Final Report to the New York Wine and Grape Foundation

Project funded in 2020-21

STUDIES PERTAINING TO ENOLOGY; WINE AND HEALTH; AND GRAPE JUICE AND HEALTH STUDIES

Title: Development of rapid approaches for quantifying key flavor compounds and their precursors in grapes

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Objectives: The overall objective of this work has been to develop new, rapid, and less costly analytical methods for characterizing odorants. Over the course of this multi-year project, we developed an approach we termed solid-phase mesh-enhanced extraction from headspace (SPMESH), in which a mesh is a coated with a thin layer of a non-polar sorbent to allow for extraction of volatile compounds from sample headspace. Following extraction, the SPMESH device can be rapidly desorbed by DART – mass spectrometry (DART-MS) to quantify targeted analytes. Over the long term, this work will make analyses of key odor active compounds in grapes more accessible to both wineries and academics. This will allow producers to better evaluate the effects of grapegrowing and winemaking practices on flavor composition, to perform quality control, or to benchmark their wines.

Our specific 2020-21 objectives were as follows:

1) Evaluate strategies for rapid, parallel off-line equilibration of volatiles in grapes prior to SPMESH extraction and DART-MS analysis
2) Validate SPMESH-DART-MS using a range of commercial grape samples

Accomplishments (Methods, Results, Analysis): This year's project concluded a multi-year project for rapid headspace extraction and analysis of volatiles (Figure 1). In our earliest work (Jastrzembski and Sacks, *Analytical Chemistry*, **2016**; Jastrzembski, Bee and Sacks, *J Ag Food Chem*, **2017**) we demonstrated solid-phase mesh-enhanced extraction from headspace (SPMESH). In this configuration, steel mesh was a coated with a thin layer of a non-polar sorbent. We subsequently adapted our approach to use SPMESH sheets in place of individual meshes to allow for parallel extraction form multi-well plates or other planar surfaces (Bee, Jastrzembski and Sacks, *Analytical Chemistry*, **2018**; Rafson, Bee and Sacks, *J Ag Food Chem*, **2019**). The SPMESH Sheet approach (Figure 2) has been validated against the "gold-standard" approach (SPME-GC-MS) for 3-isobutyl-2-methoxypyrazine (IBMP, "green pepper") and linalool ("floral, Muscat") (Bee-Digregorio, Feng, Pan, Dokoozlian, and Sacks, *Foods*, **2020**). For IBMP, good linearity was observed in the 2-1000 ng/L concentration range. When coupled to DART-MS, SPMESH Sheets could analyze 24 samples in <17 min, or approximately 50x faster than conventional SPME-GC-MS approaches.

Figure 1- Overview of project accomplishments over last five years

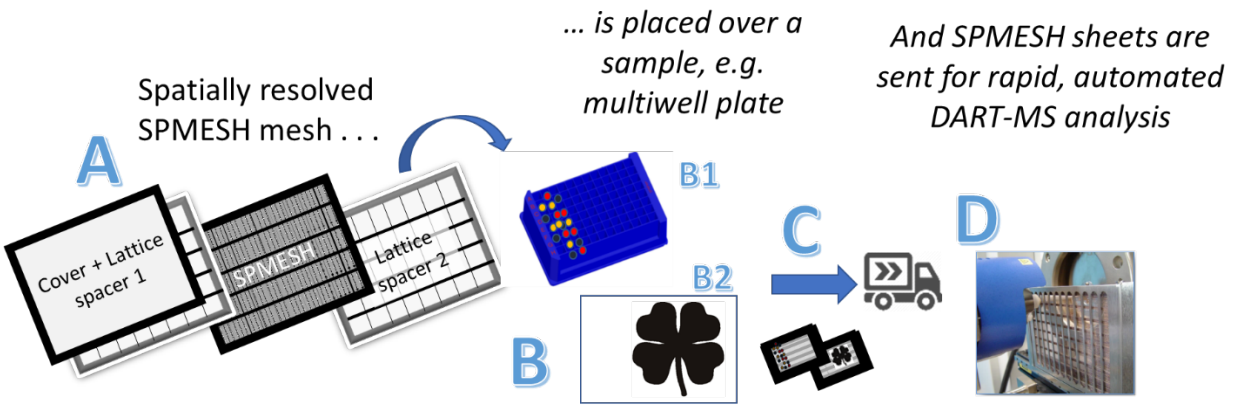


Figure 2 - Overview of newly developed approach: SPMESH Sheets coupled to DART-MS via an automated positioning stage. The approach can achieve low ppt detection limits for 24 samples in 17 min

In the most recent 2020-21 funding cycle we made further progress in developing SPMESH.

