NYWGF RESEARCH - FINAL REPORT

Funding for fiscal year: 2023-24

SECTION 1:

Project title: Expanding the range of rapid analysis approaches to semi-polar volatiles and non-volatile precursors in grapes

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New Research ☐ **Continued Research** ☒

Amount Funded **$** 115,126

SECTION 2: (This section should be in depth and akin to an academic report)

Project Summary Impact Statement: Targeted measurements of volatiles or volatile precursors in grapes are useful for grape and wine quality evaluation, but these analyses are often slow and prohibitively expensive for routine use. Over the last several years, the Sacks lab developed a new approach for increasing throughput and decreasing costs for routine volatile analyses by using sorbent sheets (SPMESH) coupled to direct-analysis in real time mass spectrometry (DART-MS).

In 2023, a commercial SPMESH-DART-MS prototype was evaluated, allowing for development of new volatile assays. Although the system showed several improvements in terms of hardware and software integration and robustness, our group determined that further modifications were necessary to achieve acceptable sensitivity for use in wine and grape analyses. Feedback was provided to the instrument manufacturer, and an updated version will be evaluated in 2024.

We also developed new SPMESH-DART-MS methods to allow for the detection of C6 alcohols ("green") and free volatile phenols ("smoky"). We expect to validate these new methods following installation of the new instrumentation.

Objectives:

Objective 1: Using a prototype turnkey commercial SPMESH-DART-MS system, validate newly developed rapid assays for analysis of volatile phenols, methoxypyrazines, and C_6 aldehydes

Objective 2: Expand the range of analytes amenable to rapid SPMESH-DART-MS analyses to include measurement of free vs. bound volatile phenols and C_6 alcohols

Objective 3: Demonstrate the appropriateness of SPMESH-DART-MS for *in situ*, real time sampling of volatiles from wines during fermentation and storage.

Materials & Methods:

For Objective 1, we attempted to validate our recently developed methods for IBMP, C6 aldehydes, and total volatile phenols using a new commercial prototype SPMESH-DART-MS. The instrument

was provided by Bruker. Evaluation used only standards in model juice and wine systems. Grape samples were collected from New York State vineyards, but were not used in analysis due to challenges with instrument performance. The SPMESH-DART-MS system was used to analyze three classes of commonly measured grape and wine odorants using recently described methods

- IBMP ("bell pepper")
- C6 aldehydes ("grassy")
- Total volatile phenols ("smoky").

For Objective 2, we attempted to expand existing the range of analytes for rapid SPMESH-DART-MS

C6 alcohols ("green") – We evaluated the use of pyridine additions to samples to enhance DART-MS sensitivity

Free vs. Bound phenols ("smoky, clove, band-aid") – Previous work had measured total (free + bound) volatile phenols only. To selectively determine free forms of these volatile phenols, the previously described acetylation approach was adapted. Parameters evaluated included:

- pH, 7-13 (lower pH is expected to avoid hydrolysis of bound forms)
- Reaction time
- Ratio of sample to derivatizing agent

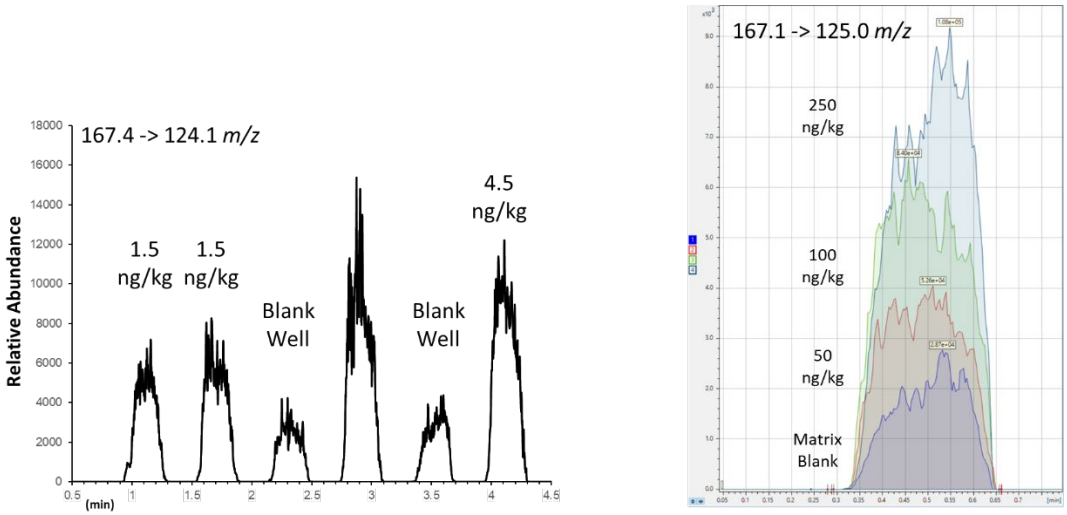
For Objective 3, we attempted to expand existing the range of analytes for rapid SPMESH-DART-MS by using six common fermentation metabolites (ethyl esters, acetate esters, and higher alcohols).

Results/Outcomes/Next Steps:

Progress and Future Work on Objective 1, validation of commercial SPMESH-DART-MS prototype. In 2023, a commercial prototype of a turnkey SPMESH-DART-MS system was produced by an industry cooperator (Bruker) and installed in the Sacks lab. We were the first lab to evaluate this system and validate its use for routine analyses.

