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Food Classification and Authenticity Testing Using a New High-Resolution LC/QTOF and Novel Classification Software

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Introduction

Routine food authenticity testing using the new MassHunter Classifier 1.0 Software.

Adulteration of food products is a growing issue and food manufacturers are increasingly interested in checking the quality and authenticity of ingredients used in products. Chemometric profiling of the ingredient's chemical components with mass spectrometry and using multivariate statistical models to classify an unknown sample is a desirable way to determine if an ingredient is pure or adulterated.

In order to simplify the analysis for a technician to routinely perform the multivariate modeling, new software has been introduced to complete the food authenticity workflow (Figure 1). A method development scientist builds the method using traditional feature extraction and statistical modeling software but then hands the analysis method and model file off to a technician to use with a simplified software for routine analysis, MassHunter Classifier.

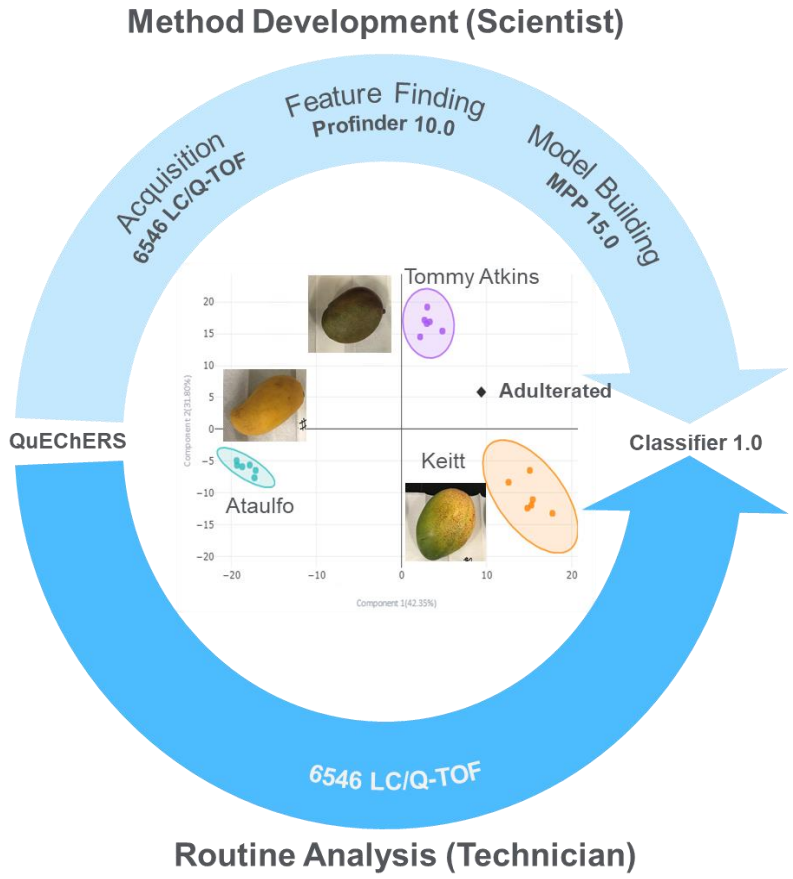

Figure 1: Complete food authenticity workflow for the method development scientist (top) and for routine analysis (bottom). The Classifier 1.0 software gives clear authenticity answer and simplified plots based on the model data given in the method (center).

This workflow was demonstrated with a proof of concept study using three mango varieties: Keitt, Ataulfo, and Tommy Atkins. The model was developed using the method development workflow (Figure 1, top) and then deployed for routine analysis in Classifier 1.0 (Figure 1, bottom).

Experimental

Mango metabolite profiling using the high resolution 6546 LC/Q-TOF.

For the model, six samples per mango variety were processed with a QuEChERS extraction kit. Positive (pure) and negative (adulterated) quality controls (QCs) were processed in the same way (Figure 2). The samples were injected on a 1290 Infinity II LC coupled to a 6546 LC/Q-TOF and full spectra data from *m/z* 50-1000 were collected. A deuterated pesticide was added as internal standard by the autosampler to monitor consistency of injections. Two weeks after the model data was collected new QCs were prepared to check model longevity.

