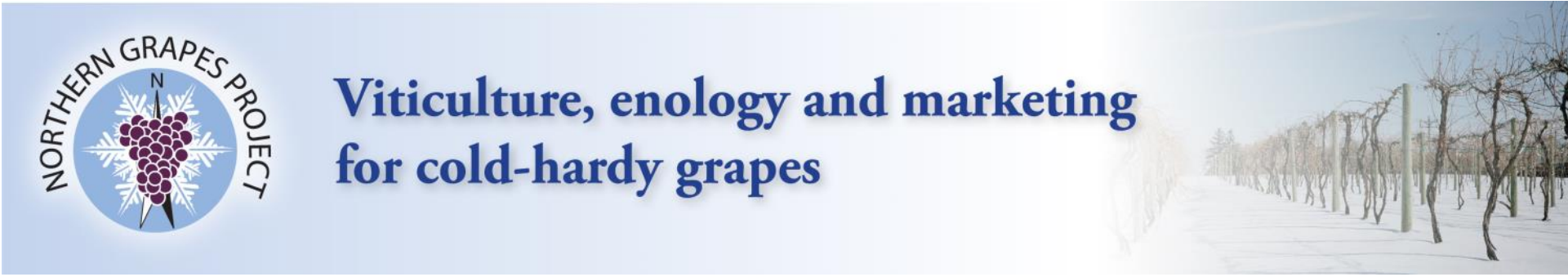



# From Vine to Glass: Understanding the Flavors and Aromas of Cold-Hardy Grapes and Wine

Anne Fennell

Plant Science Dept.

South Dakota State University

The Northern Grapes Project is funded by the USDA's Specialty Crops Research Initiative Program of the National Institute for Food and Agriculture, Project #2011-51181-30850 www.northerngrapesproject.org

**Comprehensively track what genes are activated, what metabolites are produced, and what sensory descriptors develop (SDSU, UMN, ISU)**

**Frontenac, Marquette, Frontenac Gris, Brianna**

**Analyses:**

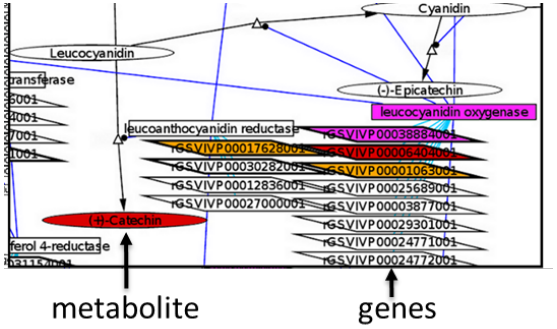
**gene expression (transcriptome)**

**volatile compounds (aroma)**

**primary metabolites (sugars, and organic and amino acids)**

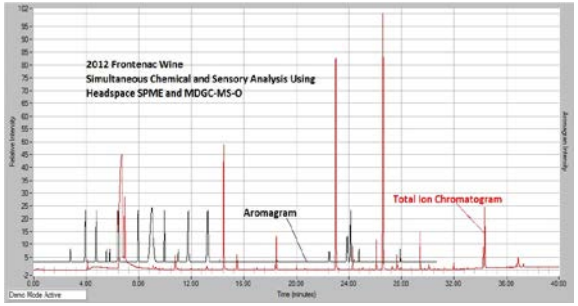
**secondary metabolites (tannins, flavonoids, anthocyanins, stilbenes, other phenolics, and terpenoids)**

**Sensory (berry and wine)**



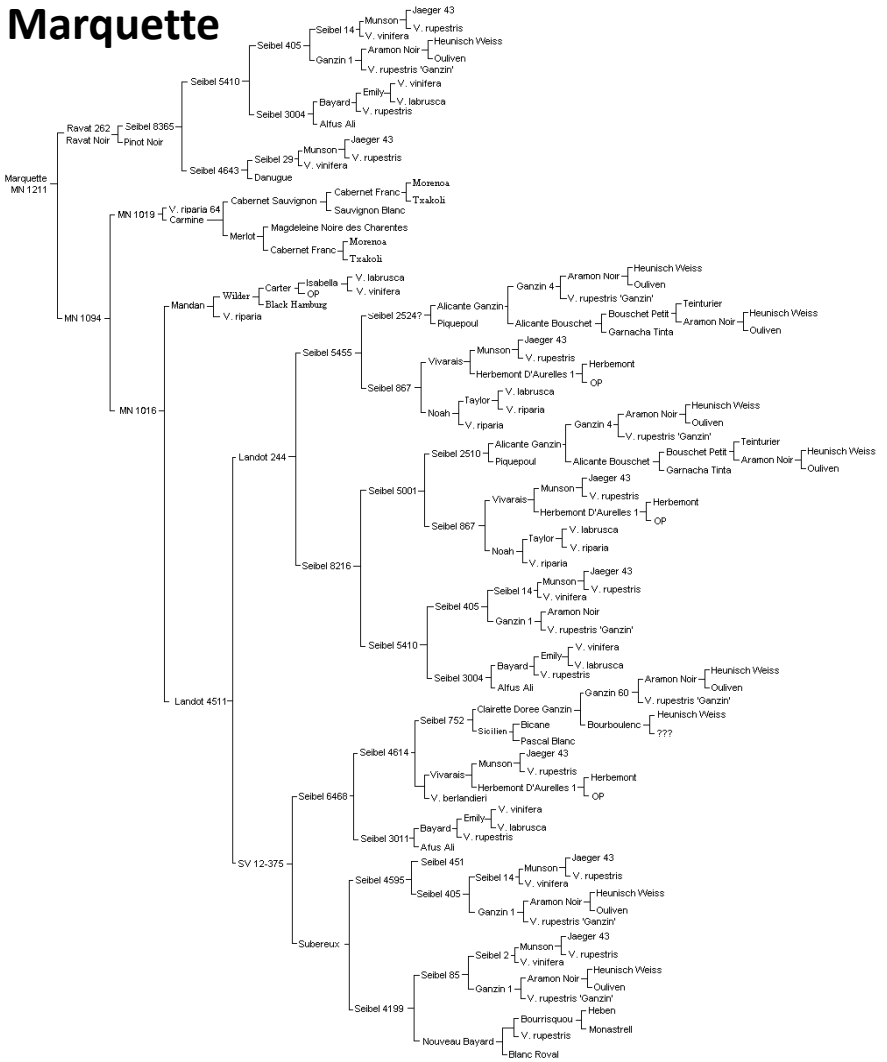






# Marquette and Frontenac

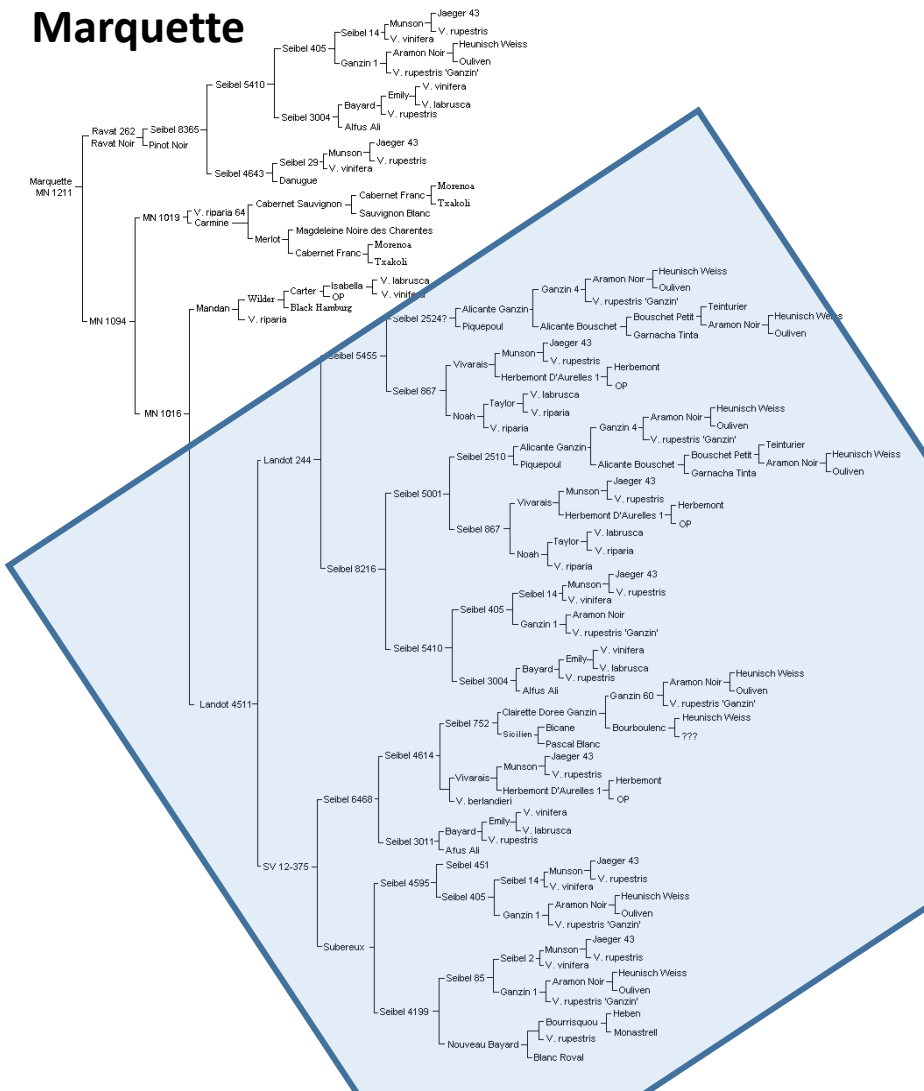
- **Marquette and Frontenac have some of their pedigree in common**



# Marquette and Frontenac

- **Marquette**
- **Frontenac (*V. riparia* x Landot 4511)**

**Pedigree in common between Frontenac (parent) and Marquette (great grandparent)**



# From the Genome to Your Senses

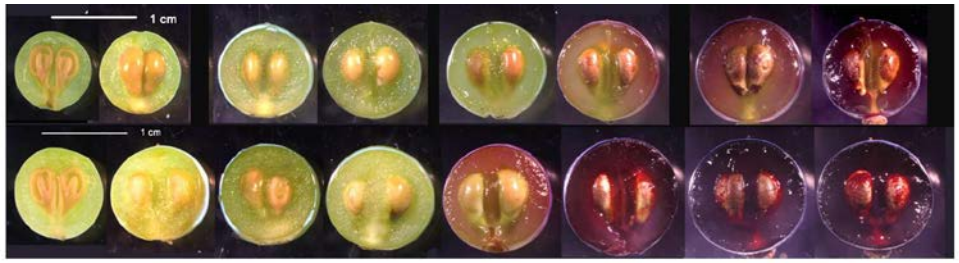



Fig. 1 Change in berry size, seed maturation and pulp pigmentation during ripening in Frontenac and Marquette.

## From veraison to harvest:

1. What genes are activated?
2. What metabolites (chemicals) are produced?
3. What sensory descriptors develop?
4. How do genes, metabolites and sensory descriptors correlate?

# Marquette and Frontenac

**Physical and molecular characterization of berry development and ripening**

- **Veraison**
- **20˚Brix**
- **22˚Brix***
- **24˚Brix***
- **26˚Brix***

***Sensory analysis of berry & wine**

- **Berries collected weekly- Brix, pH, TA**
- **Whole berries- sensory and aroma analysis**
- **Berries separated into skin and pulp – gene expression and metabolite analysis**
- **Wine**

**Berry Samples**
**20 to 26°Brix**

↓

# Transcriptome

**(gene expression)**

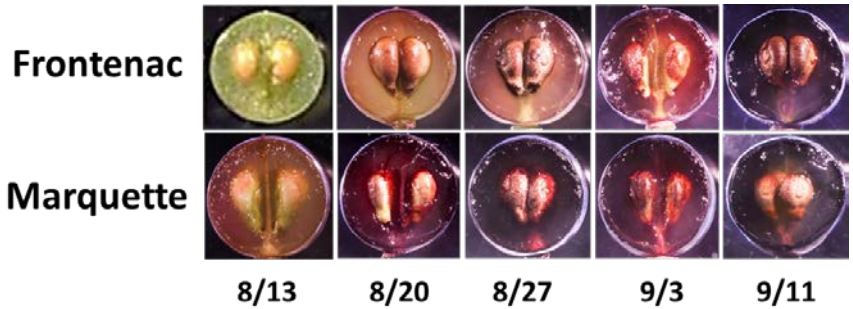
↓

# Metabolome

**(sugars, acids, flavors, aromas anthocyanins, tannins)**

↓

# Sensory descriptors



**Berry Samples**
**20 to 26°Brix**

↓

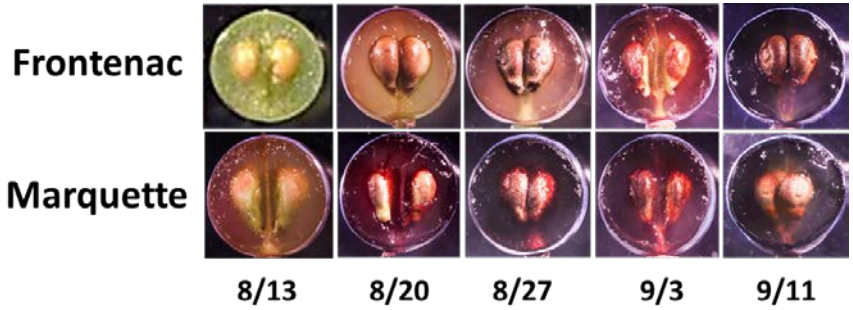
**Transcriptome**
**(genes)**

↓

**Metabolome**
**(sugars, acids, flavors, aromas anthocyanins, tannins)**

↓

**Sensory descriptors**




- Continuous changes in genes expressed during ripening.
- Greater number of genes with increasing or decreasing expression in the berry skin than in the pulp

Berry Samples
20 to 26°Brix

↓

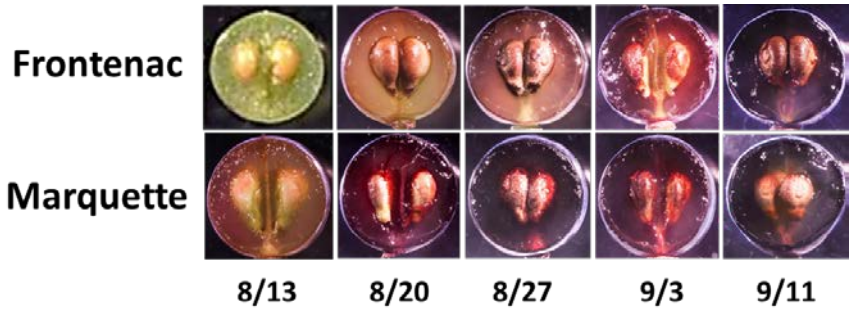
**Transcriptome**
(genes)

↓

**Metabolome**
(sugars, acids, flavors, aromas
anthocyanins, tannins)

↓

**Sensory descriptors**




A. Number of genes expressed differently between skin and pulp of Marquette or Frontenac at harvest
B. Number of gene expressed differently between skin and pulp across both cultivars at harvest

Berry Samples
20 to 26°Brix

↓

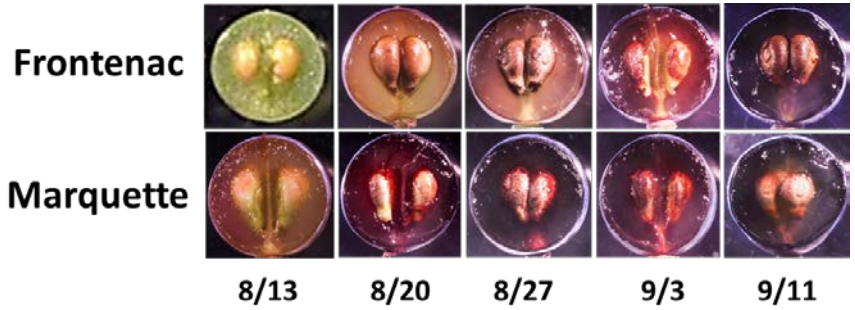
**Transcriptome**
(genes)

↓

**Metabolome**
(sugars, acids, flavors, aromas
anthocyanins, tannins)

↓

**Sensory descriptors**




- Color development: anthocyanin biosynthesis
- Aroma development: terpenoid biosynthesis; terpenes contribute to scent, flavor and color

