NYWGF RESEARCH - REPORT

Funding for fiscal year: 2022-23

SECTION 1:

Project title: Expanding the range of rapid analysis approaches to semi-polar volatiles and non-volatile precursors in grapes and quantifying the risks and benefits associated with utilizing microbial terroir in winemaking.

Principal Investigator with contact info: Dr. Gavin Sacks, Professor, Department of Food Science, 251 Stocking Hall, Cornell University, Ithaca, NY. Email: gls9@cornell.edu. 607-255-2335;

Co-PI Collaborators with contact info: Dr. Patrick Gibney, Assistant Professor, Department of Food Science, 347 Stocking Hall, Cornell University, Ithaca, NY. Email: pag235@cornell.edu. 607-255-3471.

New Research ☐ **Continued Research** ☒

Amount Funded **$** 150,876

SECTION 2:

Project Summary Impact Statement: Targeted measurements of volatiles or volatile precursors in grapes and wines are useful for quality evaluation, e.g. volatile phenols can be markers of microbial spoilage, and C6 aldehydes markers of grape maturity. However, analyses of these volatiles are typically slow, and their routine analysis by commercial labs is prohibitively expensive for most wineries. Two strategies for increasing throughput (and thus decreasing costs) of these volatiles include sorbent sheet extraction (SPMESH) followed by direct-analysis in real time mass spectrometry (DART-MS) and low-pressure gas chromatography mass spectrometry (LP-GC-MS) were evaluated. We determined that chemical transformation ("derivatization") of aldehydes and phenols prior to SPMESH-DART-MS resulted in excellent detection limits and good agreement with the "gold standard" GC-MS method. The new SPMESH-DART-MS approach increased throughput by 10-fold over the existing method. Follow-up studies will invite NY State winemakers to submit samples for further validation. Additionally, although there is strong interest among NY State winemakers in the flavor potential of "native" microorganisms, there is little data on the risks and benefits associated with utilization of these native microbes. By collaborating with winemakers in NY, we have collected vineyard samples (in duplicate) from roughly 16 different locations. The grapes were pressed and native microbes were collected by centrifugation, followed by inoculation into sterile Chardonnay juice. Fermentation kinetics were monitored by weight, and samples were taken periodically to evaluate the changes in microbial population. Additionally, post-fermentation samples were collected for standard and volatile chemical analysis. A subset of samples were selected for larger-scale fermentations which were processed identically and will be additionally used for

quantitative, descriptive sensory evaluation. By evaluating different microbes in identical juice and fermentation conditions, we were able to quantify the risks associated with utilizing only native microbes (e.g. the probability of stuck, sluggish, or spoiled fermentation) and the benefits (increased positive product differentiation). Follow-up studies will seek to identify individual microbial strains that have potential for positive contributions in winemaking (e.g. increased positive flavors/aromas, decreased off-flavors/aromas, improved fermentation kinetics, etc.).

Objectives:

Objective 1: Evaluate and validate pre-extraction derivatization of volatile phenols and herbaceous-smelling C6 aldehydes to increase the amenability of these compounds for rapid analysis by DART-MS

Objective 2: Evaluate low-pressure gas chromatography mass spectrometry (LP-GC-MS) as a rapid tool for quantitating semi-polar and volatile precursor fractions in grapes and wines.

Objective 3: Evaluate the fermentation kinetics, volatile aroma compound level, and sensory effects of different inoculation strategies using microbes isolated from wine regions in NY.

Materials & Methods:

For Objective 1, Development of Rapid DART-MS Analysis of Volatile Phenols and Aldehydes

a) Methods development and validation for volatile phenols ("VPs").
    - Standards of major VPs (guaiacol, 4-methylguaiacol, cresol) were prepared in grape juice and wine. Deuterated guaiacol was used as an internal standard.
    - Three approaches to derivatization were initially evaluated: silylation by MSTFA, acylation by ethylchloroformate, and acylation by acetic anhydride. The last approach (acetic anhydride) was selected due to its relatively benign derivatization conditions.
    - Acylation conditions were optimized by varying acetic anhydride concentration and pH
    - SPMESH extraction conditions were optimized by varying extraction time, temperature, and sample volume
    - DART-MS settings were optimized by changing DART temperature and voltage settings, and selecting MS/MS ion transitions which maximized signal-to-noise ratio,
    - Figures of merit (linearity, detection limits, precision) were determined in grape juice and wine over a range of 4 – 250 µg/L
    - To determine accuracy, VPs in five grape samples were analyzed by both the newly developed SPMESH-DART-MS and a "gold standard" acid hydrolysis method followed by SPME-GC-MS.
b) Methods development and validation for C6 aldehydes
    - Standards of the two major C6 aldehydes (hexanal and (E)-2-hexenal) were prepared in commercially prepared grape juices lacking detectable C6 adehydes