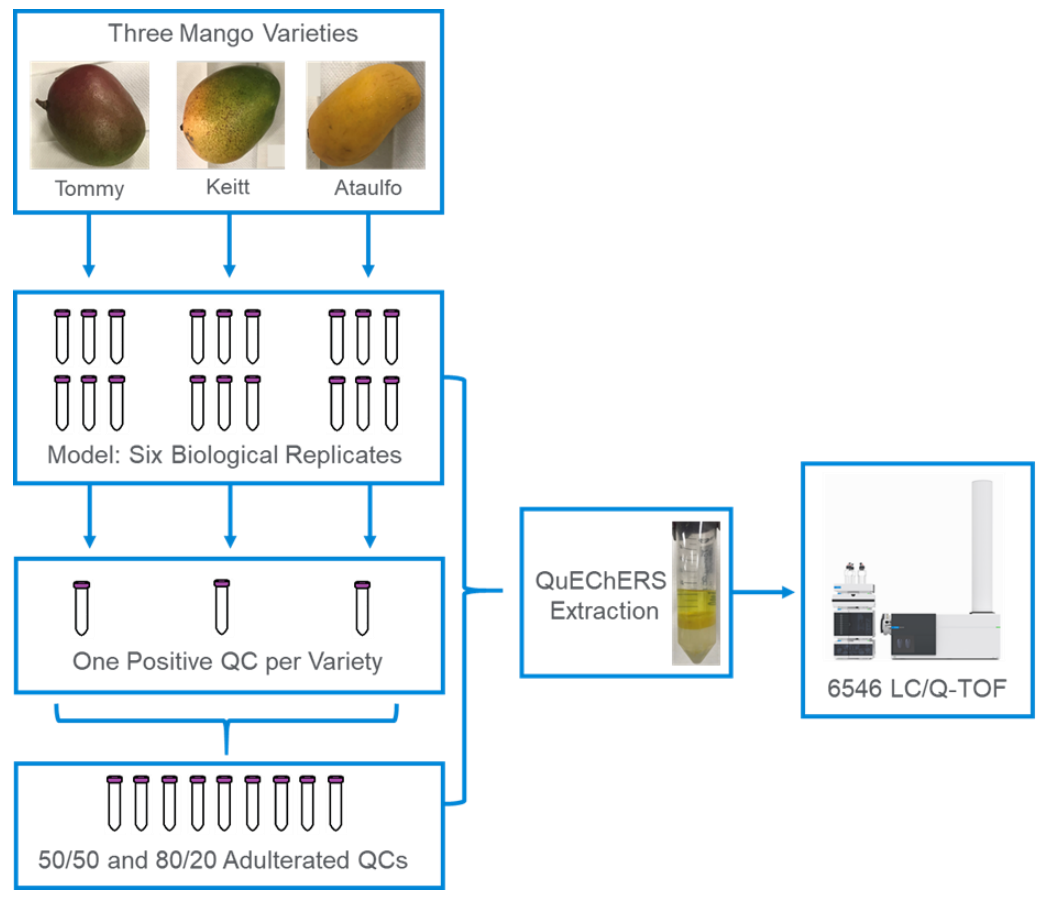

Figure 2: Sample and QC preparation scheme. Six mangos per variety were homogenized and processed with a QuEChERS EN kit. A positive QC was made for each variety by pooling the remainder homogenate. Then the positive QCs were mixed at different ratios to create negative QCs. The QCs were processed separately from the model samples. All were randomized and injected on the LC/Q-TOF.

To develop the routine method, the model samples were processed with Profinder 10.0 and Mass Profiler Professional (MPP) 15.0. Batch recursive feature extraction was used in Profinder and, after filtering for significant features, a Partial Least Squares Discriminant Analysis (PLSDA) model was built and exported. Then the developed model and method were used with Classifier to analyze pure and adulterated QC samples.

