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

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Plant Science Dept.
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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 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 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

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

Veraison
20°Brix
22°Brix*
24°Brix*
26°Brix*

*Sensory analysis of berry & 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.

Frontenac
Marquette

8/13  8/20  8/27  9/3  9/11
Berry Samples
20 to 26°Brix

Transcriptome
(genomes)

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
(genomes)

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

Sensory descriptors

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

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

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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 biosynthes gene expression

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

Frontenac Pinene synthase: eucalyptus and camphoraceous note with a spicy peppery and nutmeg nuance
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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Chemical and Sensory profile analysis (ISU)

- 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
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
Notes:
1: MultiTrax Controller
2: Precolumn Sniff Port Selector
3: Heartcut Valve
4: CO₂ Cryotrap
5: Precolumn Backflush
6: Solenoid
7: Filter
8: Injector
9: Backflush Sweep
10: Fixed Restrictor to Inlet
11: Liquid CO₂ Feed
12: CO₂ 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)

Aroma descriptor of AromaTrax (MOCON, Round Rock, TX)
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.
Modified 2 mL glass vial with septa, held by negative pressure. Photo by Somchai Rice
MDGC-MS-O Jason Vallone analyses in vivo gas emissions from Frontenac grape clusters at 24° Brix.
Photo by Somchai Rice
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

- Benzeneethanol (CAS)
- Ethanol (CAS)
- Hexanal (CAS)
- Styrene
- Benzaldehyde (CAS)
- Pentanal 1 (CAS)
- Pentanal 2 (CAS)
- 1-Hexanol (CAS)
- Heptanal (CAS)
- 1-Octanol (CAS)
- Benzoic acid, 2-hydroxy-, methyl ester (CAS)
- Benzeneacetaldehyde (CAS)
- Nonanal (CAS)
- beta.-Cyclocitral
- Butanal, 3-methyl- (CAS)
- 2-Hexenal, (E)- (CAS)
- beta.-Damascenone
South Dakota State University Frontenac 2012
In-Vivo Target VOCs from Veraison to Harvest

Chemical and Sensory profile analysis (ISU)
Chemical and Sensory profile analysis (ISU)

Total Target VOCs from crushed berries - SDSU Frontenac 2012

- Theaspirane A
- Theaspirane B
- Ethanol (CAS)
- Hexanal (CAS)
- Styrene
- Benzaldehyde (CAS)
- Pentanal 1
- Pentanal 2
- 1-Hexanol (CAS)
- Heptanal (CAS)
- 1-Octanol (CAS)
- Benzoic acid, 2-hydroxy-, methyl ester (CAS)
- Benzeneacetaldehyde (CAS)
- Nonanal (CAS)
- .beta.-Cyclocitral
- Butanal, 3-methyl- (CAS)
- 2-Hexenal, (E)- (CAS)
- .beta.-Damascenone
• 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)
• 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)

- Almond, burnt sugar
- Floral, honey, rose, fruity apple
- Fruity, green, apricot, pear, banana
- Fruit, fat, leaf
- Fruit, raspberry, pear
- Minty, peppermint, Apple peel, fruit
- Honey, spice, rose, lilac
- Apple peel, fruit, vinegar, grape, mint, wine
- Fruit, apple, banana
- Chocolate, peach, fatty
- Almond, burnt sugar
- Fruity, green, apricot, pear, banana
- Fruity, raspberry, pear

F1 (48.09 %) vs. F2 (30.62 %)
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

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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
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.
Grape Berry Metabolic Profiling by LC-MS:

**Organic acids:**
- malic acid
- tartaric acid

**Polyphenolics:**
- resveratrol
- quercetin
- polydatin

**Anthocyanins:**
- malvidin

Retention time (minutes)
Berry Samples
20 to 26° Brix

Transcriptome
(gene expression)

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

Sensory descriptors

Frontenac
Marquette
Workflow

Skin

Pulp & seed → Extract with ethanol

Homogenize

Centrifuge

Retain supernatant

Analyze by LC–MS (liquid chromatography–mass spectrometry)

http://license.umn.edu/

http://www.thermoscientific.com/
Chromatogram

Relative intensity

Time (min)

Orange: Positive ions
Green: Negative ions
Comparisons of Extracted Ion Chromatograms

Relative intensity

Time (min)

Marquette

Caffeic acid

Myricetin-3-O-glucuronide

Myricetin-3-O-glucoside

Cyanidin-3-O-glucoside

Delphinidin

Coutaric acid

Peonidin-3-O-glucoside

Petunidin-3-O-acetylglucoside

Frontenac
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
Comparisons of Extracted Ion Chromatograms

Marquette

Caffeic acid
Myricetin-3-O-glucoronide
Myricetin-3-O-glucoside
Delphinidin
Coutaric acid
Peonidin-3-O-glucoside
Petunidin-3-O-acetylglucoside

Relative intensity

Time (min)
Stilbenoids

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

Cis-stilbene \rightarrow \text{isomerization} \rightarrow \text{stilbene} \rightarrow \text{methoxylated stilbene} \rightarrow \text{glycosylated stilbene} \rightarrow \text{combretastatin A4

Downy and Powdery Mildews


Disease Identification Sheet No. 102GFSG-D2 by NY State IPM
## Stilbenoids versus *P. viticola*

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<th>Compounds</th>
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<th>Zoospore mobility</th>
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Effective dose (ED$_{50}$): Dose causing 50% of the maximum biological effect.  
Inhibitory concentration (IC$_{50}$): Concentration causing 50% inhibition.
Isolation & Identification of Stilbenoids

Cane collection

Extraction & Partitioning

Fractionation

Isolation

Identification

Standards for profiling

Bioassay testing
Viticulture, enology and marketing for cold-hardy grapes

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