- Derivatization of aldehydes to oxime derivatives was optimized by varying PFBHA concentration and reaction time.
- SPMESH extraction conditions and DART-MS settings were optimized as described for volatile phenol analyses
- Figures of merit (linearity, detection limits, precision) were determined in grape juice and wine over a range of 50 – 500 µg/L
- To determine accuracy, eight grape samples were macerated, and C6 aldehydes analyzed by both the newly optimized SPMESH-DART-MS method and a "gold standard" acid hydrolysis method followed by SPME-GC-MS.

For Objective 2, Evaluation of Low Pressure Gas Chromatography Mass Spectrometry (LP-GC-MS)

a) Samples were prepared by spiking juice with the following common odorants
    - C6 compounds: hexanol, hexanal, (E)-2-hexenal
    - Monoterpenes: linalool, geraniol
    - Other: damascenone, 3-isobutyl-2-methoxypyrazine
    - Phenols: guaiacol, cresol
b) A new low-pressure gas chromatography (LP-GC-MS) method was developed used a short restrictor column (1 m x 0.1 mm) coupled to a wide-bore analytical column (15 m x 0.53 mm, DB-5 stationary phase). Extractions were performed by SPME Arrow.
c) The following parameters were optimized
    - SPME Arrow extraction times
    - GC oven ramp rate
    - Initial GC oven temperature and hold time
d) Figures of merit (detection limits, linear range, recovery, reproducibility) were determined, analogously to Objective 1.
e) To determine accuracy, commercial juice samples were analyzed by the LP-GC-MS method and compared against gold standard SPME-GC-MS methods

For Objective 3, Evaluate the fermentation kinetics, volatile aroma compound level, and sensory effects of different inoculation strategies using microbes isolated from wine regions in NY

a) Microbial sample collection
    - Duplicate grape samples have been collected in collaboration with roughly 20 vineyards spread throughout NY state winemaking regions (this collection plan has been performed for 2 consecutive years)
    - Grape samples were individually pressed to collect juice, which was then centrifuged at low speed to remove grape solids. Native microbes were then collected by high speed centrifugation before inoculation into sterile Chardonnay juice (roughly 100 mL small-scale fermentations).
b) Monitoring fermentation kinetics

    - Fermentation kinetics were monitored by weighing each fermentation flask every day until the completion of fermentation (weight loss is equivalent to glucose consumption, in which one mole of consumed glucose produces two moles of carbon dioxide gas); fermentation completion was defined using an industry standard for dryness (between 1-3 g/L sugar)

c) Metagenomic analysis of microbial community changes during fermentation
    - Samples of from each fermentation were collected at 100, 75, 50, 25, and 0% sugar remaining to evaluate changes in the microbial community composition
    - Samples are currently being processed (optimization of genomic DNA extraction and subsequent PCR); once conditions are optimized, microbial identification PCRs from these mixed populations will be sequenced at the Cornell University Institute of Biotechnology Sequencing Core on an Illumina HiSeq.
d) Standard and volatile chemical analysis from fermented wines
    - Samples from each completed fermentation were collected for standard chemical analysis (sugar level, alcohol level, titratable acidity, pH, volatile acidity, and yeast assimilable nitrogen) and common aromatic volatile analysis using gas chromatography-based mass spectrometry (chemical analysis will be performed by collaborators at E&J Gallo Winery in Modesto, CA).
e) Large-scale fermentation trials and sensory evaluation
    - For year 2 of this study, 6 grape samples were collected for larger-scale fermentations (1 gallon each) which will be evaluated as above. Additionally, these samples included variations in inoculation timing with a robust commercial strain (not inoculated, delayed inoculation, immediate inoculation). These wine samples will be evaluated using quantitative, descriptive sensory analysis in Spring 2023.