The 6546 LC/Q-TOF yielded reproducible data for building a model to classify mangos.

The internal standard had less than 6% area drift and 2 ppm mass error over the experiment without any daily cleaning or calibration needed (Figure 3). The retention time did not shift significantly over the whole experiment.

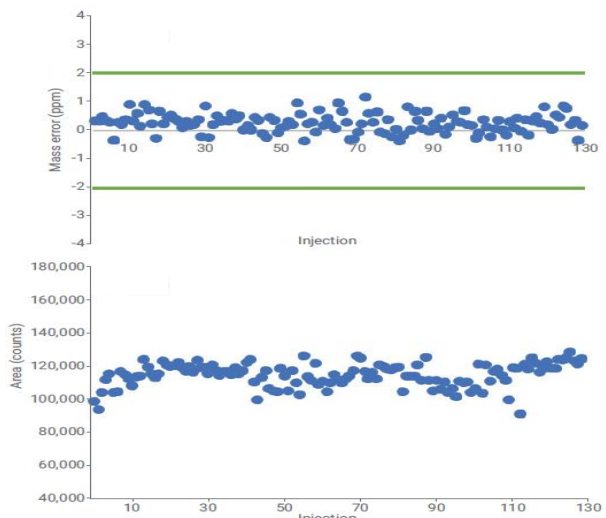

Figure 3: Reproducible internal standard results for the samples. Each injection contained 50 ppb dimethoate – D_6. It had a mass error less than 2 ppm and an area count variation of 6%. A robust analytical platform like the 6546 helps models last with longitudinal sample sets.

Profinder found a total of 4,185 features between the three mango varieties (n = 18). Because of the high quality of data, no normalization was needed on this data set. The statistical parameters and number of important features remaining after each step are described in Figure 4. A total of 481 features were found to be suitable and these were used to build the PLSDA model. High signal thresholds and strict statistical parameters were used to find robust features to differentiate the mango groups. Using robust and consistently detected features the model has the ability to last for a longer period of time.

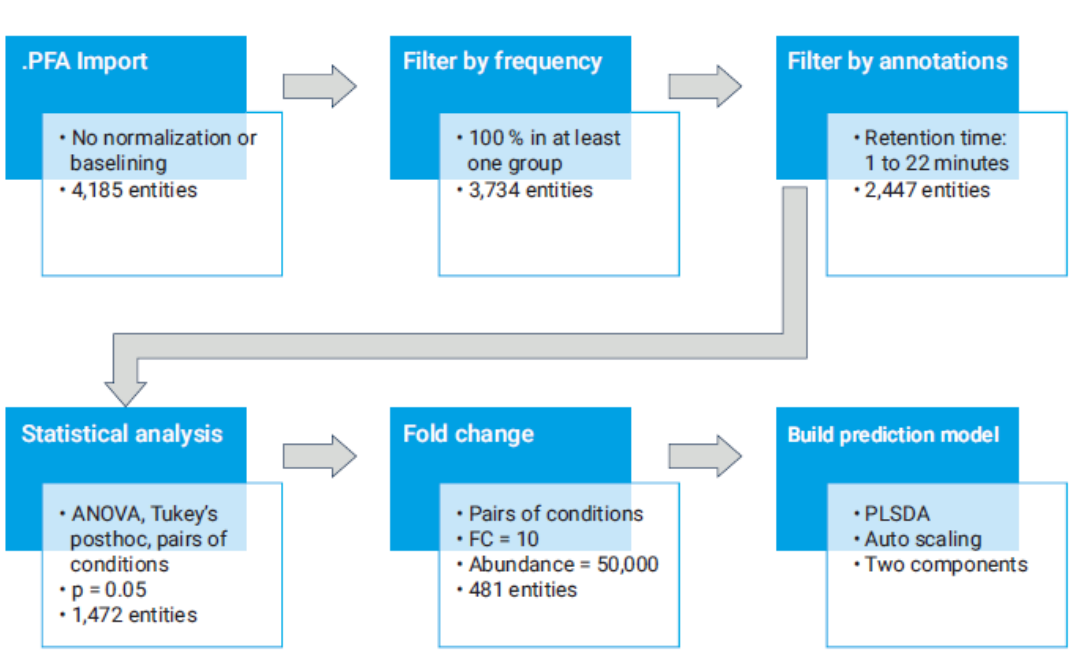

Figure 4: MPP parameters used to determine the 481 differentiating features used to build the model.

