Real-Time *In Situ* Detection of Volatile Profiles for the Prevention of Aflatoxin Fungal Contamination in Pistachios

Tiziana Bond, PhD, Senior Engineer, Material Engineering Division, Lawrence Livermore National Laboratory
Allan Chang, Engineer, Center of Micro and Nanotechnology, Lawrence Livermore National Laboratory
Jenny Zhou, Student, Lawrence Livermore National Laboratory

INTRODUCTION

The objective is to demonstrate the feasibility of a Raman, *in situ* warning system for detecting and removing developing fungal hot spots from pistachio stockpiles and transit containers, thus decreasing health risks and product loss because of contamination. The proposed project has the following goals: to calibrate the Raman fingerprinting of biomarkers, stand-alone and in premixed samples; to build a database with the vibrational profiles distinctive to the signatures of the bouquet emitted by the (contaminated or not) pistachios; and to test the improvement in the detection of the detectable markers with enhanced Raman on a small probe. Specifically, for this first year, we have been focusing on providing Raman system calibration, single and mixed gas-samples signatures.

RESULTS

The project started in June 2017, when DoE approvals were received. The progress reported is for the period June-September 2017. We have worked closely with our collaborator, Dr. John Beck, at USDA-ARS, who has developed volatile emission profiles for pistachios and almonds in different humidity conditions, which evolution could be associated to fungal growth and therefore provide early-warning detection signals. We have selected the most significant components of the bouquet gathered by Dr. Beck's team, i.e., the most significant markers of growth conditions, and investigated their Raman signatures. We initially started our measurements with samples provided by Dr. Beck's team and subsequently procured our own. We have examined isobutyraldehyde, methyl salicylate, ocimene, limonene, pentanal, specifically and also analyzed 5-methyl-furfural, isobutanol, heptanal for almonds as both nuts suffer from similar diseases and commonalities could help support any conclusions.

First, we took a Raman measurement of all the analytes in liquid phase to retrieve and identify the basic spectrum signature, as the signal would be stronger. This was done for each analyte separately. For these measurements we used a portable Raman system, a Delta-Nu Inspector Raman that was positioned on a stand in our lab for the tests but which was designed to be used also as a battery-powered hand-held system for *in-situ* real-time measurements. Power required for the operation was <30mW. The wavelength of operation was 785nm, selected for reducing the fluorescence background, which is also removed by postprocessing during which careful selection of the averaging parameters must be chosen to avoid removing the signal itself or, on the contrary, including too much noise. In the typical Raman plots that we analyzed, the reported spectrum intensity vs. Raman shifts represented the peculiar molecules-excited roto-vibrational modes, scattering the incident light back inelastically and thus with an inherent slight wavelength shift.

Once we had verified we had signals for the samples under consideration, in liquid phase, we proceeded to test 2uL droplets on metallic nanostructured substrates (by Silmeco) for Surface

Enhanced Raman Spectroscopy (SERS) signal. These samples provided enhancement of *both* the incident and the scattered electric field *amplitude* providing a power of 4 light *intensity* increase. All SERS measurements were between 0.5 and 5 secs long (10x-100x shorter than in normal Raman) and averaged over >5 regions of each Silmeco slide and several repetitions. We have taken measurements at various time intervals, from hours to days, to monitor temporal evolution of the signal. All substrates presented a strong signal after they were exposed to the chemicals ("initial reading") as expected since the droplet provided a large volume contributing to the bulk-like Raman signal.

Figure 1. SERS signals for several analytes

However, we observed some peak intensity increase, most likely to be attributed to the enhancement of the signals from the molecules in proximity of the nanostructure (SERS). Five out of eight chemicals—methyl salicylate, ocimene, heptanal, 5-methylfurfaral and isobutyl aldehyde, (only 4 in Fig. 1)—show a strong signal after several days, indicating a strong affinity of the molecules to the substrate and suggesting the potential of the technique for gas phase detection. We were also able to measure nixed signal, specifically methyl

Figure 2 Gas-phase metylsalycilate

salicylate on an isobutyl aldehyde-coated sample, indicating that the former has a higher vapor pressure. Thus, we have performed a very simple experiment to prove gas-phase detection of the methyl salicylate. We have filled a vial and taped the substrate on its cap so that only the vapor from the analyte could reach the substrate, once the vial was capped (Fig. 2). The signal appears very strong after hour one (first measurements) and increases until it reaches some saturation at about three days, afterwards the sample is moved away from the vial and the signal decays as expected. We postulate that the first hour a partial monolayer is created and then continues to rearrange, even in multiple layers, until the molecules are too far removed from the surface and cannot exploit the enhancement induced by nanostructures, that afterwards, the molecules tend to diffuse away since the adsorption is only based on VanderWaal forces (physisorption) and not covalent ones (chemisorption).
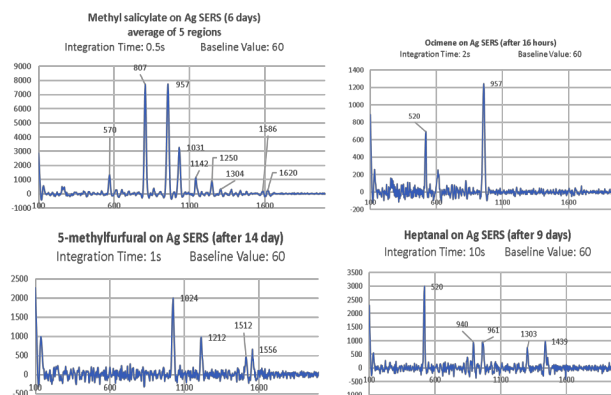
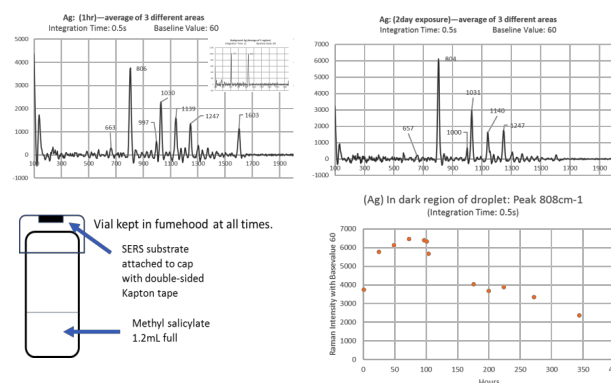## CONCLUSION AND APPLICATIONS

We verified both Raman and SERS signatures of various analytes representative of the pistachios' (and almonds') breaths and fungi growth markers when related to humidity conditions. For the rest of the funding period we are planning to provide gas-phase analysis for the same group of chemicals. Preliminary results for methyl salicylate vapors provide confidence on the technique. We will aim at controlling temperature and humidity, and providing data with enough spatial and temporal granularity to understand the evolution of the signal, which is critical information for technology development for any field application. For the

analytes not providing strong SERS signals, we postulate that either the equilibrium vapor pressure is very high and leading to quick evaporation, or the affinity between the metallic nanopillars and the molecules is low. We will further investigate these cases since a higher volatility could be beneficial in the gas-phase detection.