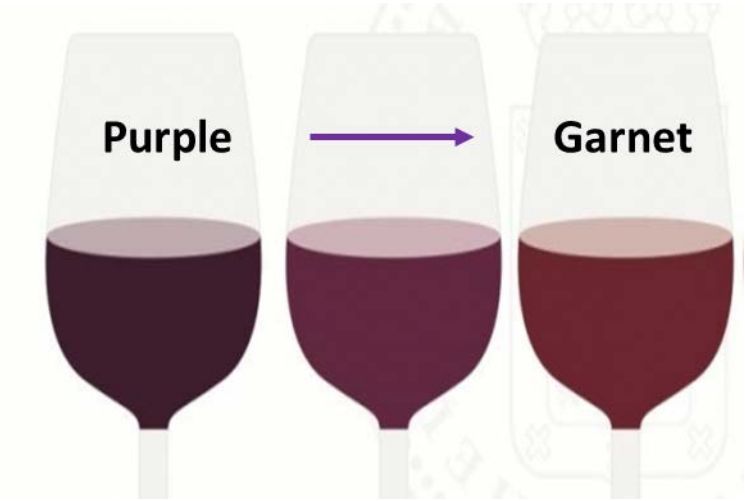
# Genomics and Fruit Composition: Link Genetics to Sensory (SDSU, UMN, IA State)

**Do gene expression differences conincide with existing knowledge?**

- Anthocyanin biosynthesis genes preferentially expressed in berry skins
- Expression of anthocyanin biosynthesis genes significantly greater expression in Frontenac than Marquette

| | Front Skin | Marq Skin |
|---|---|---|
| VIT_05s0062g00720 | 36 | |
| VIT_12s0034g00080 | 47 | |
| VIT_02s0033g00450 | 159 | 42 |
| VIT_02s0033g00390 | 165 | 84 |
| VIT_05s0049g01020 | 429 | 120 |
| VIT_02s0033g00380 | 531 | 219 |

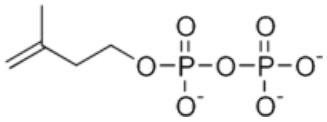
**Several anthocyanin biosynthesis genes highly expressed in Frontenac in comparison to Marquette**



# Genomics and Fruit Composition: Link Genetics to Sensory (SDSU, UMN, IA State)

## Are differences in gene expression related to aroma or flavor?

- > number of gene expression differences in the berry skins than the berry pulp
- Cultivar specific expression patterns for terpenoid (aroma) biosynthesis genes



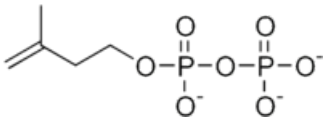
**Terpenoid biosynthesis gene expression**

| | Front Skin | Marq Skin |
|---|---|---|
| VIT_12s0134g00030 | 0.0 | 8.5 |
| VIT_06s0004g06480 | 0.1 | 2.0 |
| VIT_17s0000g05580 | 0.2 | 1.4 |
| VIT_01s0010g02320 | 0.5 | 2.5 |
| VIT_13s0067g00380 | 0.6 | 0.1 |
| VIT_15s0046g03600 | 0.8 | 3.5 |
| VIT_13s0067g00370 | 0.8 | 0.2 |
| VIT_19s0135g00200 | 1.9 | 4.9 |
| VIT_00s0253g00140 | 1.9 | 0.4 |
| VIT_19s0135g00190 | 2.4 | 5.2 |
| VIT_05s0049g00400 | 3.6 | 1.4 |
| VIT_15s0046g03570 | 3.7 | 1.2 |
| VIT_08s0032g00240 | 5.0 | 2.4 |
| VIT_15s0021g01060 | 6.7 | 3.2 |
| VIT_11s0016g01290 | 7.4 | 1.2 |
| VIT_19s0015g02500 | 9.1 | 1.9 |
| VIT_17s0000g09610 | 13.3 | 1.7 |
| VIT_15s0048g01490 | 22.3 | 4.8 |
| VIT_02s0025g04880 | 119.5 | 35.3 |

# Genomics and Fruit Composition: Link Genetics to Sensory (SDSU, UMN, IA State)

**Are differences in gene expression related to aroma or flavor?**

- **> number of gene expression differences in the berry skins than the berry pulp**
- **Cultivar specific expression patterns for terpenoid (aroma) biosynthesis genes**



**Terpenoid biosynthesis gene expression**

**Marquette E-beta-ocimene synthase: Terpy, woody green, vegetable nuances**

| | | |
|---|---|---|
| VIT_13s0067g00380 | 0.6 | 0.1 |
| VIT_15s0046g03600 | 0.8 | 3.5 |
| VIT_13s0067g00370 | 0.8 | 0.2 |
| VIT_19s0135g00200 | 1.9 | 4.9 |
| VIT_00s0253g00140 | 1.9 | 0.4 |
| VIT_19s0135g00190 | 2.4 | 5.2 |
| VIT_05s0049g00400 | 3.6 | 1.4 |

**Frontenac Pinene synthase: eucalyptus and camphoraceous note with a spicy peppery and nutmeg nuance**

| | | |
|---|---|---|
| VIT_02s0025g04880 | 119.5 | 35.3 |

# Genomics and Fruit Composition:

- **Distinct cultivar differences exist in gene expression patterns**
- **Differences in expression of genes related to aroma and flavor are found between Marquette and Frontenac.**
- **Results will be correlated with volatile compound and other metabolites.**

# Viticulture, enology and marketing for cold-hardy grapes

**Kalley Besler, Padmapriya Swaminathan, Anne Fennell – SDSU**

**Somchai Rice, Jacek Koziel, Murli Dharmadhikari, Devin Maurer – ISU**

**Emily Del Bel, Soon Li Teh, Bety Rostandy, Jenna Brady, Zata Vickers, Adrian Hegeman, Jim Luby – UMN**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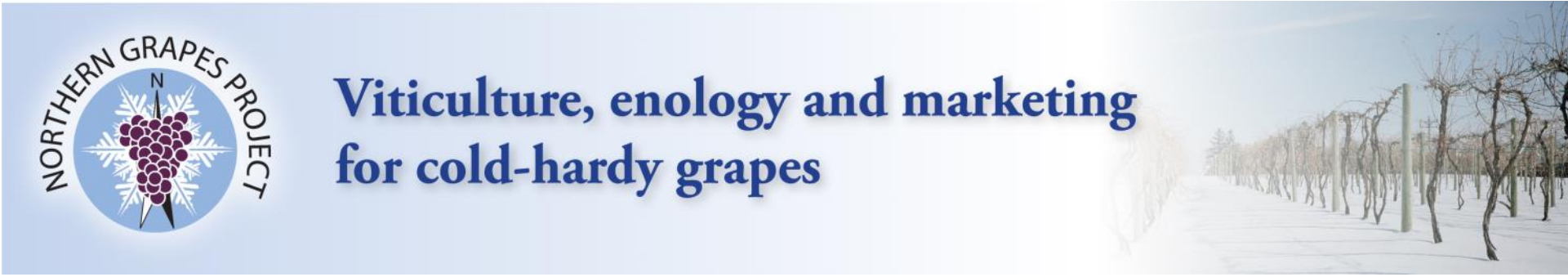


# From Vine to Glass: Understanding the Flavors and Aromas of Cold-Hardy Grapes and Wine

Somchai Rice
Agricultural and Biosystems Engineering
Toxicology
Iowa State University

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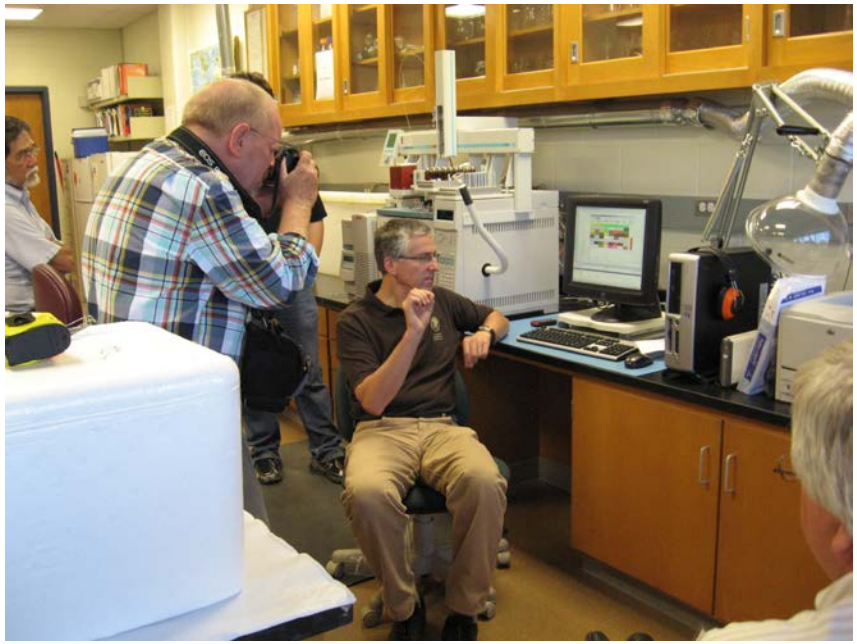
- 1 cm stationary phase fused to silica fiber
- Bonded to stainless steel plunger
- Holder operates like microliter syringe

- Analytes adsorb/absorb to the coating
- Fiber is then thermally desorbed into GC inlet, and is cleaned for re-use

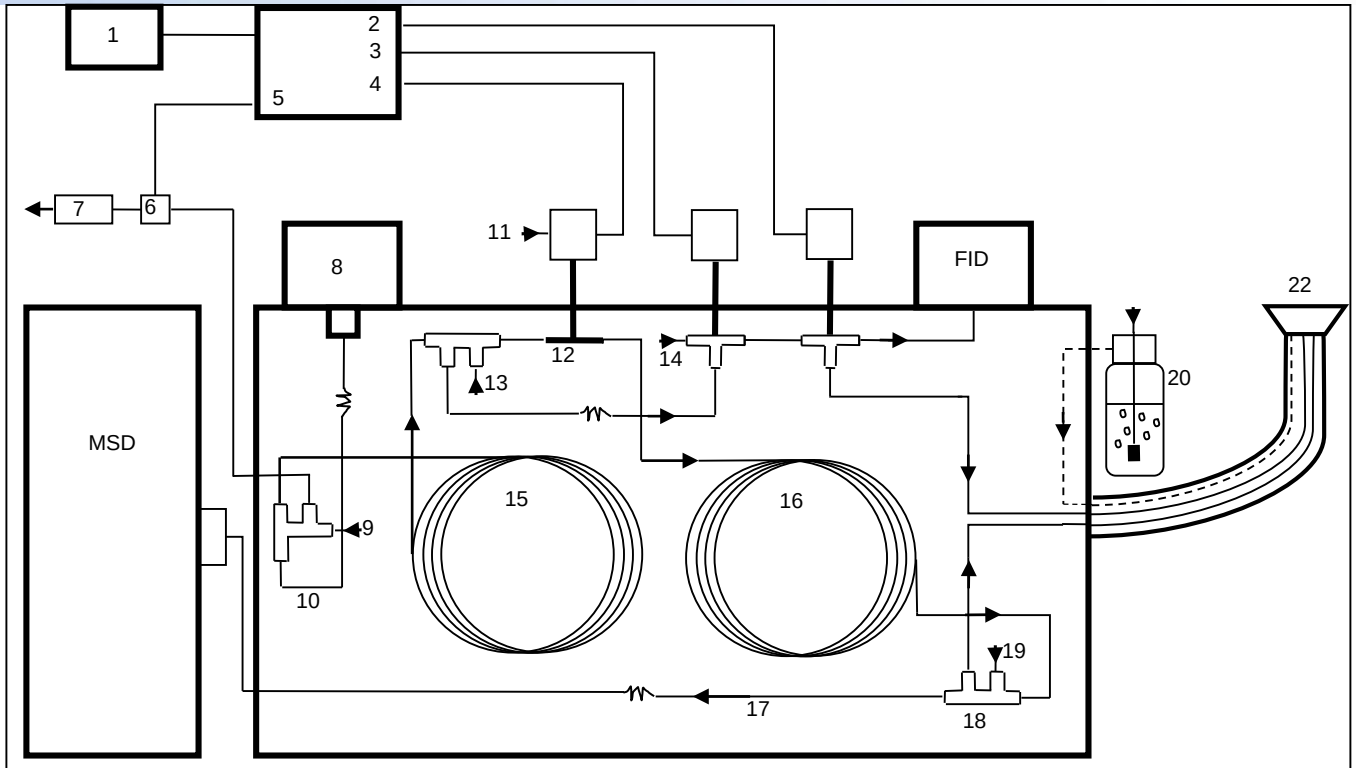
**Solid phase microextraction (SPME) fiber**

# Chemical and Sensory profile analysis (ISU)



**Multidimensional gas chromatography – mass spectrometry – olfactometry (MDGC-MS-O)**
Left to Right: Paul Domoto (ISU), Timothy Martinson (Cornell), Jason Vallone (ISU), Jacek Koziel (ISU). Photo by Somchai Rice

# Chemical and Sensory profile analysis (ISU)




Notes:

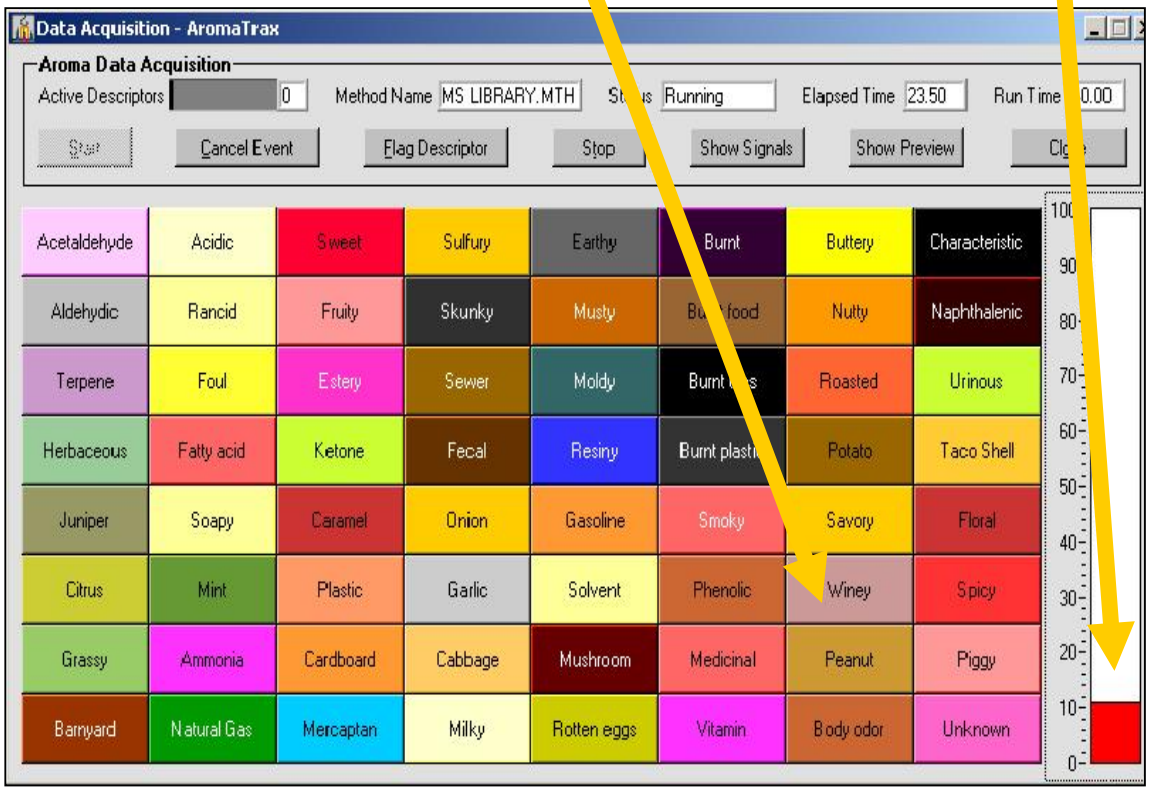
1: MultiTrax Controller
2: Precolumn Sniff Port Selector
3: Heartcut Valve
4: $CO_2$ Cryotrap
5: Precolumn Backflush
6: Solenoid
7: Filter
8: Injector
9: Backflush Sweep
10: Fixed Restrictor to Inlet
11: Liquid $CO_2$ Feed
12: $CO_2$ Cryotrap
13: Midpoint Pressure
14: Heartcut Sweep
15: Non-Polar column
16: Polar Column
17: Fixed Restrictor to MSD
18: Open Split Interface (OSI)
19: OSI Sweep
20: Humidifier
21: Air in
22: Sniff Port

**Schematic of MDGC-MS-O (MOCON, Round Rock, TX)**

# Chemical and Sensory profile analysis (ISU)

Odor Character

Odor Intensity




**Aroma descriptor of AromaTrax (MOCON, Round Rock, TX)**

# Chemical and Sensory profile analysis (ISU)

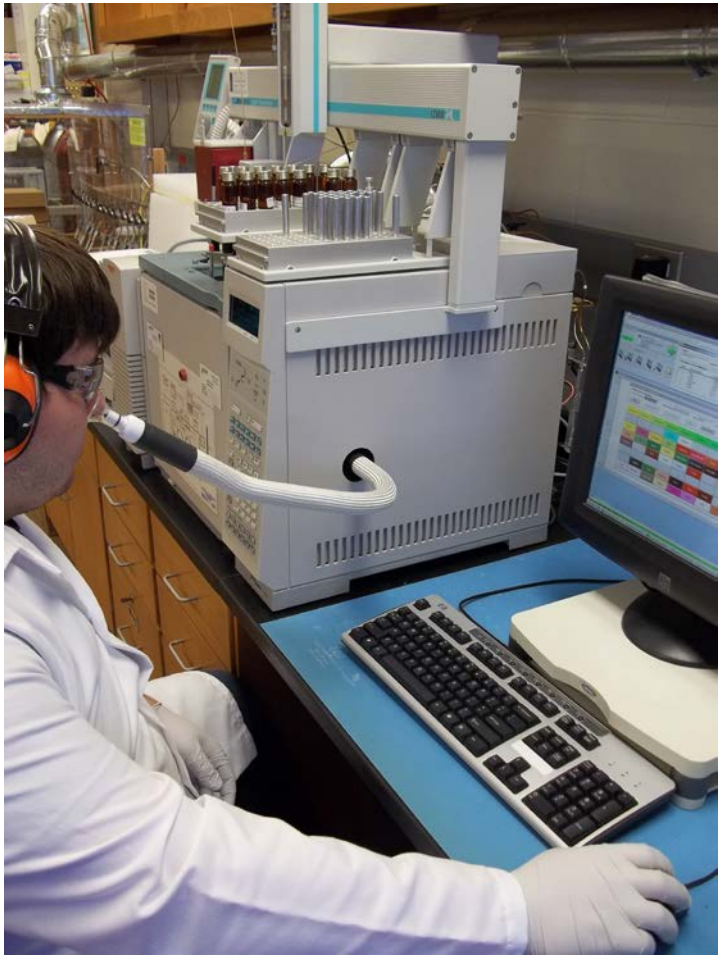
Marquette cluster in polyvinyl film (Tedlar) enclosure with stainless steel cage and solid phase microextraction (SPME) port.  Photo by Somchai Rice.