Results/Outcomes/Next Steps:

<u>Objective 1, “New Rapid DART-MS following Derivatization” - Results and Outcomes</u>

- In initial work, we confirmed that direct SPMESH extraction followed by DART-MS analysis of both volatile phenols and C6 aldehydes resulted in very poor detection limits (> 1 mg/L), well over sensory thresholds.
- Follow up work indicated that this poor sensitivity was likely due to the relatively high polarity of the target analytes, a problem which can be overcome by derivatization.
- For volatile phenol analysis, acetic anhydride derivatization was selected over alternatives (silylation, chloroformates) because of the relatively low danger associated with the reagents.
- Conditions for volatile phenol derivatization were optimized, along with SPMESH-DART-MS parameters. The optimized workflow is shown in Figure 1.
- Figures of merit were determined in juice and wine. Detection limits for target compounds were ~ 1 µg/L for the three target VPs, below sensory threshold. Good linearity and reproducibility were also achieved (Table 1).
- Similar outcomes were achieved for analysis of C6 aldehydes (hexanal, (E)-2-hexenal) using PFBHA derivatization. Detection limits under 50 µg/L could be achieved, below sensory thresholds for these compounds.
- The optimized methods can analyze 24 samples in ~60 min, which includes derivatization and extraction time. This is approximately 10-fold greater throughput than the

conventional GC-MS methods used in commercial labs.

- For validation, juice and wine samples were sourced from an industry cooperator. Samples were analyzed by both the new SPMESH-DART-MS method and the gold standard GC-MS method. Good agreement was observed between the two methods for all analytes (R^2 > 0.7-0.9). Representative correlation plots are shown in Figure 2.

- Next Steps: The new SPMESH-DART-MS methods will be validated with a larger number of samples. We intend to invite New York State winemakers and growers to submit samples for analysis in 2023-24 for a nominal fee.

Objective 2, "Evaluation of LP-GC-MS for rapid volatile analyses"- Results and Outcomes

- Conditions for low pressure gas chromatography – mass spectrometry (LP-GC-MS) of commonly measured grape and wine volatiles were optimized.
- Excellent detection limits and reproducibility could be achieved for most target analytes using LP-GC-MS. The resulting method required a total of 10 min per analysis, including extraction and GC oven cooling. This is approximately, twice as fast as the existing gold standard GC-MS method (20-25 minute cycle times per analysis).
- However, LP-GC-MS resolution and performance for early eluting C6 alcohols and aldehydes was very poor. This was hypothesized to be due to poor focusing of these highly volatile compounds at the start of the GC run.
- Attempts to improve resolution of these compounds by decreasing the initial over temperature and increasing GC column film thickness were unsuccessful.

- Next Steps: In the coming year, we intend to evaluate active cooling of the GC oven to sub-ambient temperatures to improve chromatography and figures of merit for early eluting volatile compounds.

Objective 3, "Characterization of NY State microbial diversity"- Results and Outcomes

- All grape samples and fermentations (both small- and large-scale) for the planned two years of this study have been collected and completed, respectively.
- Samples for standard/volatile chemical analysis from year 1 small-scale fermentations have been sent to our collaborator for processing. Samples from year 2 (both small- and large-scale fermentations) were recently completed, stabilized against oxidation or further microbial activity with sulfite, and will be sent to our collaborator for processing in Spring 2023.
- The graduate student performing this project has assembled and been training a descriptive sensory panel under the supervision of our department's sensory science professor, Robin Dando. Over half of the required training hours have been completed and both training and sensory analysis of large-scale fermentations will be completed in Spring 2023.
- Genomic DNA extraction and PCR conditions are currently being optimized for the metagenomics analysis. We expect the optimization period to be complete by February 2023, then to proceed with sequencing (~1 month) and data analysis (~1-2 months).
- Next Steps: In the coming semester, we intend to complete remaining experiments,

collect and analyze the data, then write the results into 1-2 manuscripts to be published in the industry standard journal, American Journal of Enology and Viticulture. In addition, we expect to produce at least one extension article summarizing the work for publication in Appellation Cornell, which is sent directly to NY winemakers.

Technology Transfer Plan: The rapid analytical tools developed in this work, especially SPMESH-DART-MS, will be of interest to existing commercial labs. We will reach these audiences by publishing in appropriate peer-reviewed analytical chemistry journals and trade journals. The Sacks lab also expect to make SPMESH-DART-MS available for fee-for-service testing for New York State wineries in 2023-24 as part of larger scale validation.

