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
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
Project 1: Comparative study of 5 techniques used to dry the grapes using Cabernet franc

Cabernet franc: 5 drying regimes compared

On-vine

Barn

Drying chamber

Kiln

Greenhouse
Project 1: 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

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

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

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

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

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

Change in soluble solids for drying conditions.

<table>
<thead>
<tr>
<th>Drying Condition</th>
<th>26°Brix Drying Period (days)</th>
<th>28°Brix Drying Period (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>On-Vine</td>
<td>30</td>
<td>43</td>
</tr>
<tr>
<td>Kiln</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Barn</td>
<td>22</td>
<td>15</td>
</tr>
<tr>
<td>Greenhouse</td>
<td>22</td>
<td>12</td>
</tr>
<tr>
<td>Drying Chamber</td>
<td>22</td>
<td>-</td>
</tr>
</tbody>
</table>
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

- 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)

- Glycerol is a byproduct of botrytis
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-O-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)

<table>
<thead>
<tr>
<th>22.5 Brix</th>
<th>26 Brix</th>
<th>28 Brix</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control – Oct 18, 2013</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Seed Analysis during Appassimento drying separated by drying technique (B. Kemp laboratory)

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

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<th>22.5 Brix</th>
<th>26 Brix</th>
<th>28 Brix</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Control – Oct 18, 2013" /></td>
<td><img src="image2" alt="Drying Chamber – Dec 9, 2013" /></td>
<td><img src="image3" alt="Drying Chamber – Jan 14, 2014" /></td>
</tr>
</tbody>
</table>

Cool Climate Oenology & Viticulture Institute
Brock University
Total Extractable seed tannin in appassimento grapes during drying process before fermentation (2013, B. Kemp Laboratory)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>22.5°Brix Epicatechin (ug/ml extract)</th>
<th>26°Brix Epicatechin (ug/ml extract)</th>
<th>28°Brix Epicatechin (ug/ml extract)</th>
</tr>
</thead>
<tbody>
<tr>
<td>On-vine</td>
<td>1230 ±59 b</td>
<td>1360 ±52 a</td>
<td>1371±55 a</td>
</tr>
<tr>
<td>Kiln</td>
<td>1230 ±59 a</td>
<td>1257 ±24 a</td>
<td>1277 ±42 a</td>
</tr>
<tr>
<td>Greenhouse</td>
<td>1230 ±59 b</td>
<td>1330 ±50 a</td>
<td>1403 ±68 a</td>
</tr>
<tr>
<td>Barn</td>
<td>1230 ±59 c</td>
<td>1394 ±77 b</td>
<td>1572 ±59 a</td>
</tr>
<tr>
<td>Drying chamber</td>
<td>1230 ±59 b</td>
<td>1320 ±19 a</td>
<td>1366 ±24 a</td>
</tr>
</tbody>
</table>
Total Extractable skin tannin in appassimento grapes during drying process before fermentation (2013, B. Kemp Laboratory)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>22.5°Brix Epicatechin (ug/ml extract)</th>
<th>26°Brix Epicatechin (ug/ml extract)</th>
<th>28°Brix Epicatechin (ug/ml extract)</th>
</tr>
</thead>
<tbody>
<tr>
<td>On-vine</td>
<td>60 ±13 a</td>
<td>49 ±9 a</td>
<td>24 ±13 b</td>
</tr>
<tr>
<td>Kiln</td>
<td>60 ±13 ab</td>
<td>41 ±14 b</td>
<td>68 ±17 a</td>
</tr>
<tr>
<td>Greenhouse</td>
<td>60 ±13 a</td>
<td>33 ±6 b</td>
<td>62 ±12 a</td>
</tr>
<tr>
<td>Barn</td>
<td>60 ±13 a</td>
<td>48 ±13 a</td>
<td>43 ±17 a</td>
</tr>
<tr>
<td>Drying chamber</td>
<td>60 ±13 b</td>
<td>60 ±20 ab</td>
<td>77 ±20 a</td>
</tr>
</tbody>
</table>
## Appassimento Wines
### High Ethanol Wines

<table>
<thead>
<tr>
<th>Drying Condition</th>
<th>26°Brix Ethanol (% v/v)</th>
<th>28°Brix Ethanol (% v/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>On-Vine</td>
<td>12.6</td>
<td>15.0</td>
</tr>
<tr>
<td>Kiln</td>
<td>14.2</td>
<td>15.1</td>
</tr>
<tr>
<td>Barn</td>
<td>14.8</td>
<td>14.9</td>
</tr>
<tr>
<td>Greenhouse</td>
<td>13.3</td>
<td>15.3</td>
</tr>
<tr>
<td>Drying Chamber</td>
<td>14.4</td>
<td>-</td>
</tr>
</tbody>
</table>