Teflon SPME port with teflon backed septum. PDMS/DVB SPME extraction, exposed fiber, 30 minutes at ambient temperature. Photo by Somchai Rice.

# Chemical and Sensory profile analysis (ISU)

Modified 2 mL glass vial with septa, held by negative pressure. Photo by Somchai Rice

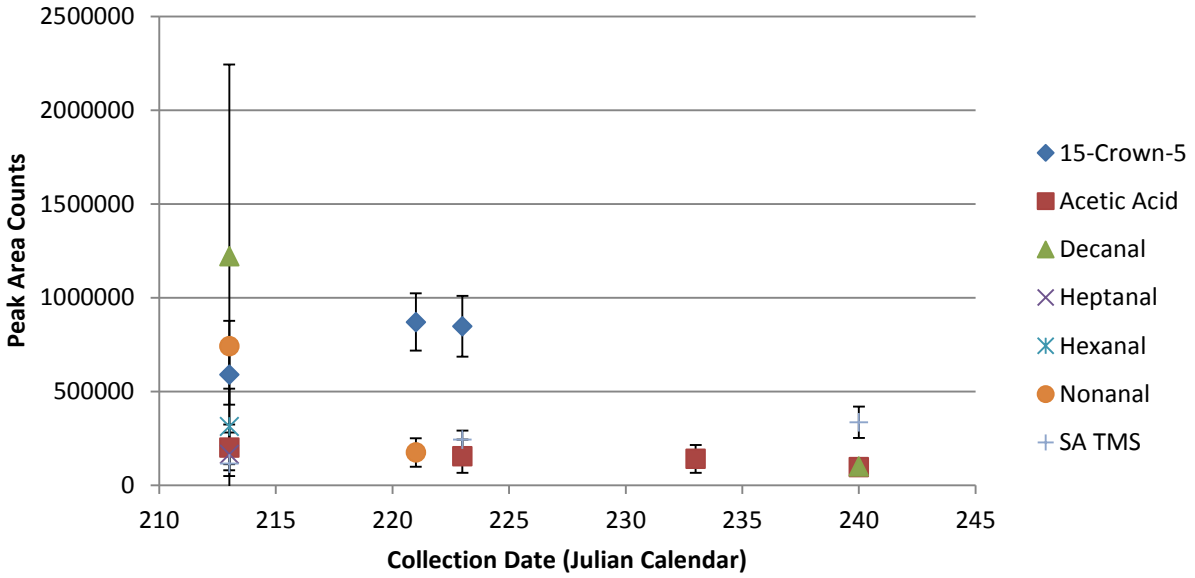
# Chemical and Sensory profile analysis (ISU)



**MDGC-MS-O** Jason Vallone analyses in vivo gas emissions from Frontenac grape clusters at 24$^{o}$ Brix. Photo by Somchai Rice

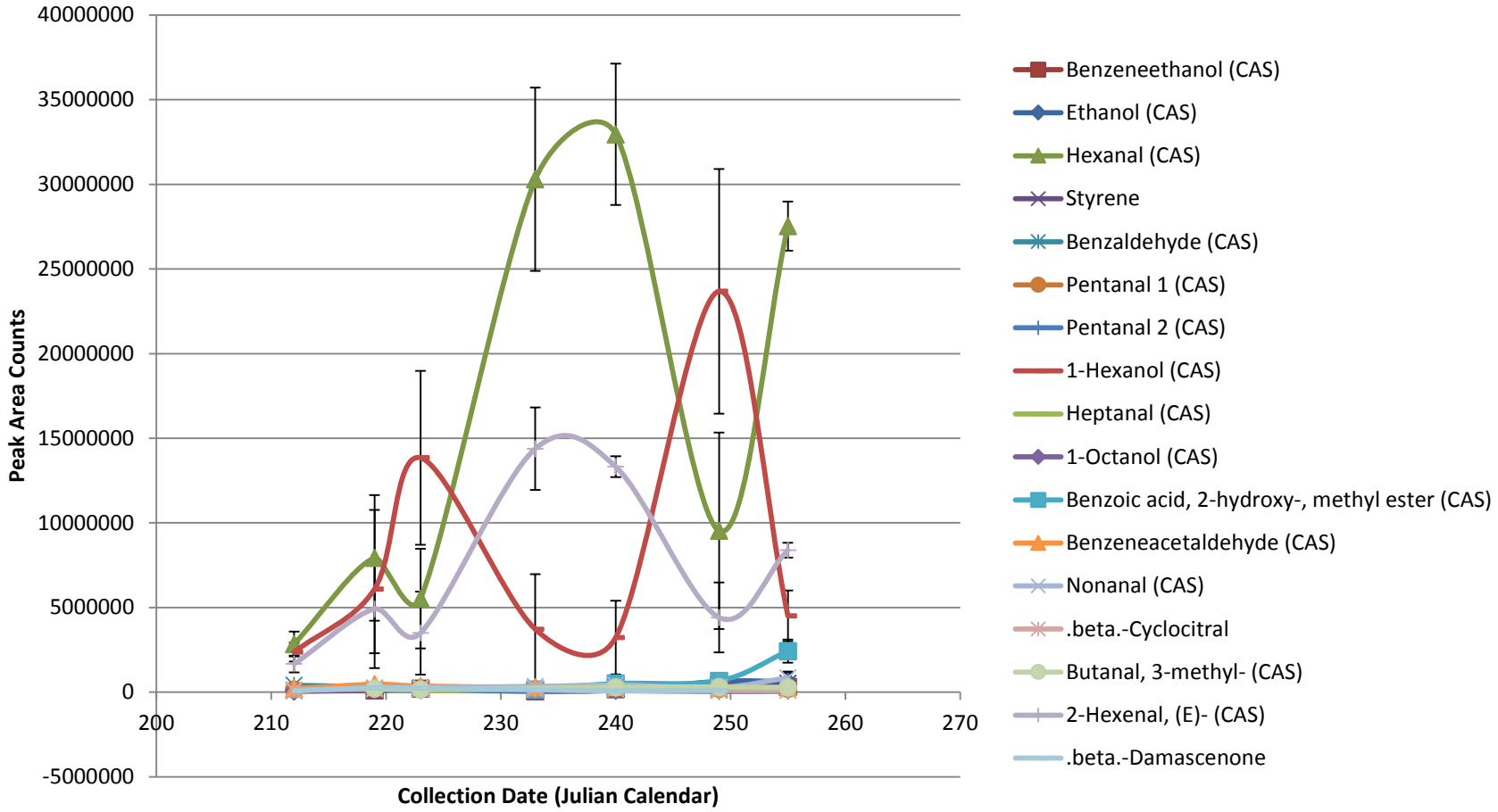
# Chemical and Sensory profile analysis (ISU)

## South Dakota State University Marquette 2012
## In-Vivo Target VOCs from Veraison to Harvest



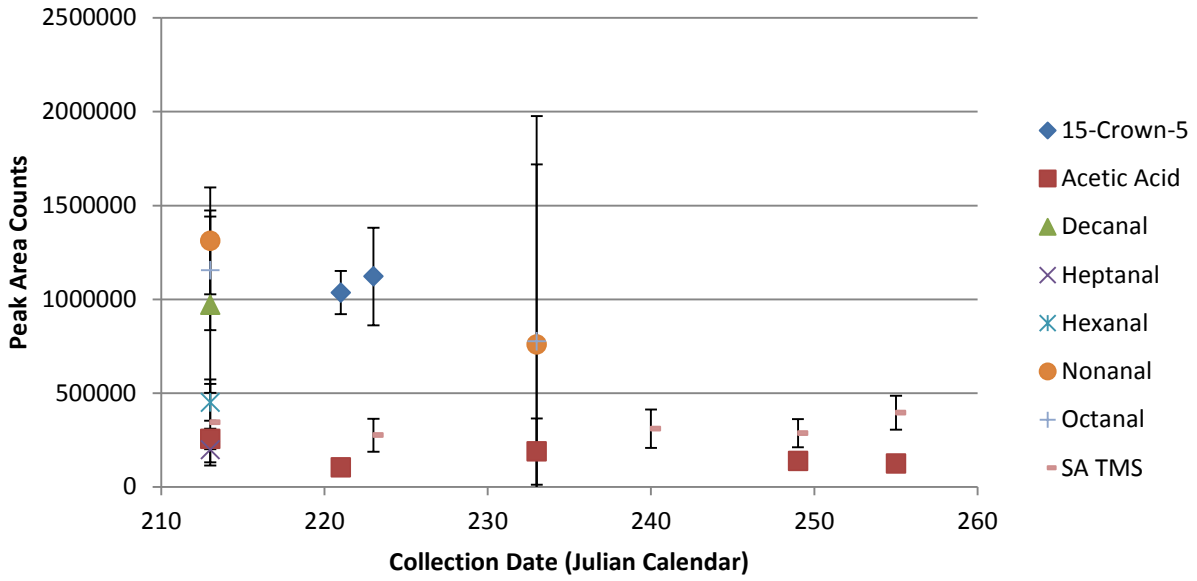
# Chemical and Sensory profile analysis (ISU)

## Total Target VOCs from crushed berries - SDSU Marquette 2012



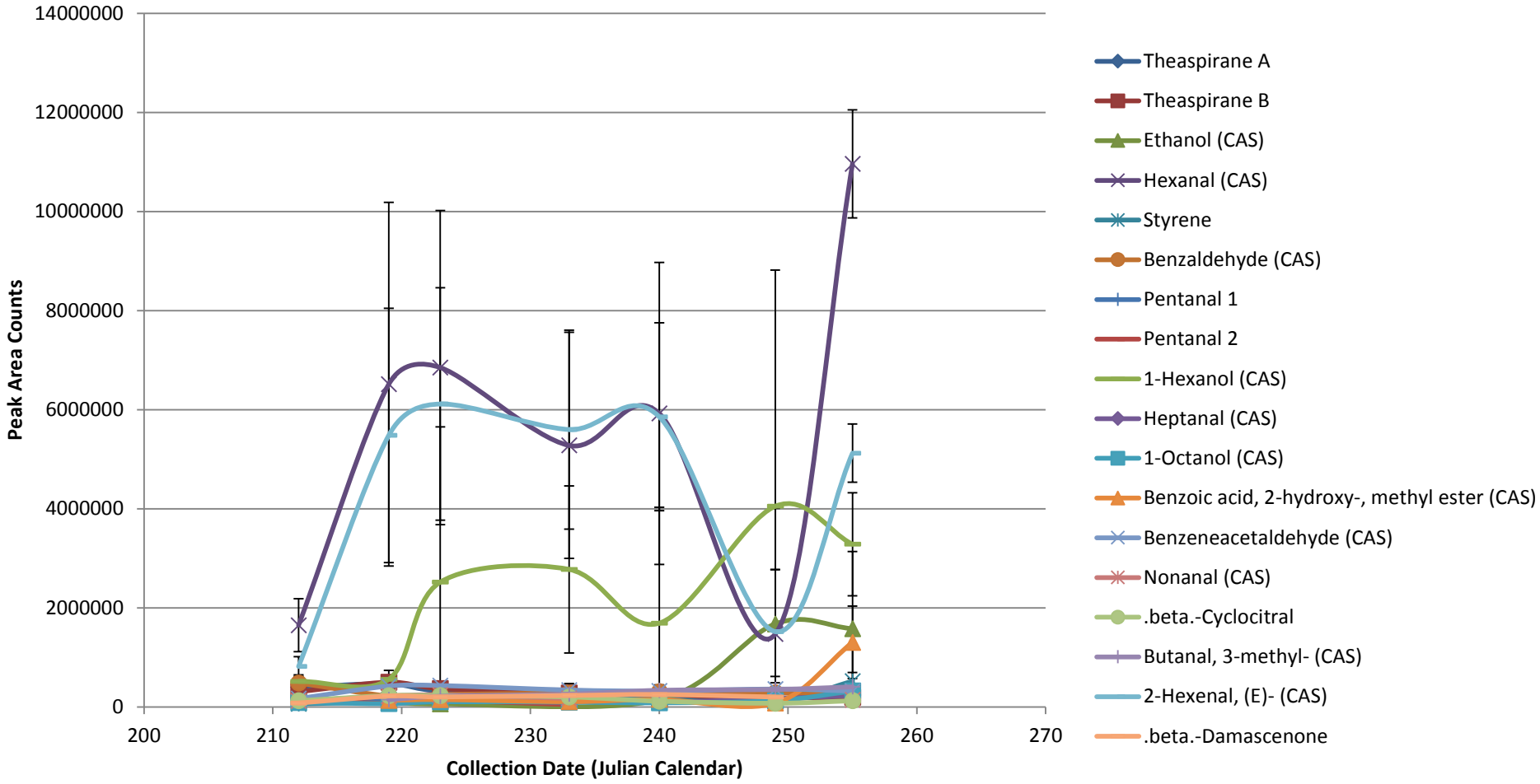
# Chemical and Sensory profile analysis (ISU)