Attachments:

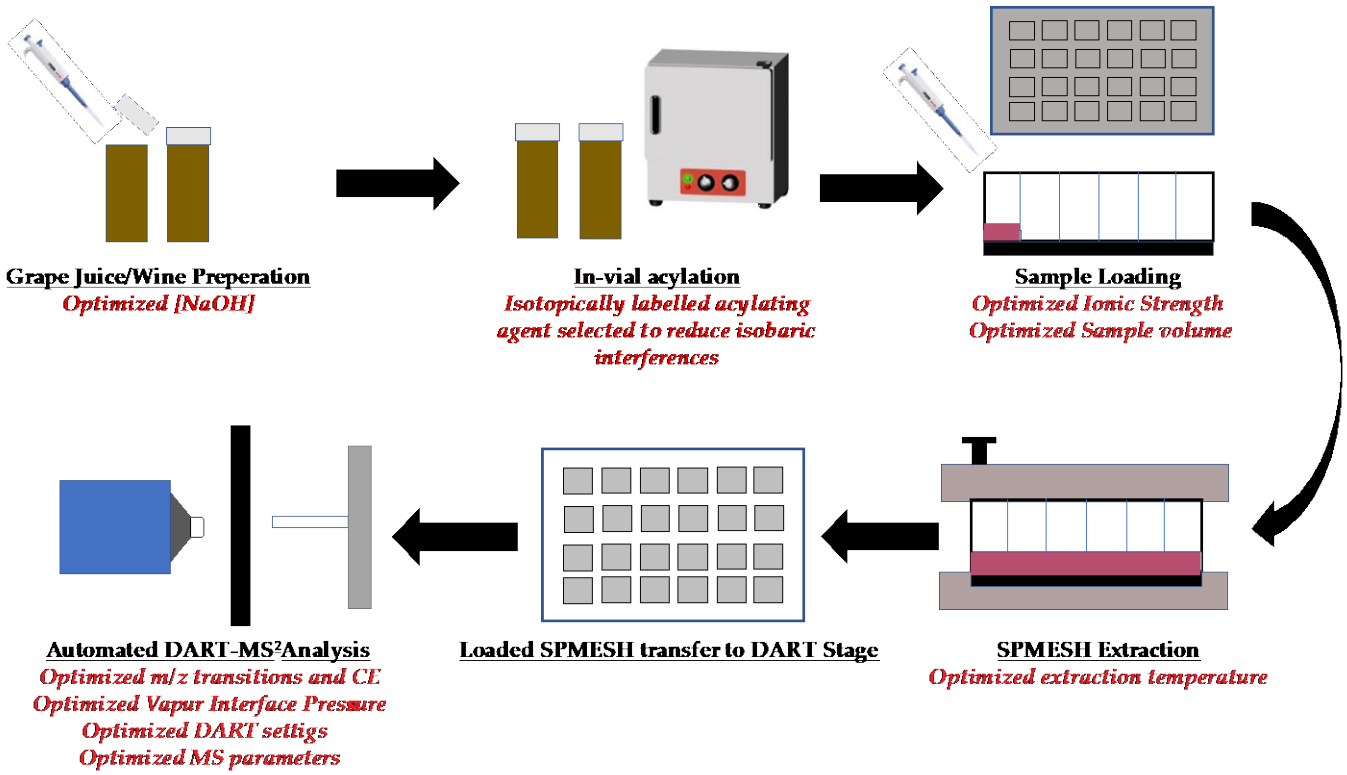

Figure 1 *– From Objective 1, optimized SPMESH-DART-MS workflow for volatile phenols. Text in red was optimized as part of the objectives. Workflow for C6 aldehyde analysis was similar, except PFBHA derivatization was used in place of acylation. Total workflow, including derivatization, extraction, and analysis, requires ~60 min for 24 samples.*

Wine

	guaiacol	*ortho-cresol*	*4-methyl-guaiacol*
Calibration Range	*6-250 µg/L*	*6-250 µg/L*	*6-250 µg/L*
R^2	*0.99*	*0.99*	*0.99*
LOD	*<1 µg/L*	*<1 µg/L*	*<1 µg/L*
LOQ	*1.6 µg/L*	*<1 µg/L*	*<1 µg/L*
Average %RSD	*6%*	*6%*	*5%*
Time for 24 analyses	*60 min*	*60 min*	*60 min*