- 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?
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
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 5x10⁶ cells/mL

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

Harvest Grapes (Cabernet Franc)

S. cerevisiae
EC1118

21.5°Brix (Control)
24.5° Brix
26.0° Brix
27.5° Brix

S. bayanus
Brock Isolate

21.5°Brix (Control)
24.5° Brix
26.0° Brix
27.5° Brix

REP 1  REP 1  REP 1  REP 1  
REP 2  REP 2  REP 2  REP 2  
REP 3  REP 3  REP 3  REP 3  
REP 2  REP 2  REP 2  REP 2  
REP 3  REP 3  REP 3  REP 3  
Fermentation Kinetics
How do the yeast species compare at each drying target?

Control (21.5 °Brix)

24.5 °Brix

26.0 °Brix

27.5 °Brix
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?

**Acetic Acid**

**Ethyl Acetate**

* *= $p<0.05$

** *= $p<0.01$

*** *= $p<0.001$
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

![Spider Plot](image)

Note: Aroma in lower case, Flavour in CAPS

Note: Red fruit= cooked/dried/fresh

**= p<0.05
***= p<0.001
PCA Chart
27.5 Brix
*S. cerevisiae* versus *S. bayanus*

Variables (axes F1 and F2: 63.10 %)
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????
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

Red

Black

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

Day 0

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

<table>
<thead>
<tr>
<th></th>
<th>Black Berries (healthy berries)</th>
<th>Red Berries (botrytis berries)</th>
<th>Sporulating Berries (botrytis berries)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brix</td>
<td>27.9</td>
<td>31.3</td>
<td>34.3</td>
</tr>
<tr>
<td>Glycerol (g/L)</td>
<td>0.1</td>
<td>9.3</td>
<td>11.1</td>
</tr>
<tr>
<td>Gluconic Acid (g/L)</td>
<td>0.1</td>
<td>1.5</td>
<td>1.4</td>
</tr>
</tbody>
</table>
Project 3: Impact of *Botrytis cinerea* on appassimento fruit - Sorting Team
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

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

![Graph showing solute solids (Brix) decrease over days for CONTROL and 10% BOTRYTIS-AFFECTED samples.](image-url)
### Project 3: Control vs 10% Botrytis Wine Analysis

<table>
<thead>
<tr>
<th>Wine Metabolite</th>
<th>Control</th>
<th>10% Botrytis</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>3.97 ± 0.03</td>
<td>4.01 ± 0.02</td>
</tr>
<tr>
<td>TA (g/L Tartaric Acid)</td>
<td>6.7 ± 0.0</td>
<td>6.4 ± 0</td>
</tr>
<tr>
<td>Ethanol (% v/v)</td>
<td>16.4 ± 0.2</td>
<td>16.4 ± 0.2</td>
</tr>
<tr>
<td>Residual Sugar (g/L)</td>
<td>0.17 ± 0.01</td>
<td>0.24 ± 0.02</td>
</tr>
<tr>
<td>Acetic Acid (g/L)</td>
<td>0.28 ± 0.00</td>
<td>0.35 ± 0.00</td>
</tr>
<tr>
<td>Acetaldehyde (mg/L)</td>
<td>108 ± 10</td>
<td>113 ± 8</td>
</tr>
<tr>
<td>Glycerol (g/L)</td>
<td>11.5 ± 0.4</td>
<td>12.7 ± 0.4</td>
</tr>
<tr>
<td>Gluconic Acid (g/L)</td>
<td>0.23 ± 0.02</td>
<td>0.34 ± 0.01</td>
</tr>
</tbody>
</table>
Project 3: 0 vs 10% Botrytis wines
Sensory Analysis

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. cerevisiae* - 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

<table>
<thead>
<tr>
<th>Overall Liking</th>
<th>p-value</th>
<th>S. cerevisiae 0% Botrytis</th>
<th>S. cerevisiae 10% Botrytis</th>
<th>S. bayanus 0% Botrytis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.16</td>
<td>6.2</td>
<td>6.1</td>
<td>6.4</td>
</tr>
</tbody>
</table>

- 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 consumer appeal

• 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)
Thank you

Cheers!

Brocku.ca/ccovi

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