## South Dakota State University Frontenac 2012
## In-Vivo Target VOCs from Veraison to Harvest



# Chemical and Sensory profile analysis (ISU)

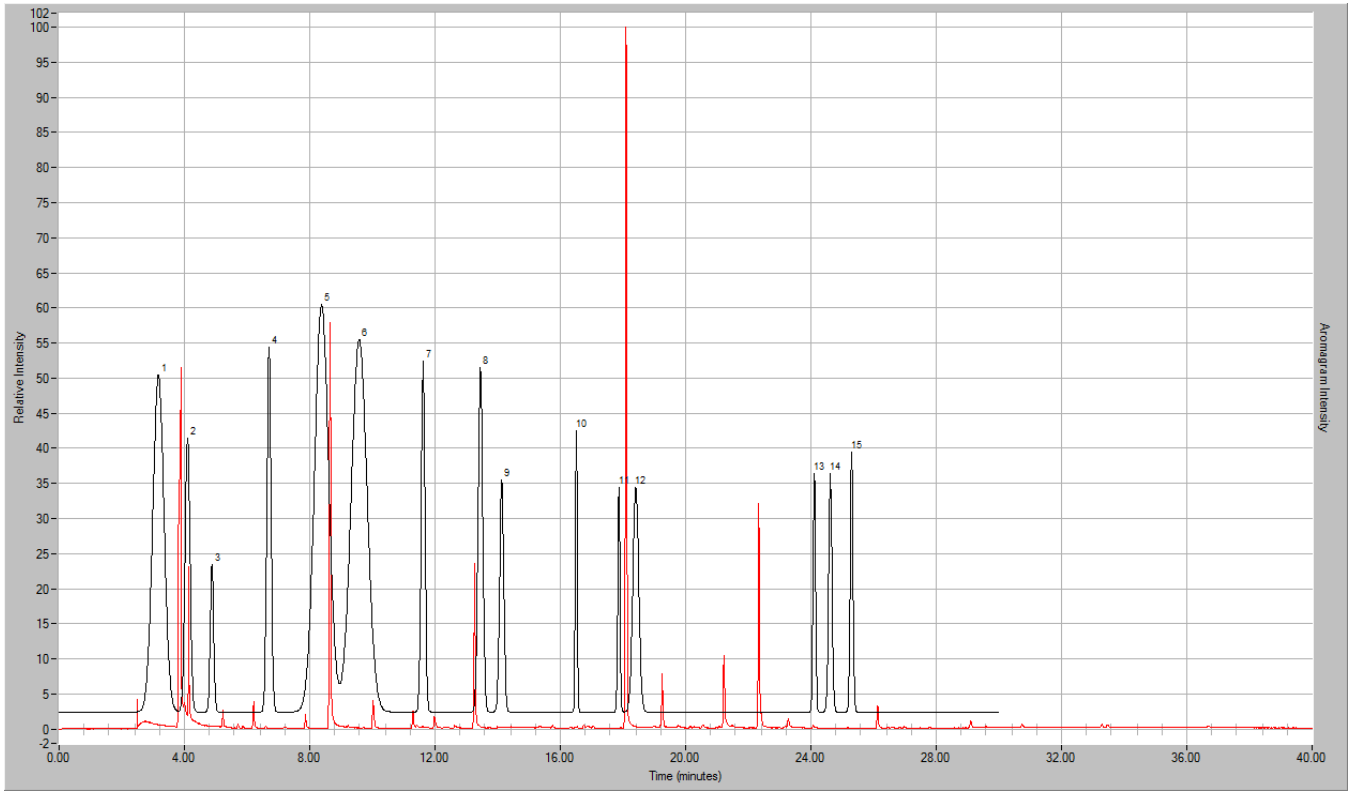
## Total Target VOCs from crushed berries - SDSU Frontenac 2012



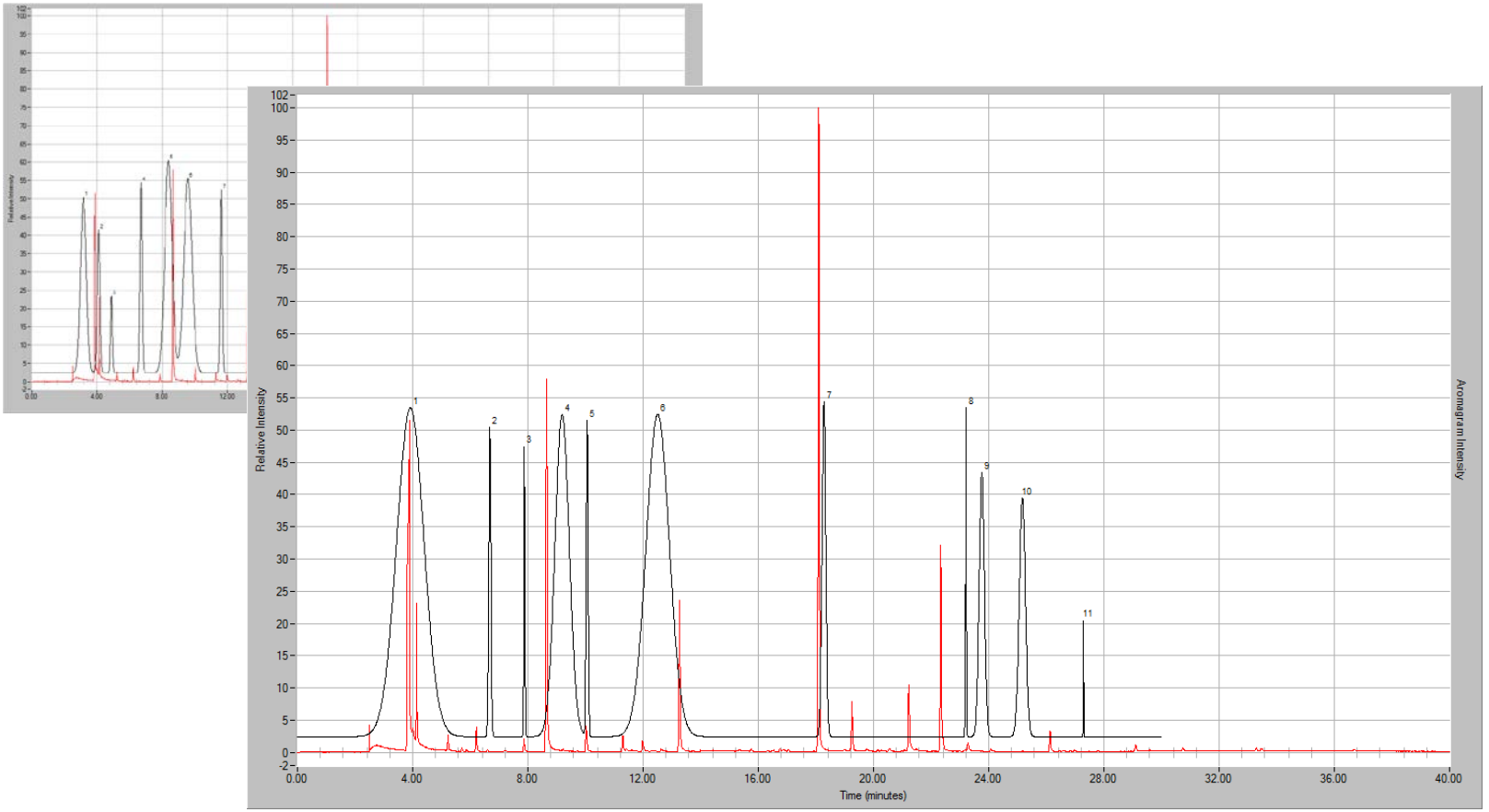
## Chemical and Sensory profile analysis (ISU)

- Aroma dilution analysis
- Successive dilutions of wine samples were analyzed to determine which compounds were most impactful in total aroma
- An automated SPME MDGC-MS-O method was developed

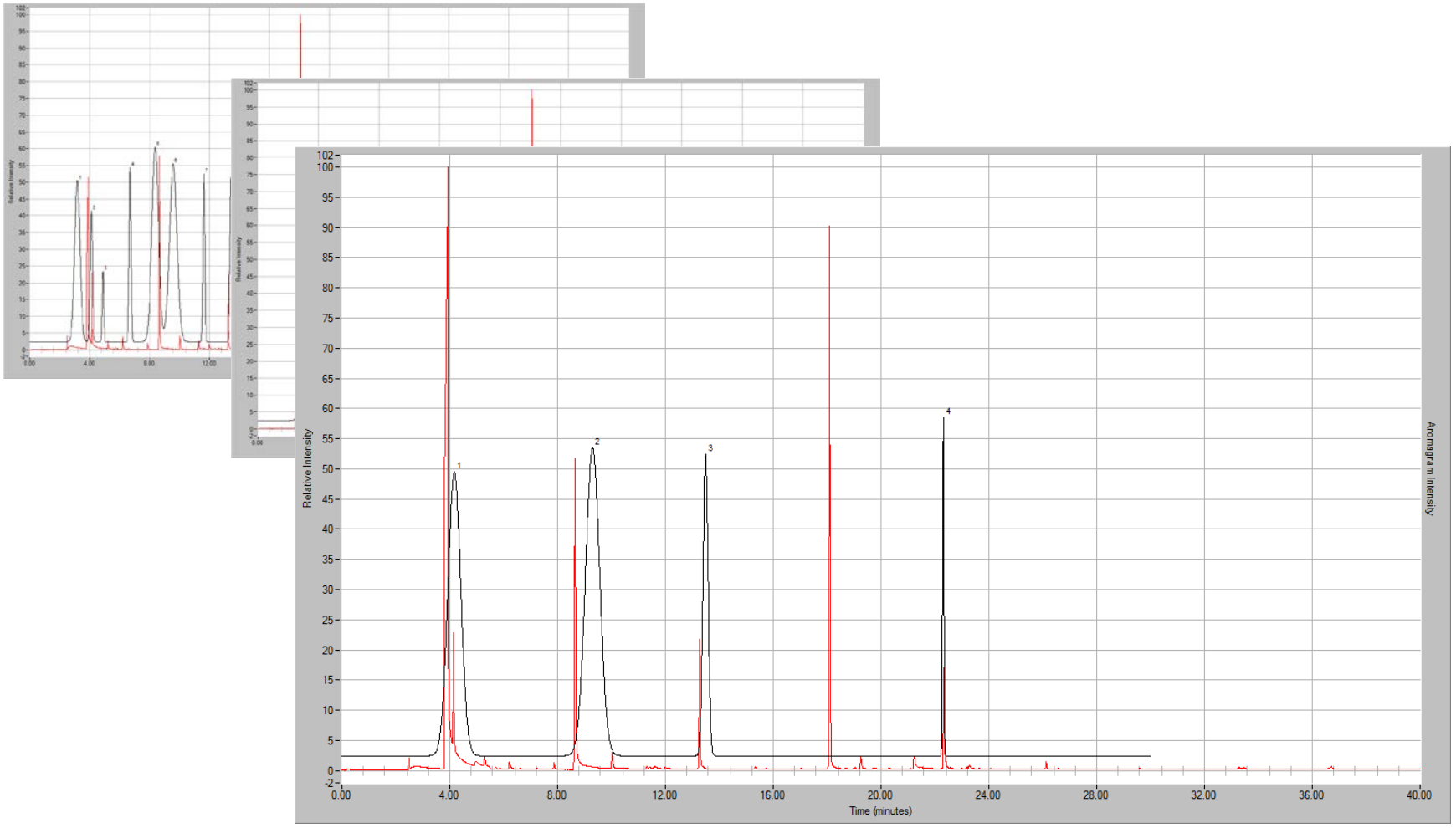
# Chemical and Sensory profile analysis (ISU)



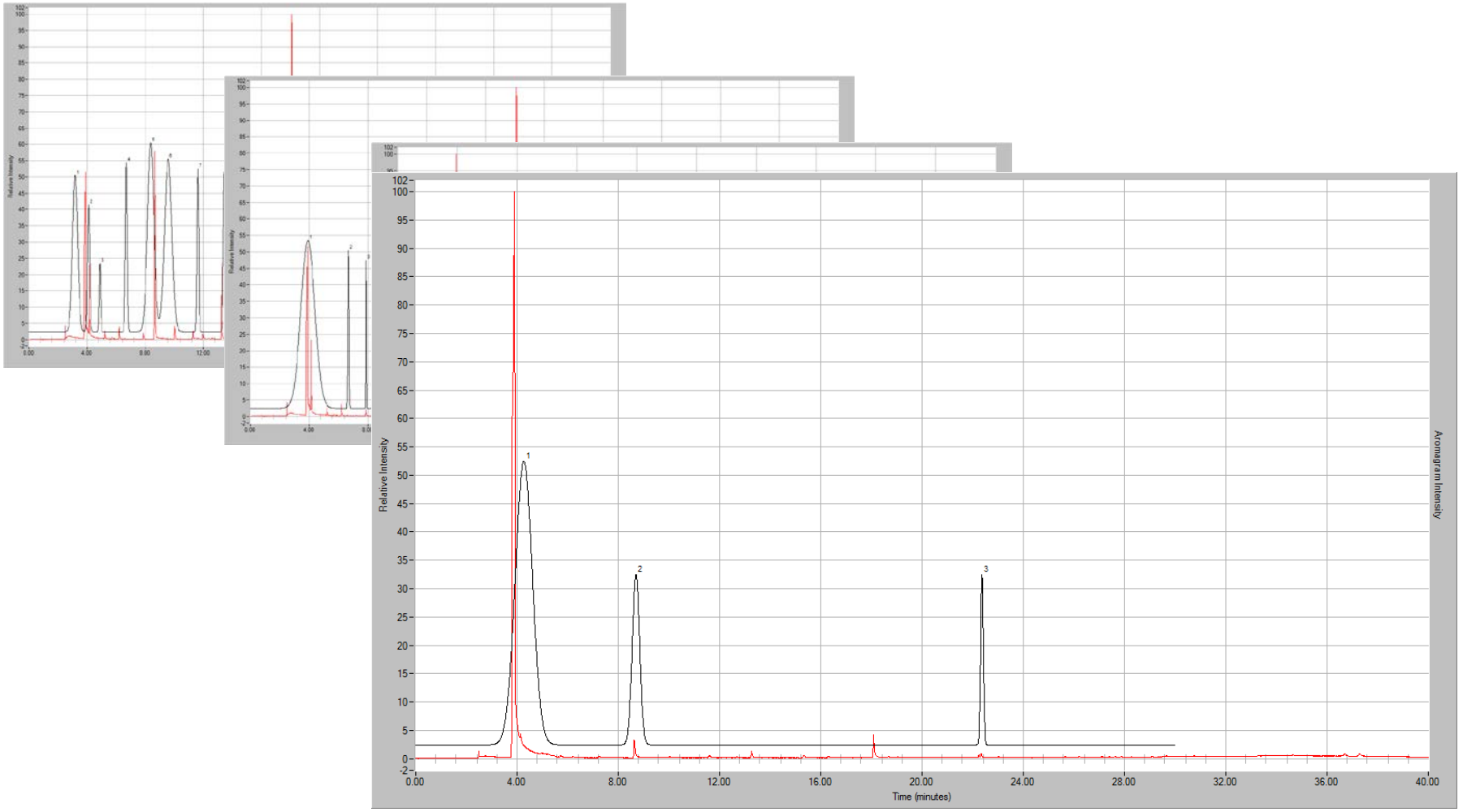
# Chemical and Sensory profile analysis (ISU)



# Chemical and Sensory profile analysis (ISU)



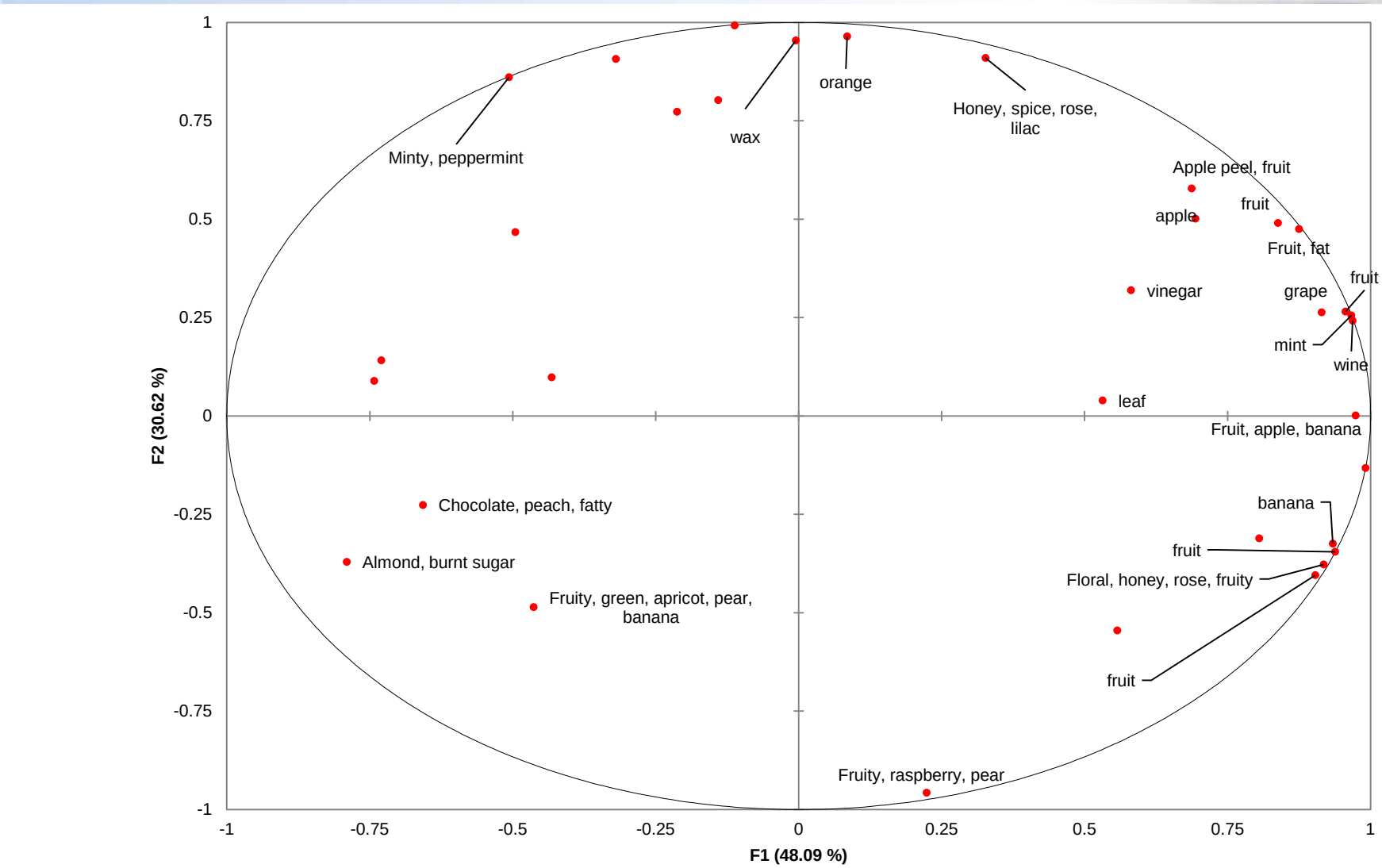
# Chemical and Sensory profile analysis (ISU)