2020-21 Objective 1: *Evaluate strategies for rapid, parallel off-line equilibration of volatiles in grapes prior to SPMESH extraction and DART-MS analysis*

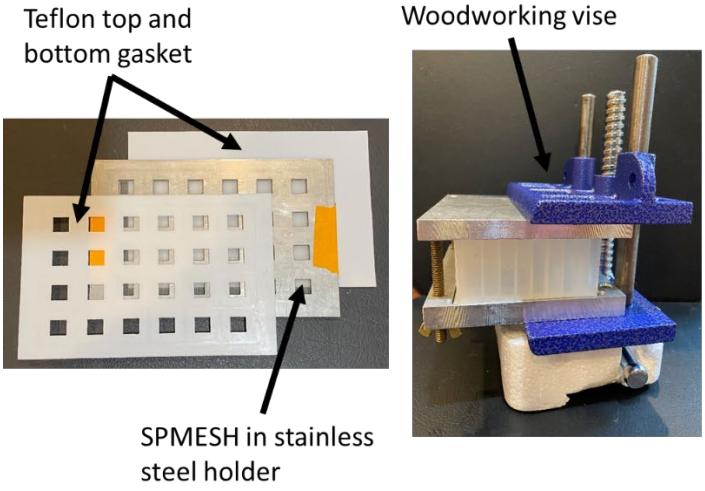
To achieve this objective, we used our SPMESH approach to analyze IBMP on Cabernet franc grapes sourced from Finger Lakes region vineyards.

- Maceration in a Ninja™ blender followed by incubation of the macerate for 30 min at 50°C is sufficient for equilibration.
- Enzymes have little effect on equilibration and are not recommended
- The presence of crushed seeds reduce signal by 3-fold, with no effect on accuracy. This signal decrease is likely due to decreased volatility of IBMP in the presence of seed lipids. Because the effect is small, and because seed removal is tedious, their removal is not recommended.
- Polypropylene multi-well plates can absorb IBMP. Plates should either be baked out or discarded following use.

2020-21 Objective 2: Validate SPMESH-DART-MS using a range of commercial grape samples

Due to Covid restrictions, we were unable to source a suitable number of commercial grape samples, as planned. However, we were able to publish work from a smaller data set of samples collected in 2019 (Bee-Digregorio, et al Foods; Figure 1). This work demonstrated good correlation between our new SPMESH method and traditional, slower SPME approaches for linalool and IBMP.

As an alternative objective, we worked to improve the SPMESH extraction set-up to minimize leakage among adjacent wells. In our initial publications, we would observe leakage of 0.5-1.0% among wells, which we referred to as crosstalk (Figure 3, top right). With the help of a local engineer, we developed new gaskets and clamps for SPMESH that decreased cross-talk to <0.1% (Figure 3, bottom right). These improvements make SPMESH better suited for real samples which can possess a wide range of volatile concentrations.



Crosstalk evaluation: Wells contained either 10 ppb IBMP (gray) or blank (yellow/orange/red)

Original SPMESH setup

2,852	482,440	1,203	764,075	1,318	608,563
552,586	3,910	498,653	12,573	585,129	5,465
1,508	422,133	11,092	713,305	10,764	1,084,836
723,382	537	873,857	2,728	760,530	1,006

SPMESH setup with 2 gaskets and vise

138	561,912	197	507,319	385	1,368,450
418,078	453	390,118	854	1,021,058	2,124
1,717	481,810	544	598,575	453	757,146
917,897	379	892,096	511	739,268	293

Figure 3 - Newly designed gaskets (left) and holder (middle) for SPMESH extraction. The new approach results in ~10-fold lower leakage or crosstalk among adjacent wells (right)

***Future Directions*:** Our work over the last five years has demonstrated that SPMESH-DART-MS can be used for rapid headspace analyses of certain volatiles such as IBMP. However, a major shortcoming is that the approach is not suitable for low volatility odorants (e.g. volatile phenols) or for extraction of non-volatiles (e.g. flavor precursors). This shortcoming decreases the likelihood of widespread adaptation by service labs. In the coming year, we are proposing to extend our headspace SPMESH-DART-MS approach to make it suitable for semi-volatile or non-volatile analytes.