	Analysis step	**Previous Thermo Discovery TSQ Max SPMESH-DART-MS**	**New Bruker EVOQ Elite SPMESH-DART-MS**	**Expected updates from Bruker in Spring 2024**
	DART Automation	Minimal = can scan SPMESH sheet in one direction at constant rate Must manually move to next row	Improved = Can scan SPMESH sheet in both X and Y directions automatically Can jump from spot to spot ("Jumpshot") Requires separate software DART and MS control	Further improvement = Fully integrated DART and MS control software
	Data collection and post-processing	Chronogram data stored as "rows" of spots Not amenable to automated post-processing or naming individual spots	Chronogram data stored for individual SPMESH spots Facilitates post-processing, naming, LIMS	

Table 1 – Summary of software and hardware performance for new commercial SPMESH-DART-MS unit as compared to earlier homebuilt systems



	Original Bruker QqQ	New Bruker QqQ
LOD	0.5 ng/kg	20 ng/kg
%RSD	4.8% @ .5 ng/kg	30.5% @ 50 ng/kg

Figure 1- Comparison of detection limits for IBMP ("green pepper" aroma) between earlier homebuilt SPMESH-DART-MS system (left; Bates and Sacks 2022) and new commercial system (right, unpublished data). The new system had unacceptably high detection limits due to issues with the DART to MS coupling, which is expected to be resolved in a newer version available in 2024.

The new SPMESH-DART-MS unit was evaluated in comparison to earlier work. For IBMP, detection limits were over an order of magnitude higher than previous work with a homebuilt unit (Figure 2). A similar loss of performance was observed for total volatile phenols (data not shown). This loss of sensitivity was determined to be due to issues in the coupling between the DART and the MS. This information was shared with Bruker, who is now releasing a new version of the mass spectrometer (EVOQ TQ+) in Jan 2024 with an improved DART to MS coupling.

As a result of the performance issues in 2023, we did not solicit grape and wine samples from NYS wineries and vineyard as originally planned. With the new instrumentation, in 2024-25, we will execute our original plan, and solicit samples from New York State producers (n=300 grape and wine samples), and analyze IBMP, volatile phenols, and/or C6 aldehydes by both our new SPMESH-DART-MS method and conventional GC-MS analyses. If successful, this method can be made available to NY State producers in future years either through their lab or through translation to commercial fee-for-service labs.

Progress and Future Work on Objective 2, developing new SPMESH-DART-MS targets. In 2023, we made considerable progress in extending the range of analytes amenable to SPMESH-DART-MS, including C6 alcohols ("grassy") and selective measurements of free vs. bound volatile phenols. These analytes had not been previously detectable in their native forms (Table 1). As a result, derivatization approaches were utilized.

- For **free volatile phenols**, a derivatization method for total volatile phenols was modified. By performing the derivatization at pH 10.5 instead of 13, free volatile phenols could be selectively converted to their acyl derivatives without hydrolysis and inclusion of bound, glycosylated phenols

- For **C_6 alcohols**, sensitivity and selectivity is improved by addition of pyridine to samples to form pyridinyl adducts in the DART-MS source.

  Preliminary work with these approaches indicates that these approaches will be sufficiently selective and sensitive, but the approaches have not been validated, and figures of merit (e.g. detection limits) have not been determined. In the coming year, we will establish figures of merit and validate these new methods with real samples.

Progress and Future Work on Objective 3, developing SPMESH for measurement of fermentation volatiles. Currently, SPMESH-DART-MS analysis has been optimized for grape juice measurements, but little work has been done on measurements in fermenting musts or wines. The impact of ethanol or other fermentation compounds on targeted measurements of important fermentation volatiles is unknown.

In 2023, we evaluated the response of several representative fermentation volatiles (ethyl hexanoate, ethyl octanoate, ethyl isovalerate, isoamyl acetate). Detection limits were typically in the range of 10 – 100 µg/L on an older DART-MS system, acceptable for monitoring these compounds during fermentation. However, because of sensitivity issues with the new DART-MS unit (see Objective 1), this initial work could not be extended. We expect to continue this work with the new, more sensitive DART-MS unit.

Technology Transfer Plan: An overview of the project was presented to the NYS wine industry at the 2023 BEV-NY conference. Additionally, it is expected that this work should lead to a publication in the American Journal of Enology Viticulture (AJEV) and to an article in Cornell Extension Enology publications (Veraison to Harvest, or Appellation Cornell).

SECTION 3:

Project summary and objectives: Measurements trace-levels of odorants or their precursors in grapes and wines are useful to winemakers, but existing analytical approaches are prohibitively expensive. We evaluated a new commercial system – based on innovations in our lab – for rapid inexpensive odorant analyses. Using this system, we also investigated new approaches to measurements of odorants in grapes, wines, and fermenting must.

Importance of research to the NY wine industry: Analysis of many components of juices and wines, such as acids, sugars and alcohol is routine. However, analysis of most odorants in grapes or wines is prohibitively expensive for many wineries. Decreasing the cost of these volatile analyses will allow for their more frequent usage by winemakers and grapegrowers in decision making.

Project Results/next steps: The new commercial system had several software improvements to improve throughput, but the sensitivity of the system was unacceptably poor for grape and wine analyses. We have provided feedback to the instrument manufacturer, who will be incorporating changes into a new instrument version for us to evaluate in 2024. We also developed new methods for measuring free volatile phenols (“smoky” aroma) and C6 alcohols (“grassy” aroma), which will be validated using the new equipment.