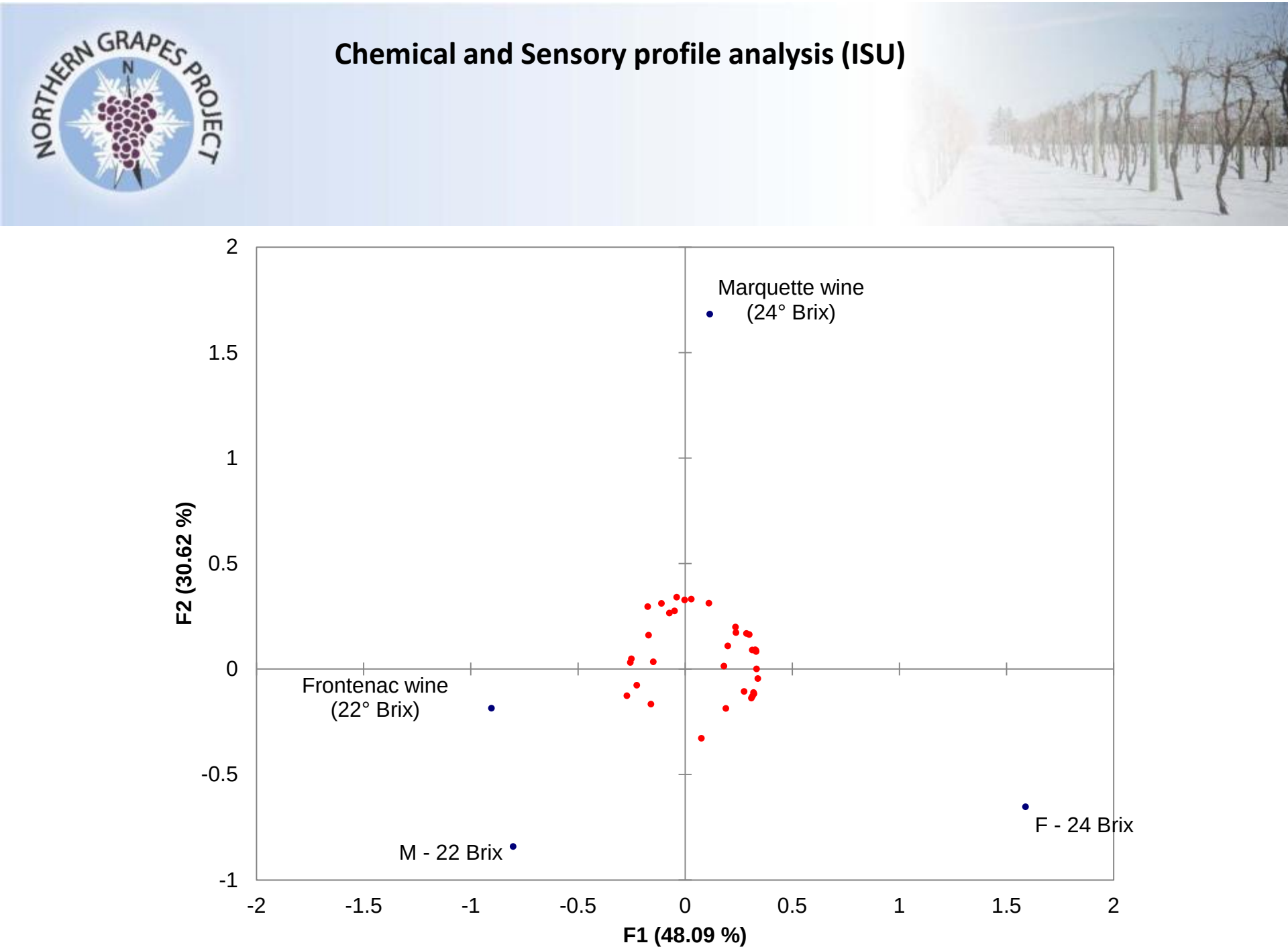
## Chemical and Sensory profile analysis (ISU)

- Marquette wine aromas developed from ‘wine, apple and fruity’ to ‘cheesy, chocolate, and strawberry’.
- ‘Chocolate and molasses’ aroma intensified, and ‘jam’ aroma was developed in Frontenac wine as sugar levels increased.

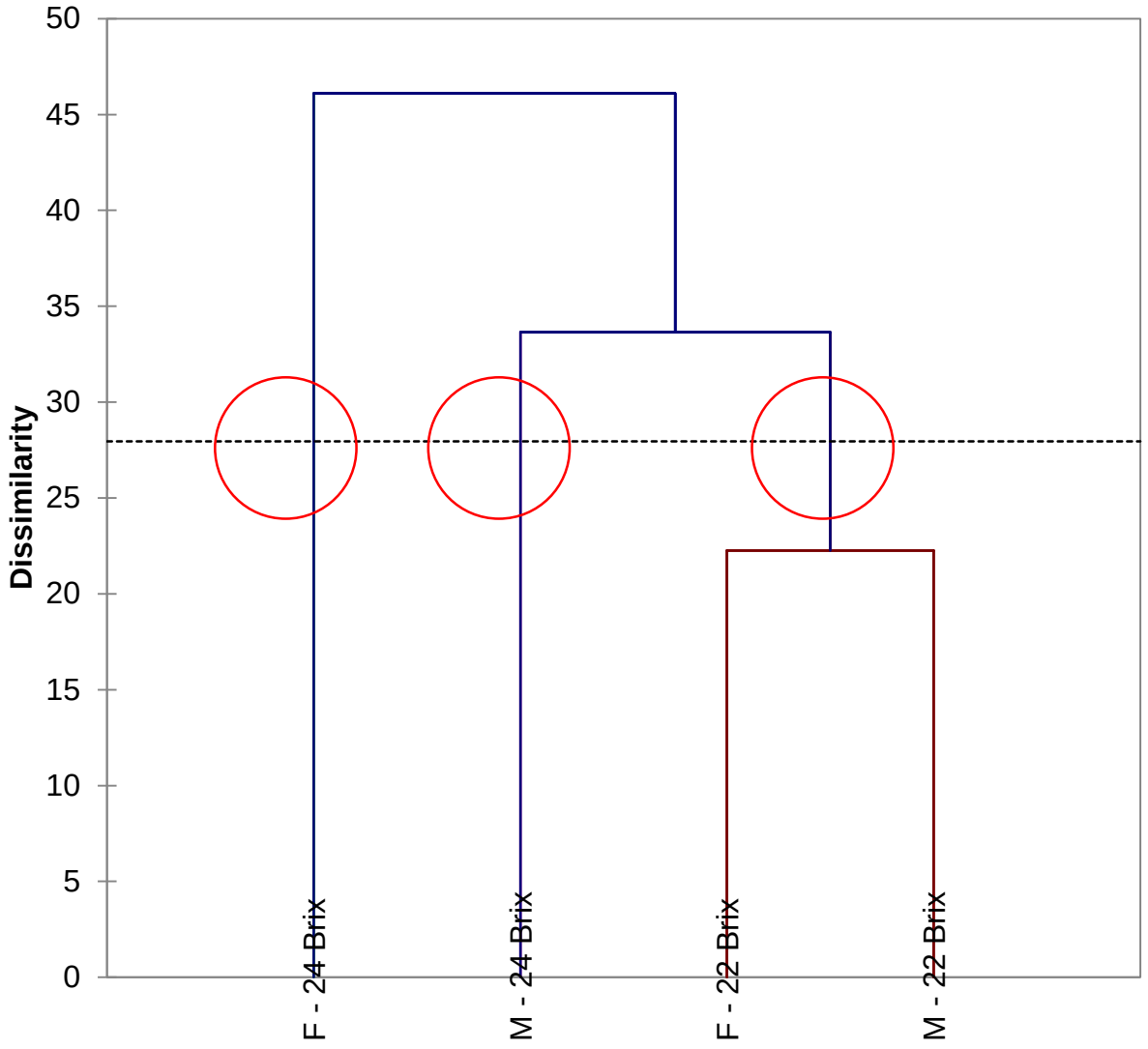
# Chemical and Sensory profile analysis (ISU)



# Chemical and Sensory profile analysis (ISU)



# Chemical and Sensory profile analysis (ISU)



## Chemical and Sensory profile analysis (ISU)

- The VOCs of Marquette and Frontenac wine made from berries harvested at 24° Brix are unique from each other and from Marquette and Frontenac wine made from berries harvested at 22° Brix.
- The VOCs of Marquette and Frontenac wine made from berries harvested at 22° Brix are most similar to each other.
- Similar work is being done on skin contact studies of La Crescent and Edelweiss wines, and harvest times of Brianna and Frontenac Gris wines.

# Viticulture, enology and marketing for cold-hardy grapes

**Kalley Besler, Padmapriya Swaminathan, Anne Fennell – SDSU**

**Somchai Rice, Jacek Koziel, Murli Dharmadhikari, Devin Maurer, Jason Vallone, Nanticha Lutt – ISU**

**Emily Del Bel, Soon Li Teh, Bety Rostandy, Jenna Brady, Zata Vickers, Adrian Hegeman, Jim Luby – UMN**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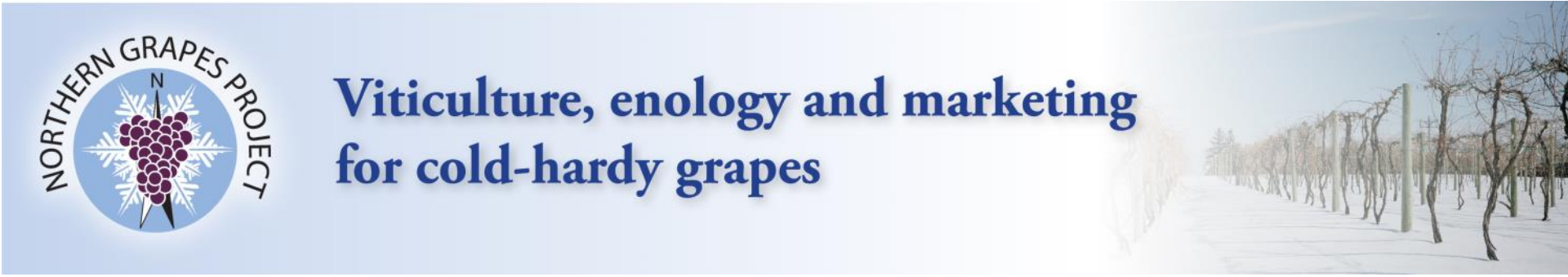



# From Vine to Glass: Understanding the Flavors and Aromas of Cold-Hardy Grapes and Wine … (Part 3!)

Adrian Hegeman

Departments of Horticultural Science and Plant Biology

University of Minnesota

The Northern Grapes Project is funded by the USDA's Specialty Crops Research Initiative Program of the National Institute for Food and Agriculture, Project #2011-51181-30850
www.northerngrapesproject.org

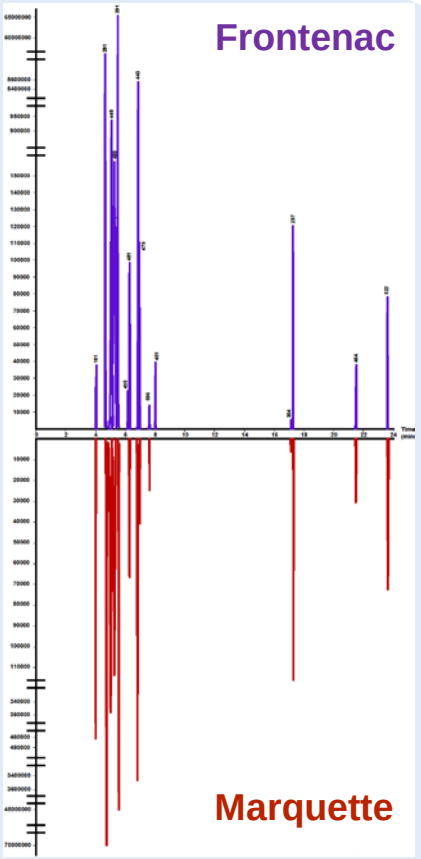
# Fruit Composition & Metabolomics

Soon Li Teh, Bety Rostandy,
Adrian Hegeman & Jim Luby – **UMN**

**Goal:**

**Identify genes that can be used as markers for selection of desirable aroma, pigmentation, organoleptic trait metabolites (and removal of undesirable traits) via marker assisted breeding**


Cultivars



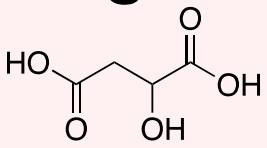

Ripening profiles

The Northern Grapes Project is funded by the USDA's Specialty Crops Research Initiative Program of the National Institute for Food and Agriculture, Project #2011-51181-30850

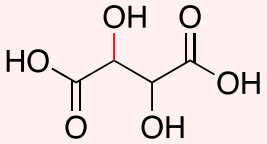
Plant Metabolomics Laboratory
Dept. Horticultural Science
University of Minnesota