Classifier 1.0 and improvements to Profinder and MPP made developing and deploying a model for food authenticity simple and straight forward.

Classifier required the PLSDA model file and Profinder method but neither program needed to be opened to classify a new sample. Instead, the Classifier interface was very simple for inputting the method and sample information (Figure 5) and produced fast classification results (Figure 6).

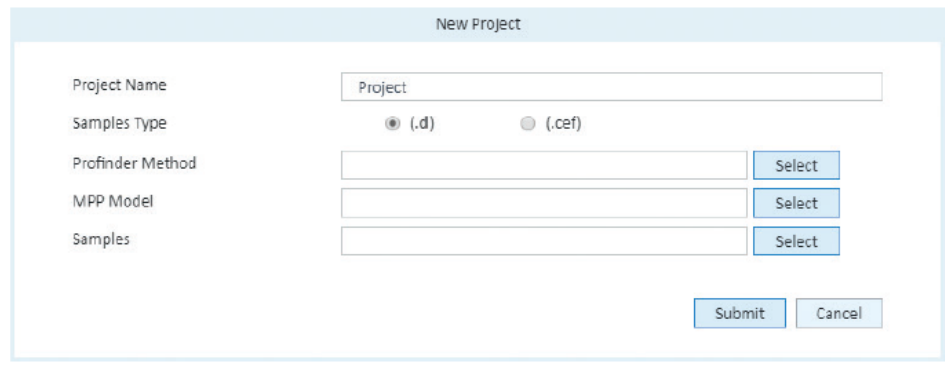

Figure 5: New Classifier project inputs require the method, model and new samples data files. This user interface is easy to use and understand.