Table 1 *– Figures of merit for SPMESH-DART-MS analyses of three volatile phenols*

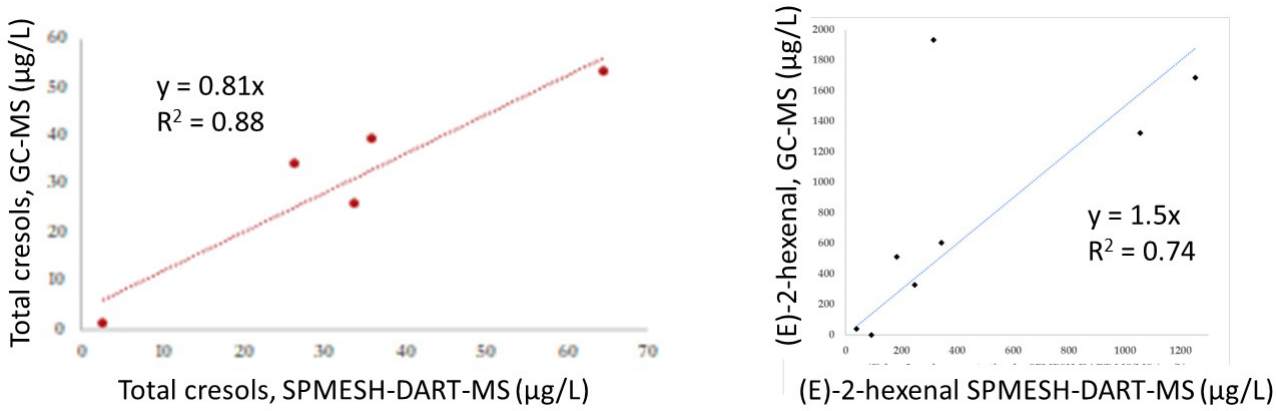

Figure 2 *– Representative validation results from Objective 1 for cresols in wine (left) and (E)-2-hexenal in juice (right). Samples were provided by an industry cooperator, and were analyzed by both the newly developed SPMESH-DART-MS approach and the "gold standard" SPME-GC-MS approach.*

SECTION 3:

Project summary and objectives: Targeted measurements of volatiles or volatile precursors in grapes and wines are useful for quality evaluation, e.g. volatile phenols can be markers of microbial spoilage, and C6 aldehydes markers of grape maturity, but existing analytical approaches are slow and expensive. We evaluated two strategies for increasing throughput and thus decreasing costs for analysis of aldehydes and phenols in juices and wines: sorbent sheet extraction (SPMESH) followed by direct-analysis in real time mass spectrometry (DART-MS) and low-pressure gas chromatography mass spectrometry (LP-GC-MS) were evaluated. Additionally, growing winemaker interest in achieving product differentiation through utilization of "native" microbes has led to development of this research

project aimed at quantifying the benefits and risks of using native microbes for wine production, especially with regard to fermentation kinetics/completion and chemical/sensory consequences on resulting wine.

Importance of research to the NY wine industry: Although analysis of non-volatile macro-components of juices and wines (e.g. TA, ethanol, sugars, malic acid) is routine for New York State winemakers, analysis of trace-level volatiles is non-routine due to its cost. Decreasing the cost of these volatile analyses will allow for their more frequent usage by winemakers and grapegrowers in decision making. Additionally, providing winemakers with quantitative data regarding risks and benefits associated with utilizing “native” microbes, also sometimes called microbial terroir, will allow winemakers to make the most resource-effective decisions for their desired wine production styles.

Project Results/next steps: Chemical transformation (“derivatization”) of aldehydes and volatile phenols prior to SPMESH-DART-MS resulted in excellent detection limits and good agreement with the “gold standard” GC-MS method. The new SPMESH-DART-MS approach increased throughput by 10-fold over the existing method. Follow-up studies will invite NY State winemakers to submit samples for further validation. food ingredients into canned wines and related beverages while maintaining their inhibitory activity. Additionally, for the microbial terroir aim, all of the grape samples and fermentations for this two-year study have been collected and completed. Samples for chemical analysis are currently being processed by an industry collaborator. Quantitative, descriptive sensory analysis will commence in Spring 2023. Metagenomic analysis will also be performed in Spring 2023, after completion of optimizing DNA extraction and PCR conditions. We expect that data analysis will be completed and final manuscripts published in 2023.