# Grape Berry Metabolic Profiling by LC-MS:

## Organic acids:

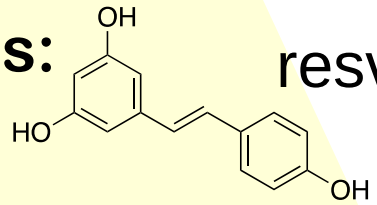


malic acid

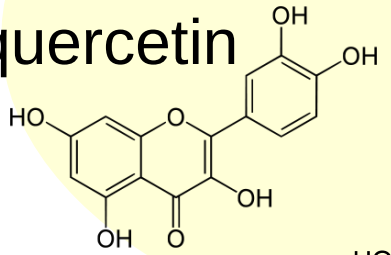


tartaric acid

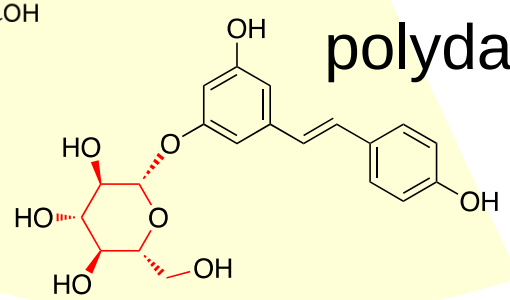
## Polyphenolics:



resveratrol

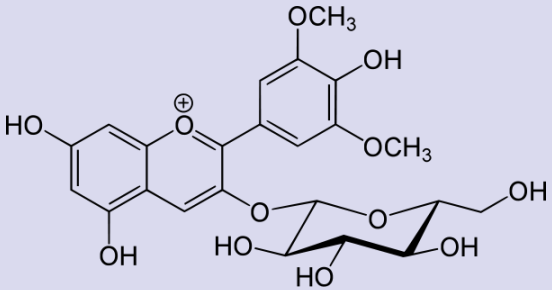


quercetin

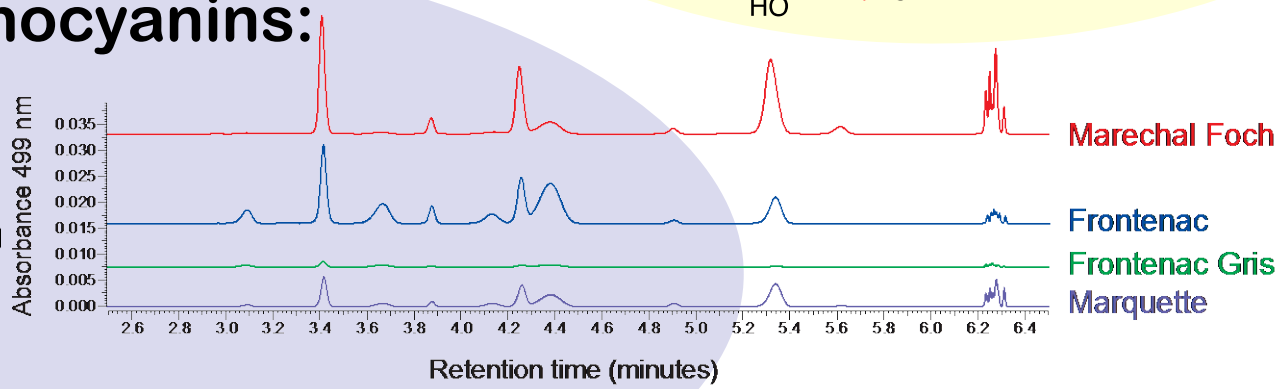


polydatin

## Anthocyanins:



malvidin



**Berry Samples**
**20 to 26° Brix**

↓

**Transcriptome**
**(gene expression)**

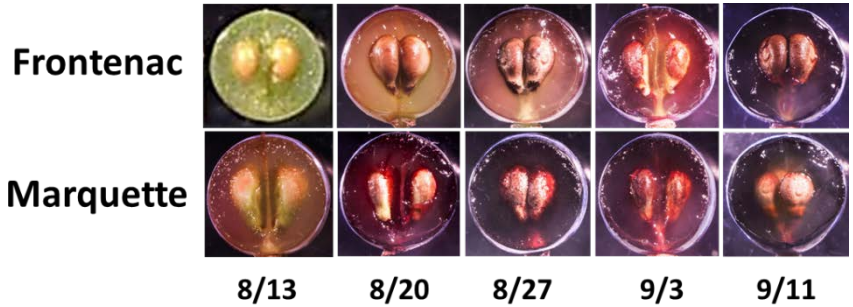
↓

# Metabolome

**(sugars, acids, flavors, aromas anthocyanins, tannins)**

↓

**Sensory descriptors**



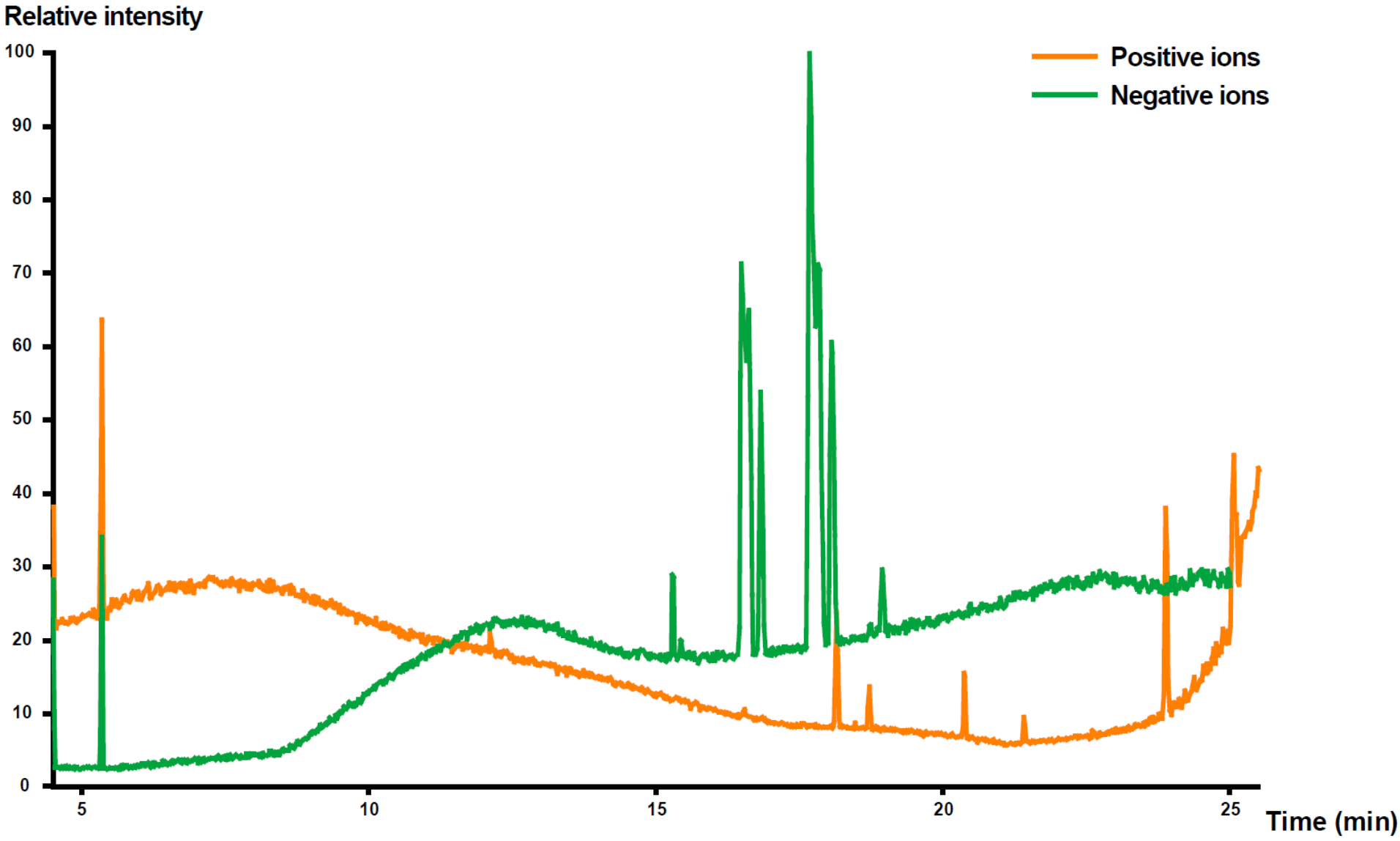
# Workflow


Skin

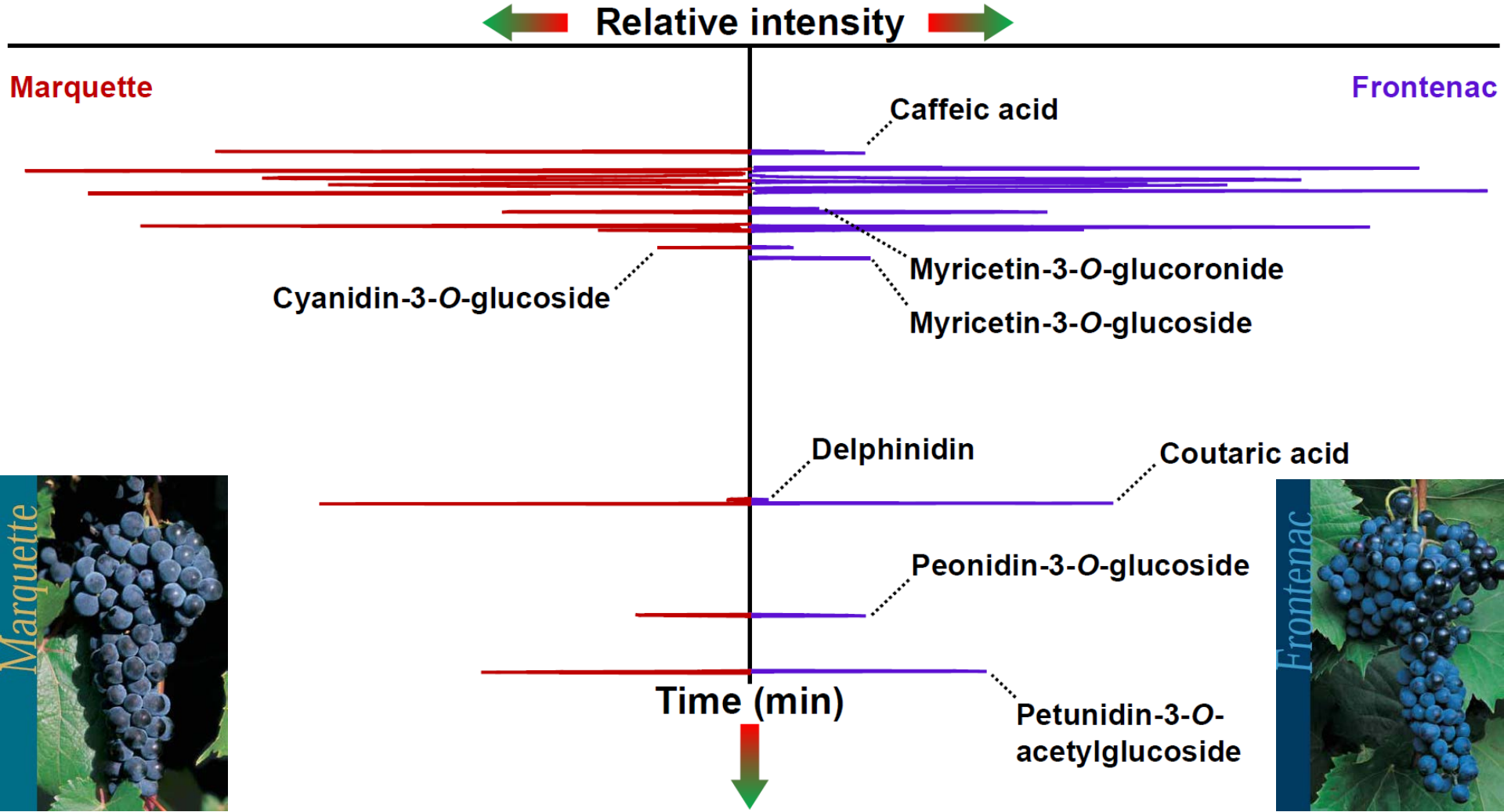
Pulp & seed → Extract with ethanol → Homogenize → Centrifuge → Retain supernatant → Analyze by LC–MS (liquid chromatography–mass spectrometry)


http://www.thermoscientific.com/

# Chromatogram



# Comparisons of Extracted Ion Chromatograms



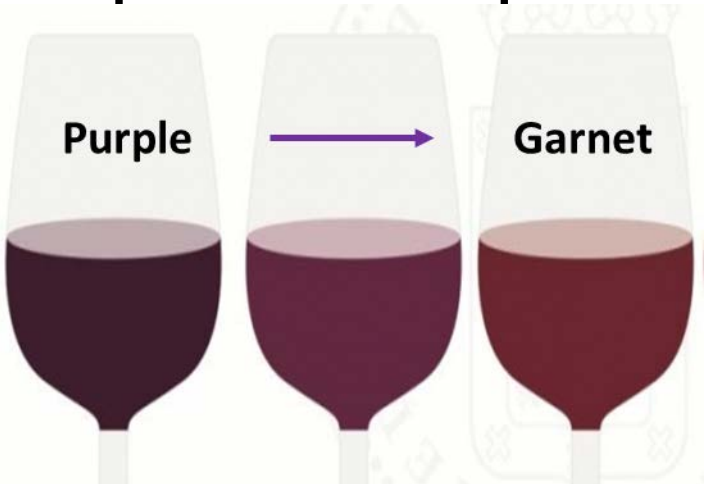
# Genomics and Fruit Composition: Link Genetics to Sensory (SDSU, UMN, IA State)

**Do gene expression differences coincide with existing knowledge?**

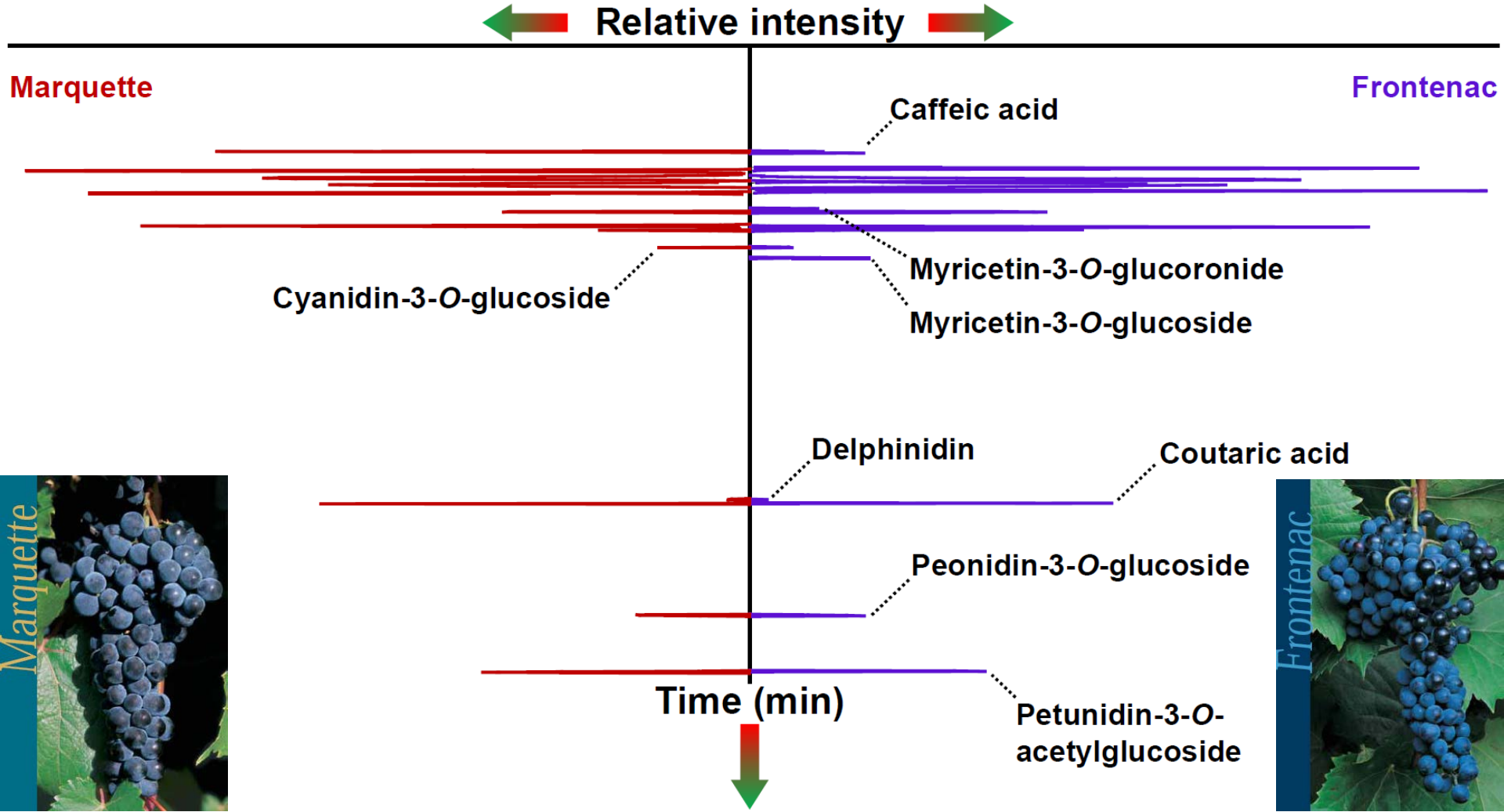
- **Anthocyanin biosynthesis genes preferentially expressed in berry skins**
- **Expression of anthocyanin biosynthesis genes significantly greater expression in Frontenac than Marquette**

| | Front Skin | Marq Skin |
|---|---|---|
| VIT_05s0062g00720 | 36 | |
| VIT_12s0034g00080 | 47 | |
| VIT_02s0033g00450 | 159 | 42 |
| VIT_02s0033g00390 | 165 | 84 |
| VIT_05s0049g01020 | 429 | 120 |
| VIT_02s0033g00380 | 531 | 219 |