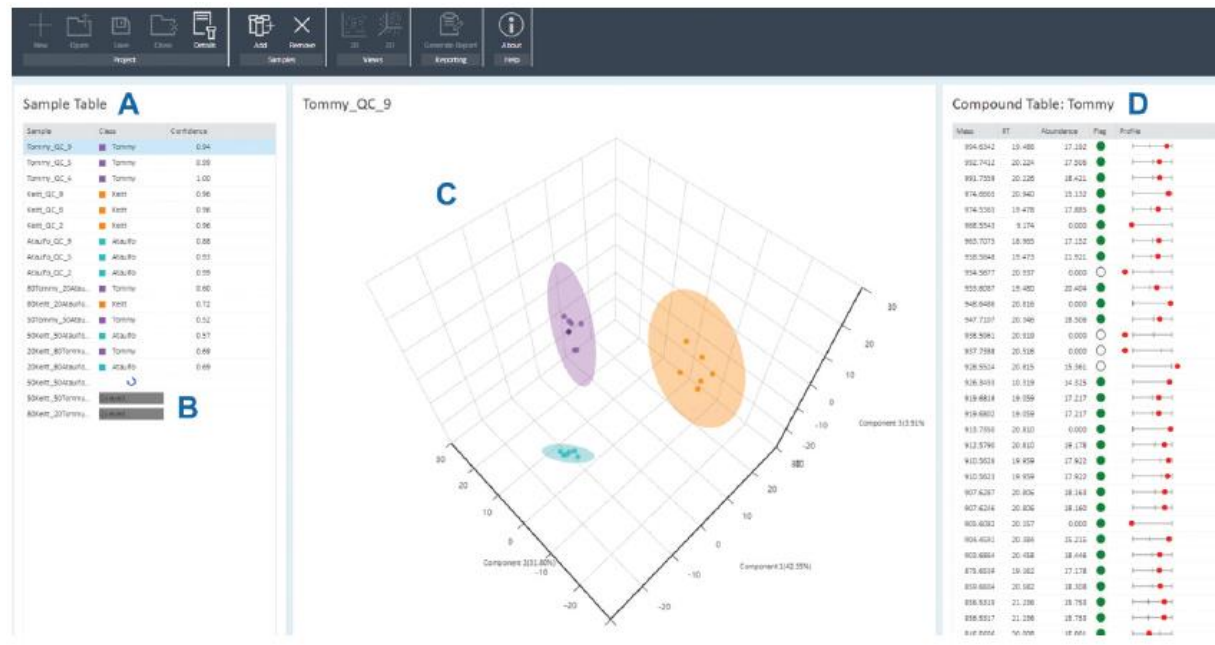

Figure 6: Classifier results show the group classification in the Sample Table (A) for analyzed samples while unanalyzed samples are queued (B). The PCA plot (2D and 3D) for the model is shown in the center (C) and the selected sample appears on the plot as a black diamond. The groups here have Hotelling's that correspond to 95% confidence interval. More detailed information for the analytes used in the model is displayed in the Compound Table (D). Features that were present in the sample within the signal range expected by the model are marked with a green dot, while those that did not or were missing, are marked with a white dot.

Results and Discussion

Confidence values are a numerical way to indicate if a sample is pure or adulterated.

In Classifier software, pure and adulterated QC samples were submitted with the PLSDA model. The software took only a minute to classify each new data file. Each sample was classified as the proper mango variety. Pure samples had high confidence scores (>0.8). In the case of adulterated samples, the software predicted the mango variety it was majority composed but gave a much lower confidence score (<0.8). When the confidence scores were plotted indicating both variety and purity, it was easy to see how they can be used to differentiate a pure and adulterated sample (Figure 7). Using a confidence threshold of 0.8, the accuracy of this model was 100% on day 1 and day 14 of using the model to classify pure and adulterated samples (n = 63).

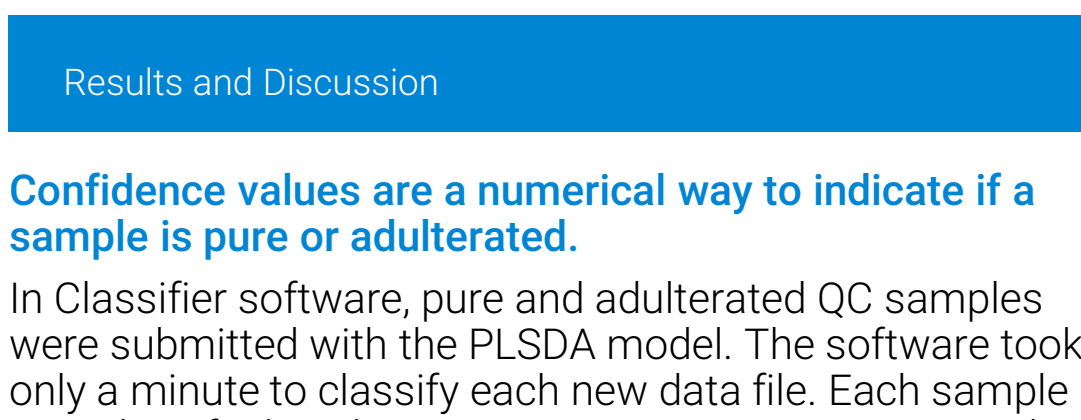

Figure 7: Keitt, Tommy, and Ataulfo mango samples were all classified in the correct group. When the sample was 100% pure a high confidence value was given. However, when the sample was adulterated, a lower confidence score was reported. The samples collected immediately after the model was built (day 1) and new samples collected two weeks later yield similar results.

Based on the confidence value alone, a determination for a sample being pure or adulterated can be made. However, more information for interpreting the results is also given. The PCA plots (2D or 3D) have 95% confidence Hotelling's surrounding the model samples for each group. This information is built into the model file. The sample that is selected from the Sample Table appears as a black diamond on the plot. If the sample is pure, it falls neatly into the 95% confidence interval of the group (Figure 8, A and B). But when it is adulterated, it falls outside of the confidence interval (Figure 8, C and D). Furthermore, the Compound Table (Figure 6, D) gives information on how the features are behaving in the unknown sample compared to the model for that group.

