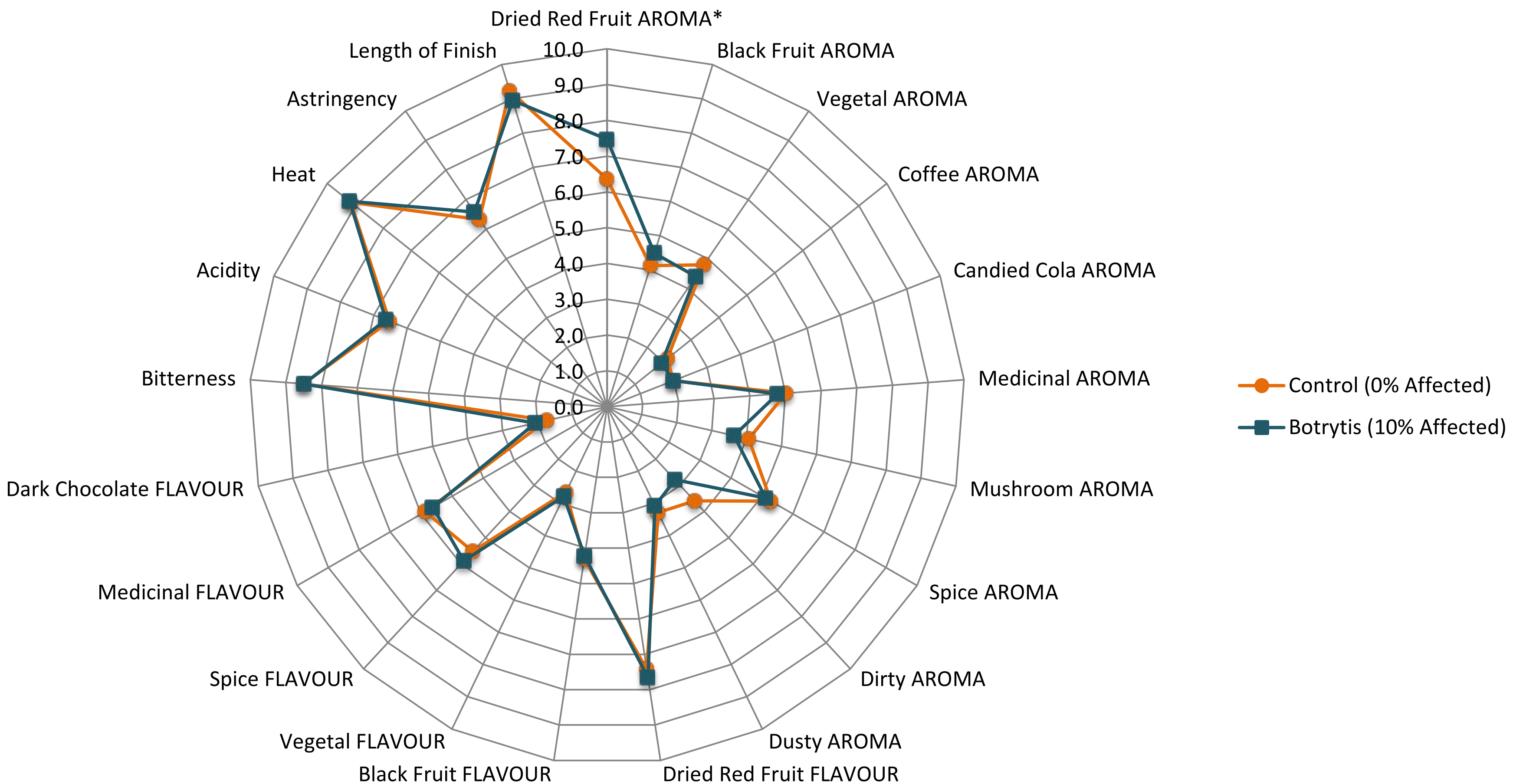
2013 Appassimento Trial: 0% vs. 10% *Botrytis cinerea* infection
Descriptive Analysis Results- All Attributes



Consumer Preference Among Appassimento Wines

- **Compared the consumer preference of Appassimento wines**
  - EC1118 *S. cerevisiae* – 0% Botrytis (27.6 Brix)
  - EC1118 *S. cerevisae* - 10% Botrytis infection (28.1 Brix)
  - *S. bayanus* Brock Isolate – 0% Botrytis (27.5 Brix)
- **Consumer Preference study carried out in Guelph at Compusense**
  - 153 consumers participated
  - Each participant received one wine at a time
  - Scored on a 9-point hedonic scale where 9="like extremely" and 1="dislike extremely"
  - Preference was determined from liking score
  - Values of 6+ are representative of "good" CONSUMER ACCEPTANCE
  - Anything over 7 is "excellent" consumer preference, but is usually reserved for products like chocolate

Consumer Preference

Means and ANOVA results

	p-value	*S. cerevisiae* 0% Botrytis	*S. cerevisae* 10% Botrytis	*S. bayanus* 0% Botrytis
Overall Liking	0.16	6.2	6.1	6.4

- Good Consumer Acceptance of Wine Style
- There was no significant difference among the three tested products

Summary

- **Ripening grapes off-vine after harvest to produce appassimento wines represents a new and exciting innovation for the Ontario wine industry**
  - overcome climatic barriers to obtaining fully ripe grapes
  - develop a unique signature wine style for Ontario.
- **Process produces full-bodied red wines of high quality and <u>consumer appeal</u>**
- **Wine flavour moderated through**
  - drying method
  - choice of fermenting yeast
  - level of botrytis infection in the fruit

Partners

VRIC

- Michael Brownbridge, Bernard Goyette, Jianbo Lu, Kimberly Cathline
- Irina Perez-Valdes (mold analysis)
- Harvest team from Cherry Ave and VRIC

Niagara College

- Terence van Rooyen, students, staff

CCOVI

- Gary Pickering, Vincenzo DeLuca, Jim Willwerth, Belinda Kemp, Debra Inglis
- Lisa Dowling (Berry sampling, analysis)
- CCOVI Harvest team
- Kyung-Hee Kim, Lisa Dowling, Tony Wang, Fei Yang, Linda Tremblay, Lynda van Zuiden (chemical analysis)
- **Fred Diprofio**, **Lisa Dowling** for wine making
- **Jen Kelly,** Ian Bock, Cristina Huber, Caitlin Heit- students

Industry

- **Pillitteri Estates Winery**
- **Cave Spring Cellars**
- **Reif Estate Winery**
- **European Planters**
- **Sunrise Greenhouses**
- **Integra (Graham Rennie)**
- **Grape Growers of Ontario**
- **Ontario Grape and Wine Research Inc**
- **Angel's Gate**

Government

- **Ontario Ministry of Research and Innovation ORF RE program**
- **Agriculture and Agrifood Canada (DIAP program)**

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Thank you

Cheers!

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