**Several anthocyanin biosynthesis genes highly expressed in Frontenac in comparison to Marquette**

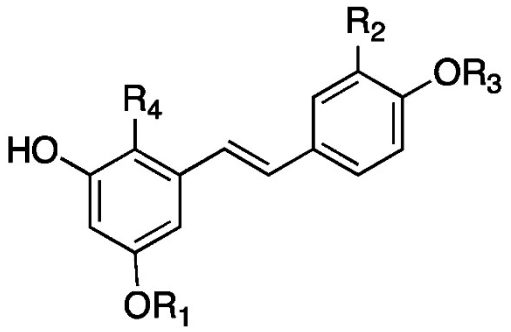


# Comparisons of Extracted Ion Chromatograms



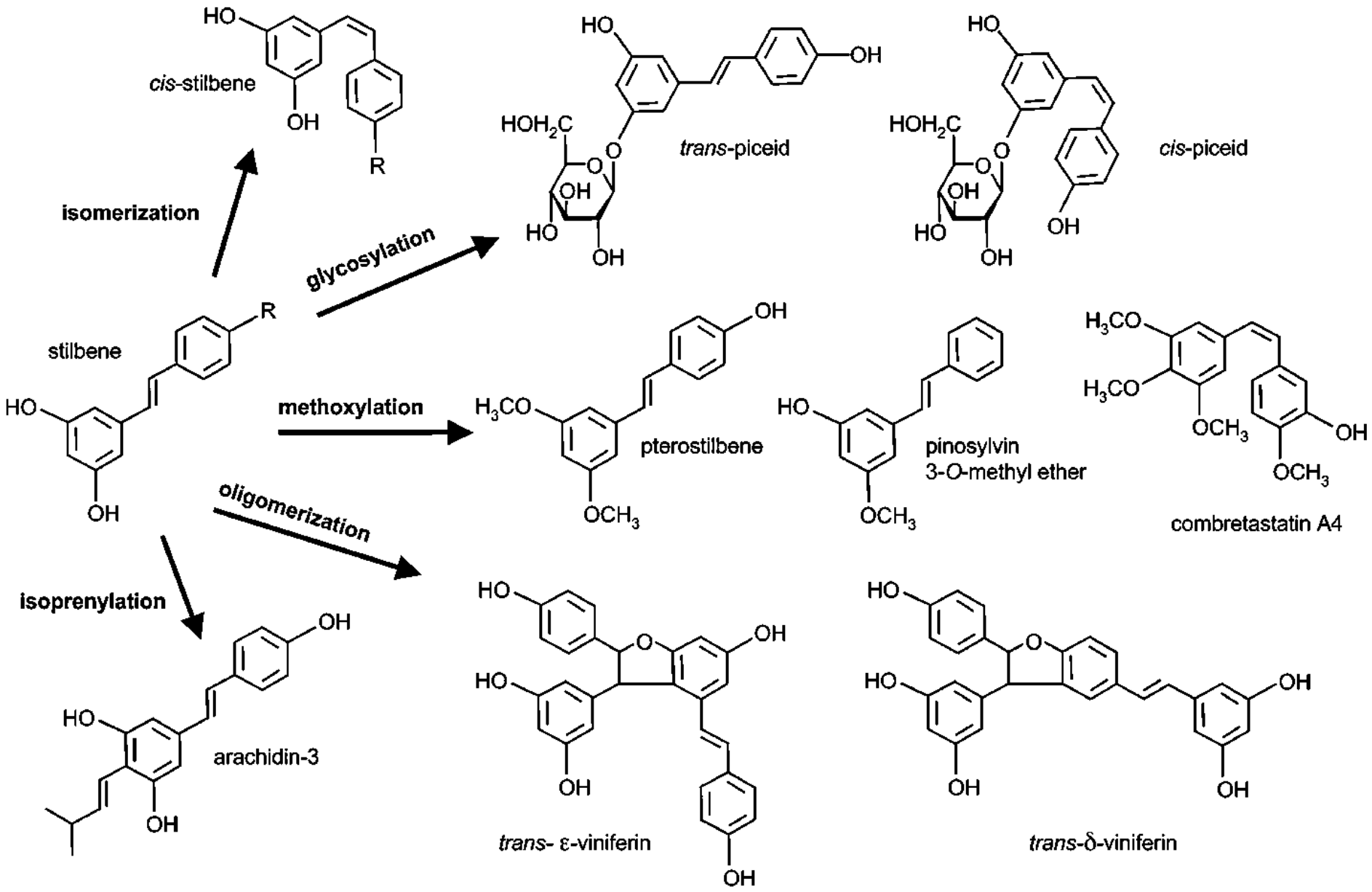
# Stilbenoids

- A class of polyphenolic secondary metabolites
- Receive much attention due to:
  - Health-promoting properties
  - Phytoalexins in *Vitaceae*



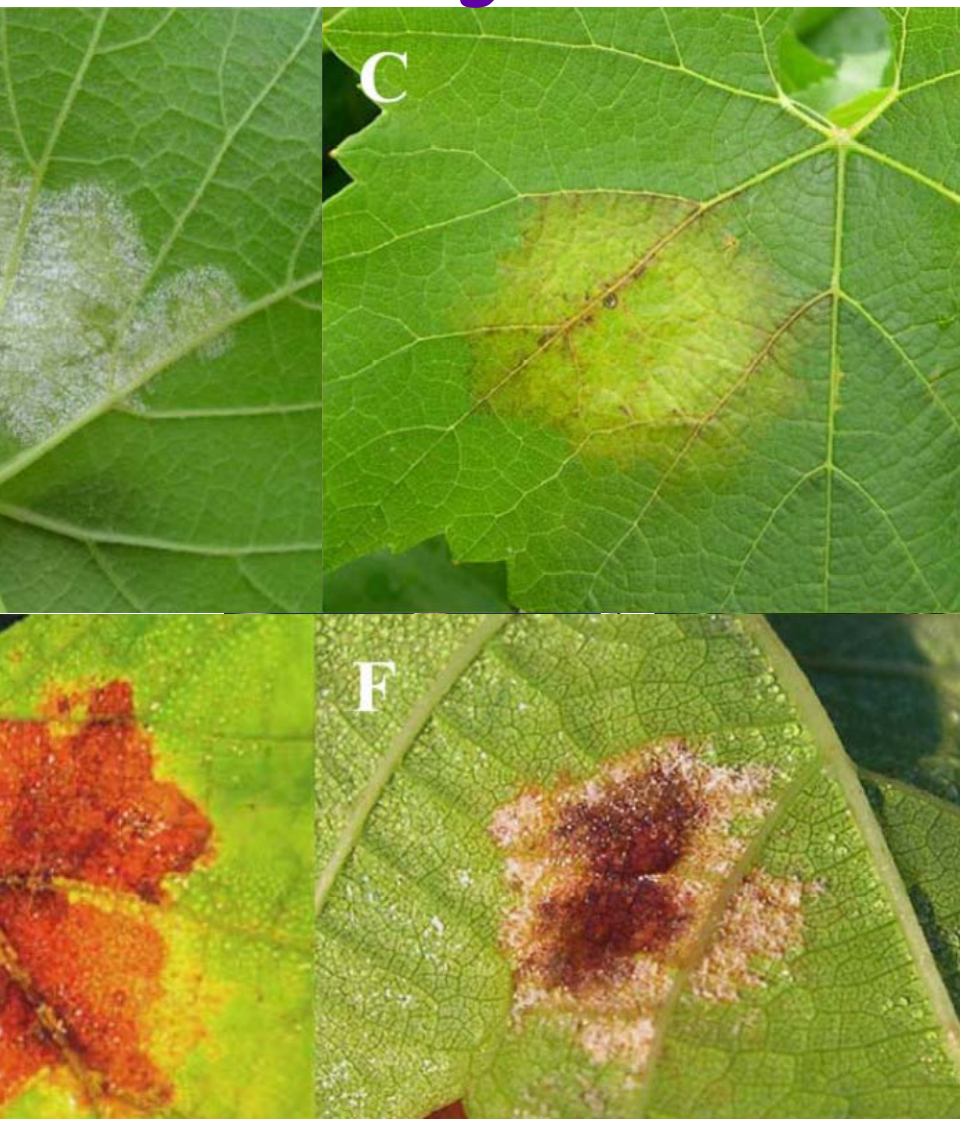

**Resveratrol**: $R_1,R_2,R_3 = H,\ R_4 = H$
**Piceid**: $R_1 = Glc\ R_2,R_3,R_4 = H$
**Piceatannol**: $R_1,R_3,R_4 = H,\ R_2 = OH$
**Resveratroloside**: $R_1,R_2,R_4 = H,\ R_3 = Glc$

Pawlus *et al*. (2013). *Journal of agricultural and food chemistry*, *61*(3), 501-511.




Chong *et al*. (2009). *Plant Science*, *177*(3), 143-155.

# Downy and Powdery Mildews




Kennelly *et al*. (2007). *Phytopathology*, *97*(4), 512-522.

PHOTO BY R. PEARSON

Fig. 3. Distortion of young leaves.

PHOTO BY R. PEARSON

Fig. 4. Dark lesions on green shoots.

PHOTO BY R. PEARSON

Fig. 7. Dark, dusty fruit infections.

PHOTO BY R. PEARSON

Fig. 8. Shriveled and cracked

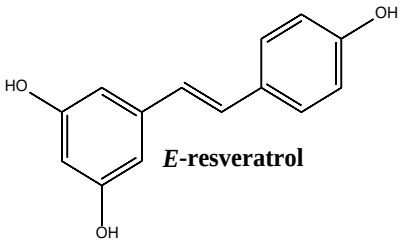
Disease Identification Sheet No. 102GFSG-D2 by NY State IPM

# Stilbenoids *versus P. viticola*

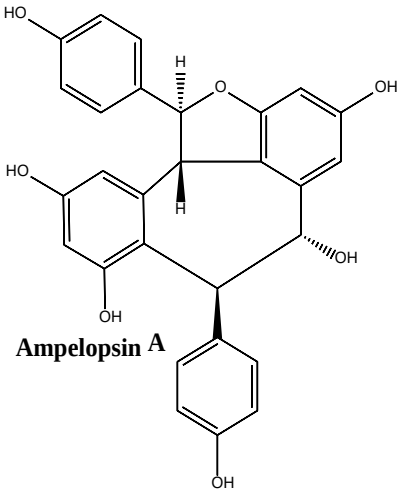
| Study | Compounds | $ED_{50}$ (µM) | |
|---|---|---|---|
| | | Zoospore mobility | Disease expression |
| Pezet *et al*. 2004 | *E*-resveratrol | 192 | 145 |
| | *E*-ε-viniferin | 73 | 74 |
| | *E*-δ-viniferin | 15 | 15 |
| | *E*-pterostilbene | 28 | 13 |

| Study | Compounds | $IC_{50}$ (µM) | |
|---|---|---|---|
| | | Zoospore mobility | Sporulation |
| Schnee *et al*. 2013 | Ampelopsin A | 124 | 282 |
| | Hopeaphenol | 17 | 26 |
| | *E*-resveratrol | 122 | 121 |
| | Ampelopsin H | 92 | 282 |
| | *E*-ε-viniferin | 66 | 63 |
| | *E*-vitisin B | 13 | 12 |

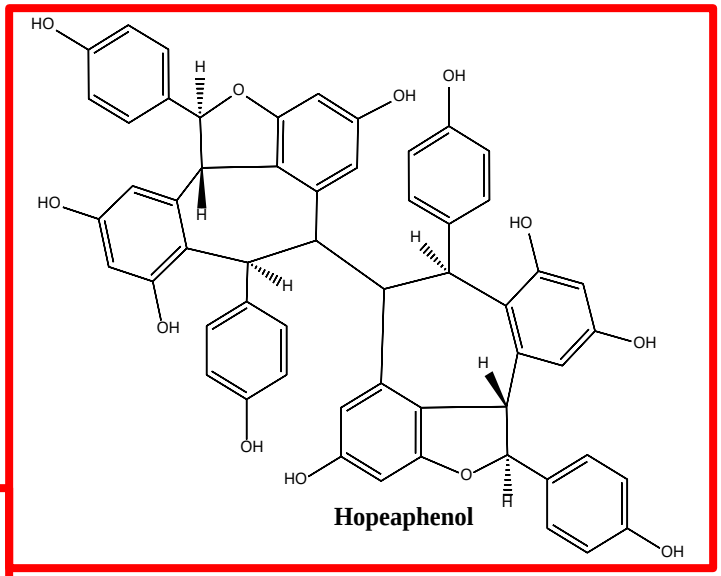
**Effective dose ($ED_{50}$): Dose causing 50% of the maximum biological effect.**
**Inhibitory concentration ($IC_{50}$): Concentration causing 50% inhibition.**



