Progress Report to the New York Wine and Grape Foundation

Project funded in 2019-20

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 proposal is to develop new, rapid, and inexpensive analytical methods for characterizing grape-derived flavor compounds or their precursors. In previous years of this project, we have 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. 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 2019-20 objectives are as follows:

1) Evaluate several strategies to improve detection limits for a new, high throughput method for trace volatile analysis (SPMESH-DART-MS)
2) Apply both SPMESH-DART-MS and traditional SPME-GC-MS to rational, targeted analysis of novel odorants in grape germplasm

Progress towards Objectives

In previous work (Jastrzembski and Sacks, *Analytical Chemistry*, 2016 and Jastrzembski, Bee and Sacks, *J Ag Food Chem*, 2017) we demonstrated solid-phase mesh-enhanced extraction from headspace (SPMESH), in which steel mesh is a coated with a thin layer of a non-polar sorbent. The coated mesh can then be positioned in the headspace of a sample, e.g. grape macerate, before being desorbed into a DART-MS system. Linear responses were observed in the 10-1000 ng/L concentration range for analysis of ultra-trace level odorants like 3-isobutyl-2-methoxypyrazine (IBMP, "green pepper"), with individual analyses requiring only 15 seconds. However, this approach was not automatable. More recently we adapted our approach to use SPMESH sheets in place of individual meshes. Volatiles could then be extracted in parallel form multi-well plates prior to DART-MS analysis. However, tour initial attempts at this approach suffered from poor sensitivity and cross-talk among wells.

In our most recent 2019-20 funding cycle, we had the following two objectives:

Previous Objective 1: *Evaluate several strategies to improve detection limits for a new, high throughput method for trace volatile analysis (SPMESH-DART-MS)*

Over the last year of funded work (2019-20), we observed that a using tandem MS (MS/MS) in combination with SPMESH could improve detection limits for a key grape odorant, 3-isobutyl-2-methoxypyrazine (IBMP, "green pepper") by an order of magnitude (Bee-DiGregario, Feng, Pan, Dokoozlian, and Sacks; *Foods;* **in review**). We also evaluated several other strategies for improving detection limits, including using solvent desorption instead of DART thermal desorption. This approach did not improve detection limits, and was not further explored (Bee-DiGregario, Rafson, and Sacks, **in preparation**)
We then evaluated the new SPMESH-DART-MS method using commercial grape samples. SPMESH-DART-MS required about 1/10th the time for analysis as SPME-GC-MS. We could also achieve good correlation between SPMESH-DART-MS and the gold standard SPME-GC-MS

method, but only when a heated equilibration step was used prior to SPMESH extraction (Figure 1) (Bee-DiGregario, Feng, Pan, Dokoozlian, and Sacks; *Foods;* **in review**).

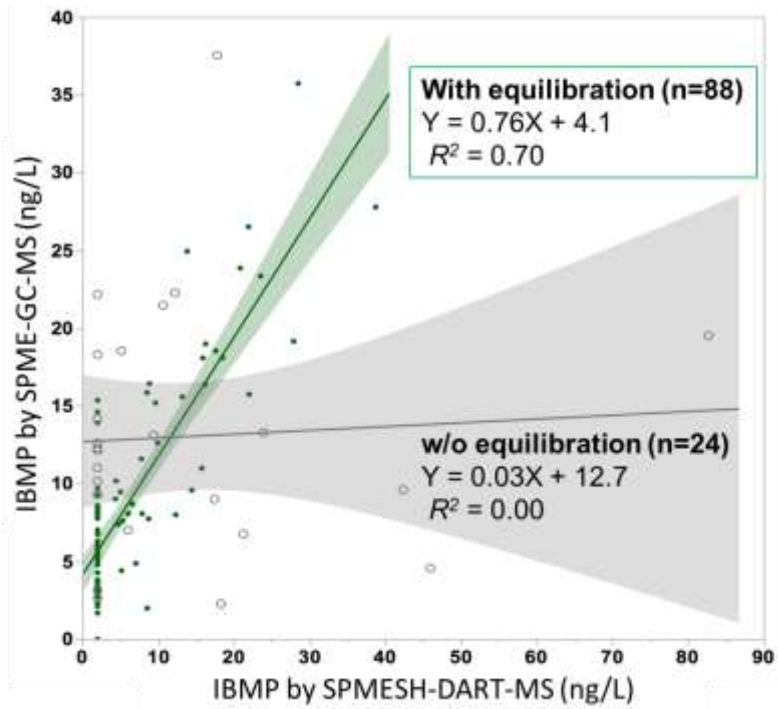

Figure 1 – Validation of SPMESH-DART-MS method (x-axis) against the gold standard SPME-GC-MS method for measurement of IBMP in commercial Merlot and Cabernet Sauvignon grape samples. Without equilibration (black line), poor agreement is observed between the methods. With equilibration (green line), much better agreement can be achieved. Image reproduced from Bee-Digregario, et al Foods, ***in review.***

