

Metabolomic Analysis of Citrus

Project Leader:

Pat Unkefer

Bioscience Division, Los Alamos National Laboratory

Metabolomics is the study of the entire metabolome within a system, allowing for a more comprehensive look at how that system functions and reacts. The overall objective of this project is to establish metabolomics as a useful tool to analyze the metabolic state of citrus (navel orange). This tool will be applied to characterize the effects of such agriculturally important factors as changes in field management practices such as nutrient regimes, growth regulator use, timing of harvest and postharvest efforts, etc.

The present project sought to develop metabolomic profiles in navel oranges. The juice and two peel fractions were the focus of the work. Samples were taken from oranges near and at the end of orange development; these oranges were also subjected to taste panel testing as well. The two sets of data are thus available for examination for potential correlations between the sets of results.

This project required that we develop methods for extraction and mass spectrometric analysis of metabolites in the orange fractions as such analyses had not been performed on oranges by any previous workers. This requirement has been met. It is a very labor-intensive process to develop such methods, and a significant amount of the funding was required for this phase.

Because of the three-month stand-down of Los Alamos National Laboratory (mid-July to mid-October 2004), all projects were delayed with respect to their calendars. In an attempt to compensate the Citrus Research Board for this delay, I discussed with several members of the Board who visited LANL in July if they would be agreeable to having the oranges analyzed on the new ICR-Mass spectrometer which would give much more information regarding the identity of the metabolites in the profile as compared to the original scope of this work which did not include accurate-mass based identification of any of the metabolites in the profile. They concurred that they would like to have this information even though they understood that the instrument was at that time being installed and would require calibration and a shake-down phase before efficient operation would be assured. The bottom line was more information for the same amount of work and expense but with a bit of a time delay.

We completed the calibration and set-up of the instrument and immediately dedicated it to the orange samples. An initial survey of the juice and peel fractions revealed approximately 300, 250, and 180 metabolites in the juice, albedo, and flavedo fractions respectively in the SPE bound fraction; this fraction ultimately gives rise to the hydrophilic fraction which can be expected to differ in its composition from the volatiles analyses such as performed by David Obenland (USDA-ARS) and others. The volatiles are those compounds known to be directly associated with such sensory-active characteristics as flavor and aroma. The hydrophilic compounds can be expected to include metabolites in central metabolism, an aspect of the overall metabolism in oranges that has not been as well investigated and characterized. Thus, these two analyses compliment each other. The SPE unbound fraction was also analyzed, even though it contained

significantly fewer metabolites. A database was created for this data set as well; it includes the FT and linear trap datasets as for the SPE bound fraction.

The first samples analyzed were the juice from the sample oranges. The juice was extracted in the absence of the peel. Mass spectral analyses have been obtained for these samples. A database of accurate mass metabolites has been created. In addition, a database of the linear-trap mass spectra of the metabolites in these samples has been created.

As mentioned above, we are attempting to deliver accurate-mass enabled metabolite identification to the CRB even though it was not in the original scope of work. A list of the metabolites with their accurate mass (to fourth and sometimes fifth decimal accuracy) is in the process of being generated for the orange metabolites. The accurate-mass-enabled metabolite identification depends on resolving some software issues with our software provider which will be done at LANL expense. The draft list with names associated for the metabolites will be derived from a comparison of the orange data with a master list of known metabolites and their respective accurate masses calculated for the mass spectral identification as ions (developed by LANL's metabolomics projects as a separate current activity).

The two orange peel fractions are being extracted; they will then be analyzed with the ICR-MS to provide accurate mass data sets for them as well.