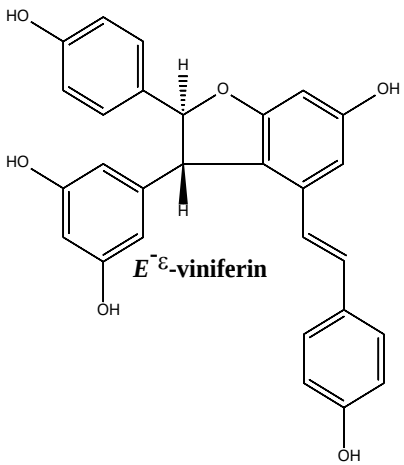
***E*-resveratrol**



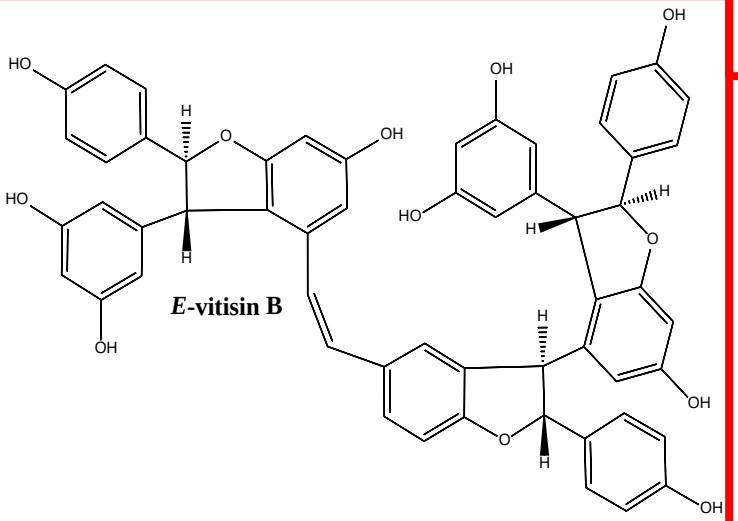
**Ampelopsin A**



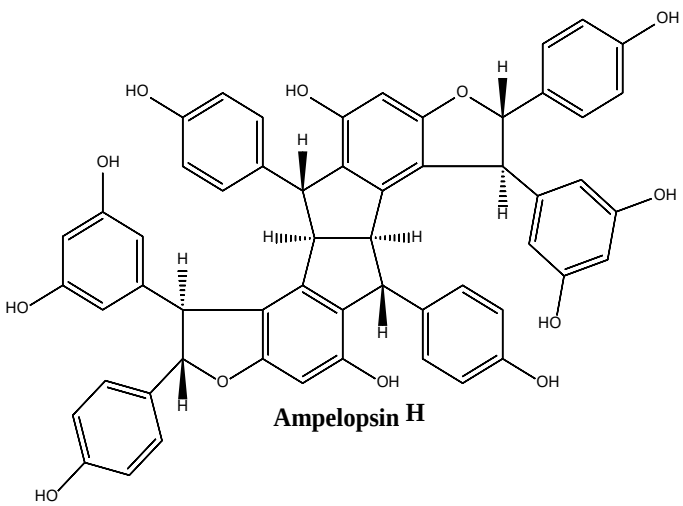
**Hopeaphenol**



***E*-ε-viniferin**

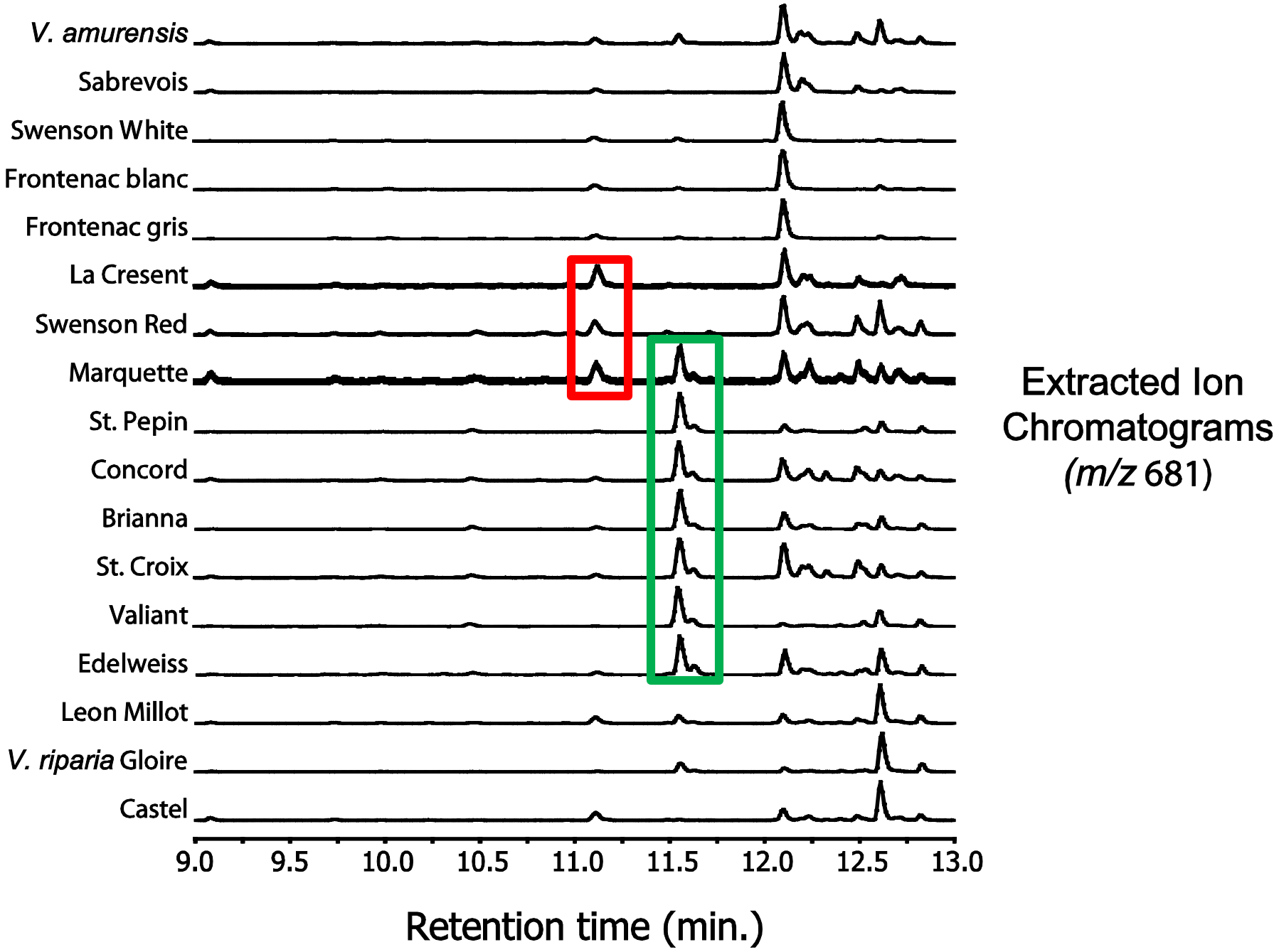


***E*-vitisin B**

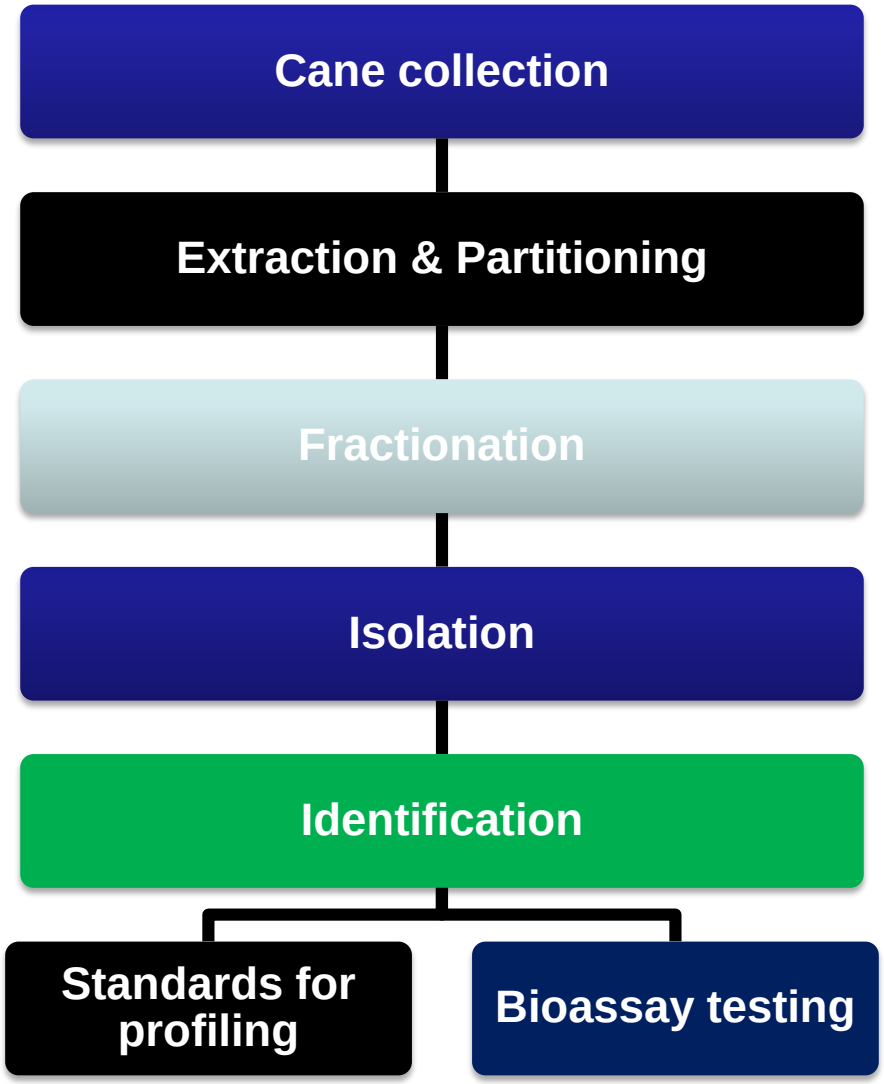


**Ampelopsin H**

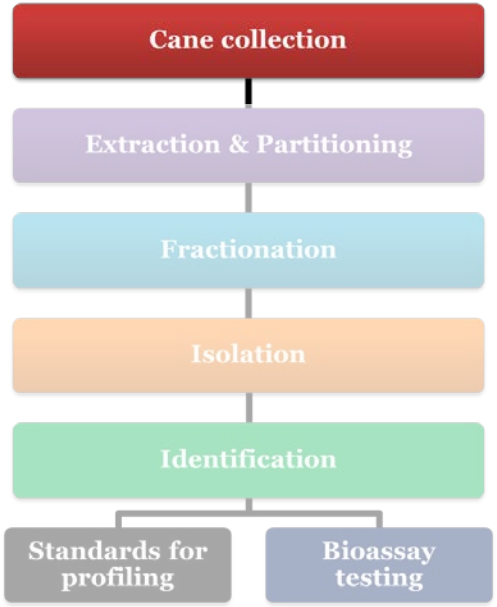
Structure credit: Alison Pawlus

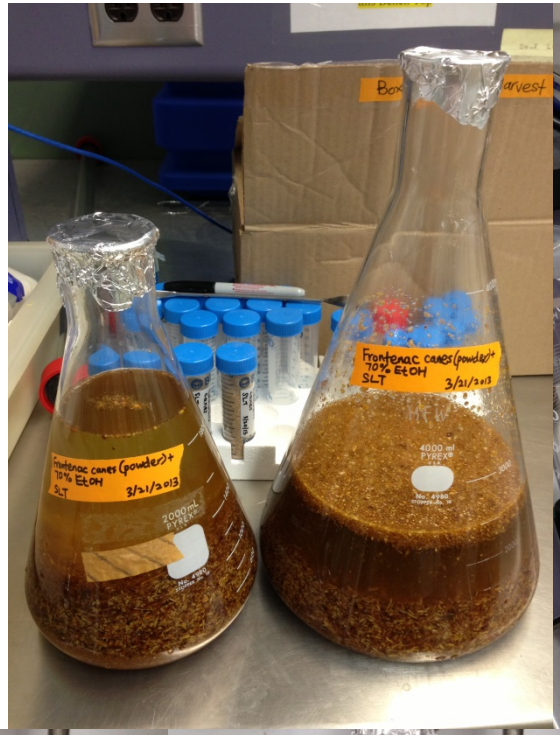

V. amurensis
Sabrevois
Swenson White
Frontenac blanc
Frontenac gris
La Cresent
Swenson Red
Marquette
St. Pepin
Concord
Brianna
St. Croix
Valiant
Edelweiss
Leon Millot
V. riparia Gloire
Castel
9.0
9.5
10.0
10.5
11.0
11.5
12.0
12.5
13.0
Retention time (min.)
Extracted Ion Chromatograms (m/z 681)

# Isolation & Identification of Stilbenoids



Cane credit:
John Thull

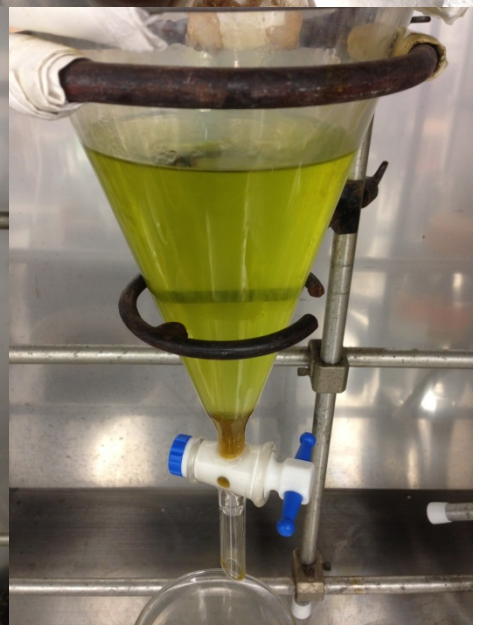


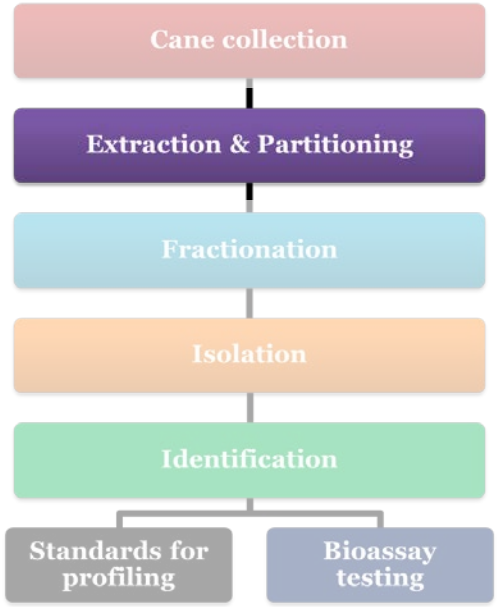

Frontenac canes (powder)+
70% EtOH
SLT
3/21/2013

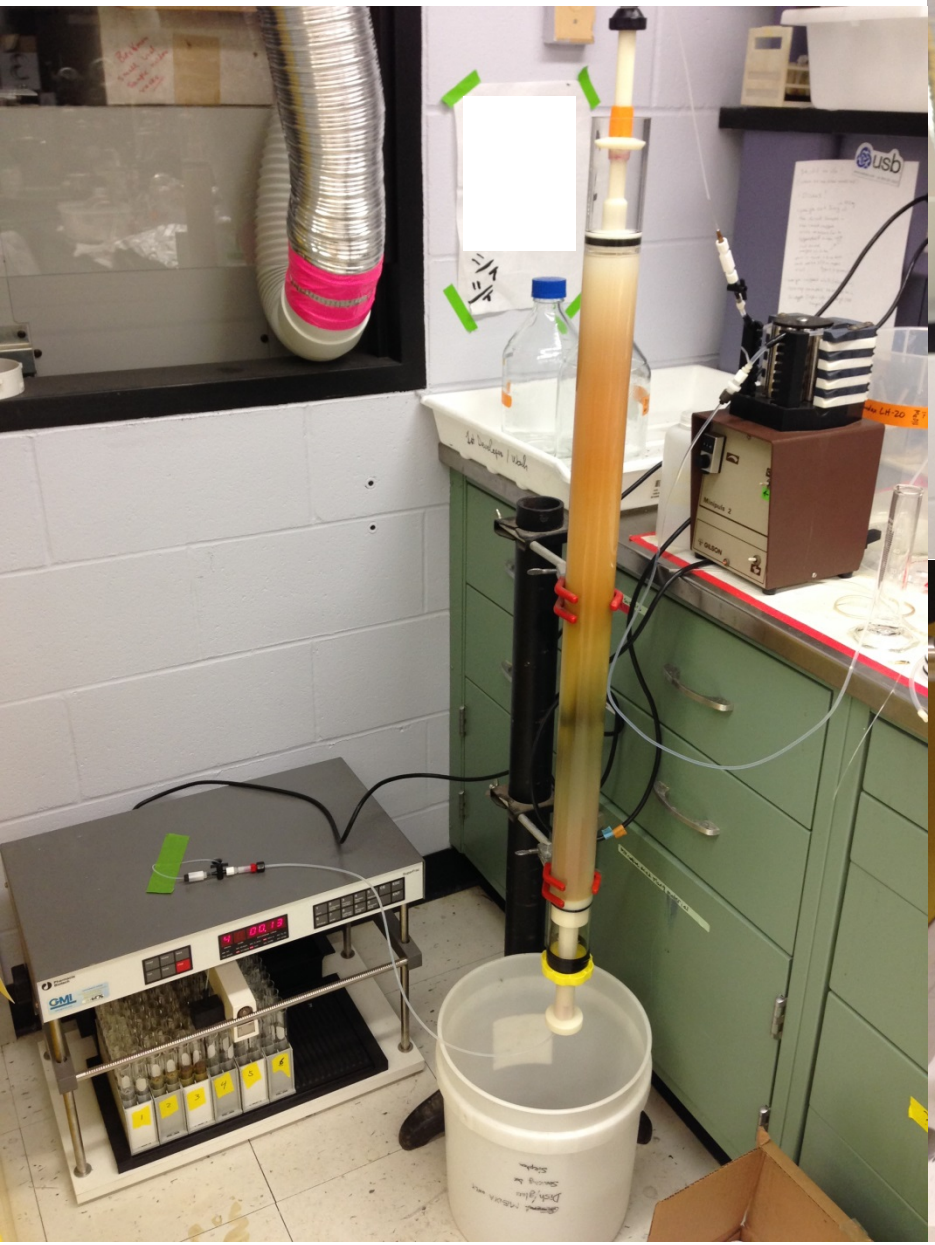

Frontenac canes (powder)+
70% EtOH
SLT
3/21/2013


2000 ml
PYREX


2000 ml
PYREX




Cane collection
Extraction & Partitioning
Fractionation
Isolation
Identification
Standards for profiling
Bioassay testing



Cane collection

Extraction & Partitioning

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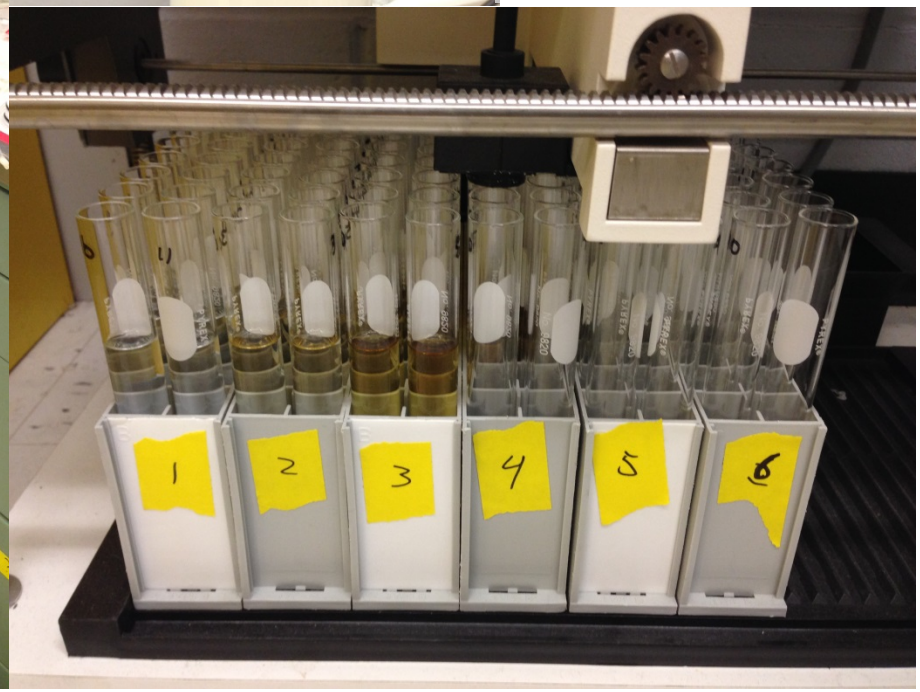
Bioassay testing



# Viticulture, enology and marketing for cold-hardy grapes

**Kalley Besler, Padmapriya Swaminathan, Anne Fennell – SDSU**

**Somchai Rice, Jacek Koziel, Murli Dharmadhikari, Devin Maurer – ISU**

**Emily Del Bel, Soon Li Teh, Bety Rostandy, Jenna Brady, Zata Vickers,**

**Adrian Hegeman, Jim Luby – UMN**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www.northerngrapesproject.org