Previous Objective 2: *Apply both SPMESH-DART-MS and traditional SPME-GC-MS to rational, targeted analysis of novel odorants in grape germplasm*

Using data from Dunkel, et al (*Angew. Chem.* **2014**), we narrowed a list of known volatiles in all foods and beverages (~10000) to about 220 compounds known to be important to aroma. This list was further curated to 74 odorants by eliminating compounds that would be unstable in a wine matrix due to reactivity at low pH with either sulfites or water, instability in the presence of yeast metabolic activity, or due to poor solubility in hydroalcoholic solutions. Finally, odorants for which good sources already exist from grape or yeast were eliminated (e.g. ethyl esters, linalool). A summary of this process is shown in Figure 2A. The final list had 26 target, novel odorants. Representative examples of these odorants are shown in Figure 2B.

Out group harvested 90 accessions from the USDA-GGRU Cold-hardy Grape Collection (Geneva, NY) in 2019. We are currently developing SPME-GC-MS based methods to screen the accessions for these compounds. If accessions with high concentrations of targets are identified, this information can be passed along to grape breeders for them to use in their breeding programs.

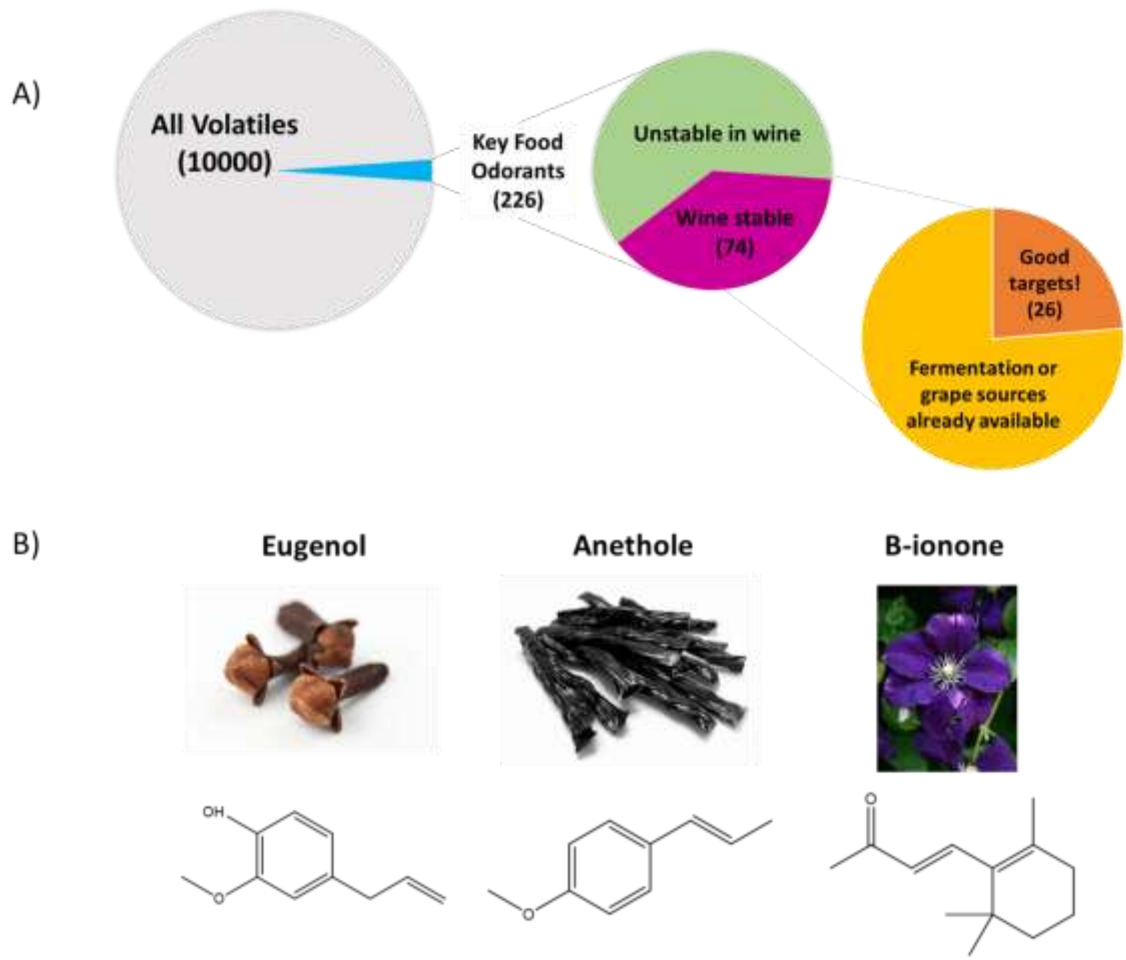

Figure 2 – A: Workflow for reducing target odorants from all known food and beverage volatiles (10000 or more) to 26 compounds that should be stable in wine, but are not currently sourced at high concentrations from grapes, yeast, or other materials. B: Three representative odorants, identified using the workflow in 2A.