PCA plots display model samples with confidence intervals and plot the new sample in the same space.

Limitations for adulteration testing still exist. Pure, authentic samples are needed for building the model and model longevity may be an issue depending on the robustness of the differentiating features. However, this workflow introduces a new software that allows for routine classification analysis to occur without needing complex statistical software every time a new sample needs to be classified.

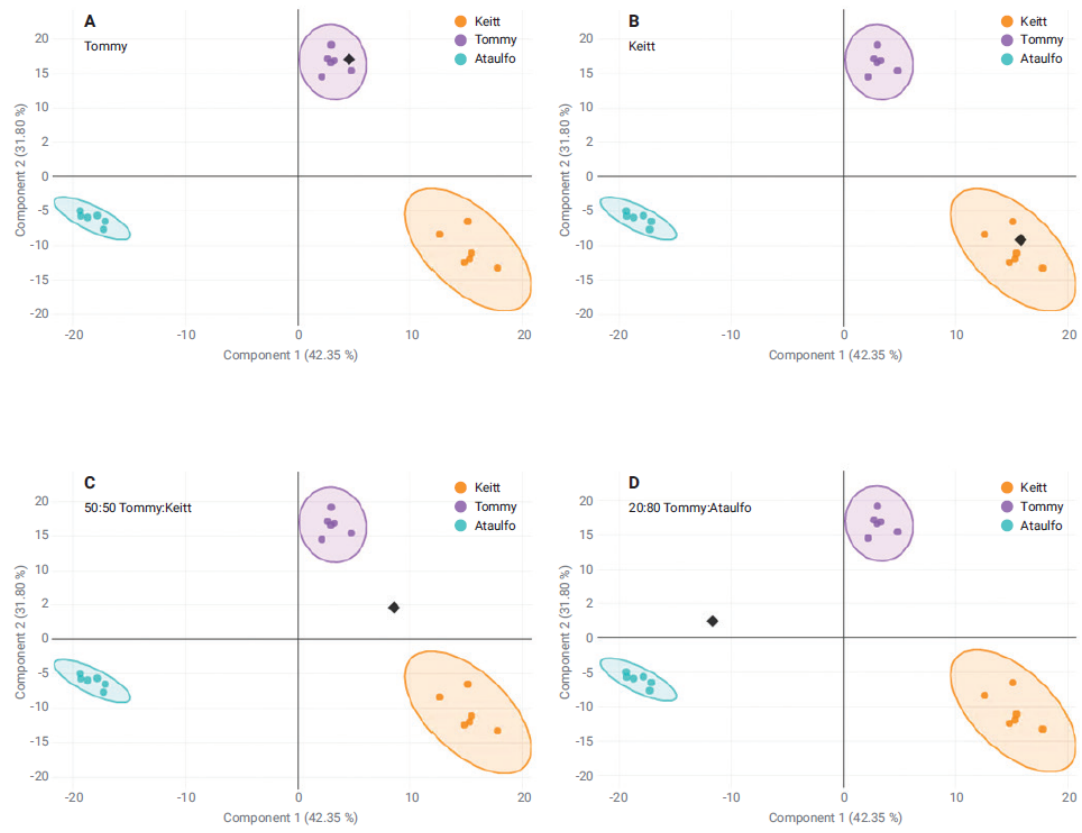

Figure 8: PCA plots for the mango varieties with four different samples plotted. A) pure Tommy Atkins, B) pure Keitt, C) 50/50 Tommy and Keitt and D) 20/80 Tommy and Ataulfo.

Conclusions

Classification can be routinely performed to determine if a food sample is pure or adulterated.

- The new MassHunter Classifier software enables user-friendly and high-throughput food authenticity analysis for lab technicians.
- Mango samples can be classified as pure or adulterated using the confidence values.
- PCA plots show how close an unknown sample is to the group of model samples.

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