Poster Reprint

**ASMS 2020**
ThP 184

# Black Pepper authenticity workflow using high-resolution GC/Q-TOF

Sofia Nieto, Melissa Churley

Agilent Technologies, Santa Clara, CA

## Introduction

Black pepper, as a highly valued commodity, is known to be subject to economically motivated adulteration [1]. Here we present a novel workflow which utilizes a high-resolution GC-QTOF and classification modeling to be able to distinguish two pepper samples from different geographic regions as well as to identify adulteration of black pepper samples. Two tested "adulterants" to black pepper were Szechuan pepper and papaya seeds, the latter of which is known to be used in the adulteration process.

1.https://www.sciencedirect.com/science/article/abs/pii/S0956713519300842

## Experimental

Adulteration of Malabar black pepper was studied. Black pepper from two different geographical regions (Malabar, from India, and Phu Quoc, from Vietnam), Szechuan pepper and papaya seeds were ground. In separate sample groups for each adulterant, Szechuan pepper and Papaya seeds were mixed in varying proportions to Malabar to mimic 5 – 50% adulteration. Pure samples of each material listed above were used to build the classification model. Positive controls consisted of pure samples, and negative controls consisted of Malabar mixed with either Szechuan or papaya seeds. All samples were extracted sequentially using hexane and acetone. Method blanks were prepared using the same solvent. The extracts were combined and filtered through 0.45 µm nylon filters and analyzed in random order using a 7890 GC coupled to a high-resolution Q-TOF MS in full acquisition mode.

| GC and MS Conditions: | Q-TOF (7250) |
|---|---|
| GC | 7890 |
| Column | 30-5MS UI, 15 m, 0.25 mm, 0.25 µm |
| Inlet | MMI, 4-mm UI liner single taper w wool |
| Injection volume | 1 µL |
| Injection mode | Split, 10:1 |
| Inlet temperature | 280°C |
| Oven temperature program | 50°C for 2 min; 10°C/min to 300°C, 10 min hold |
| Carrier gas | Helium |
| Column flow | 1.2 mL/min |
| Transfer line temperature | 300°C |
| Quadrupole temperature | 150°C |
| Source temperature | 200°C |
| Electron energy | 70 eV |
| Emission current | 5 µA |
| Spectral acquisition rate | 5 Hz |
| Mass range | 45 to 650 m/z |

Table 1. GC/Q-TOF acquisition parameters.

## Experimental

The retention indices were calculated based on the alkane ladder to ensure correct compound identification. The GC/Q-TOF data were processed using the Unknown Analysis tool of MassHunter Quantitative Analysis Software 10.1, Mass Profiler Professional 15.1 and Classifier 1.1. The conditions are described in detail in Table 1.

## Results and Discussion

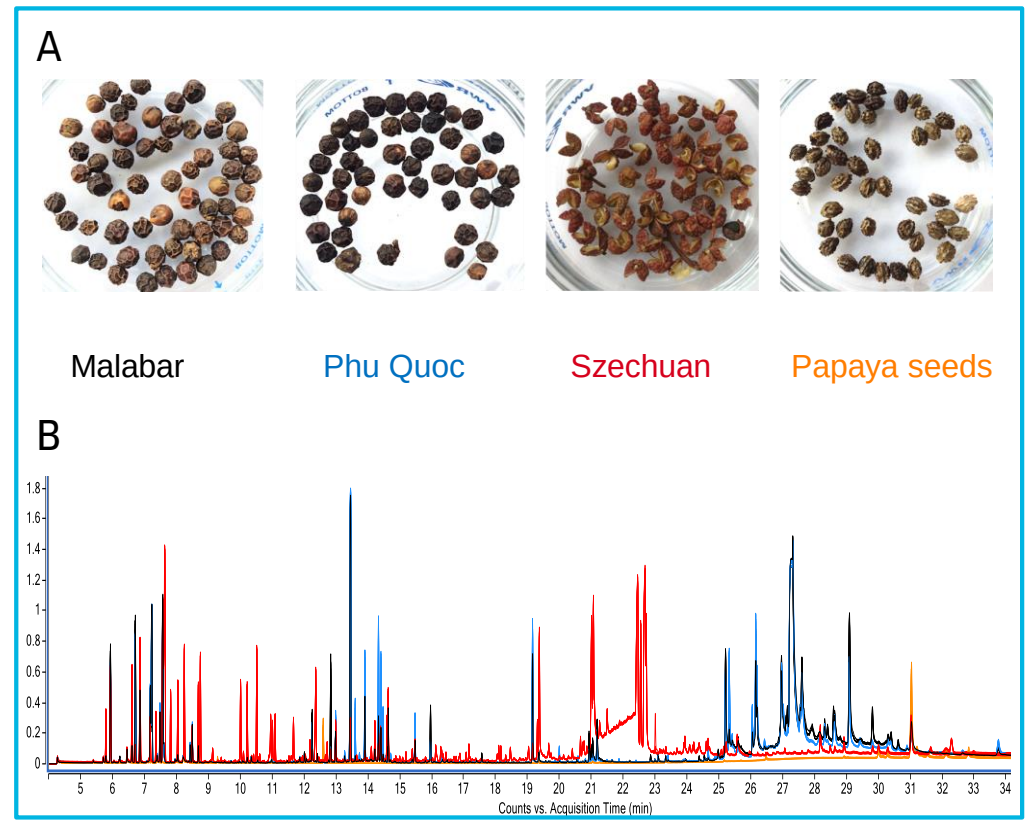


Figure 1. A) Pepper and papaya seeds samples B) Chromatogram overlay of 6 replicates of each extract. The colors in the chromatogram in B correspond to the colors shown in A. Papaya seeds extract (orange color trace) was found to be relatively clean versus the more complex pepper samples.

### Classification Model Building

Six replicate extracts of each type of pepper – Malabar, Phu Quoc, Szechuan, as well as papaya seeds were used to build the classification model in Mass Profiler Professional (MPP). General workflow for building a classification model and processing unknown samples is shown in Figure 2. The first step involves data acquisition for the pure unadulterated samples as well as positive and negative controls. The accurate mass GC/Q-TOF data were processed in the Unknowns Analysis to perform chromatographic deconvolution and NIST17 library search (Figures 2 and 3). Next, the data were exported from Unknowns Analysis to MPP.

## Results and Discussion

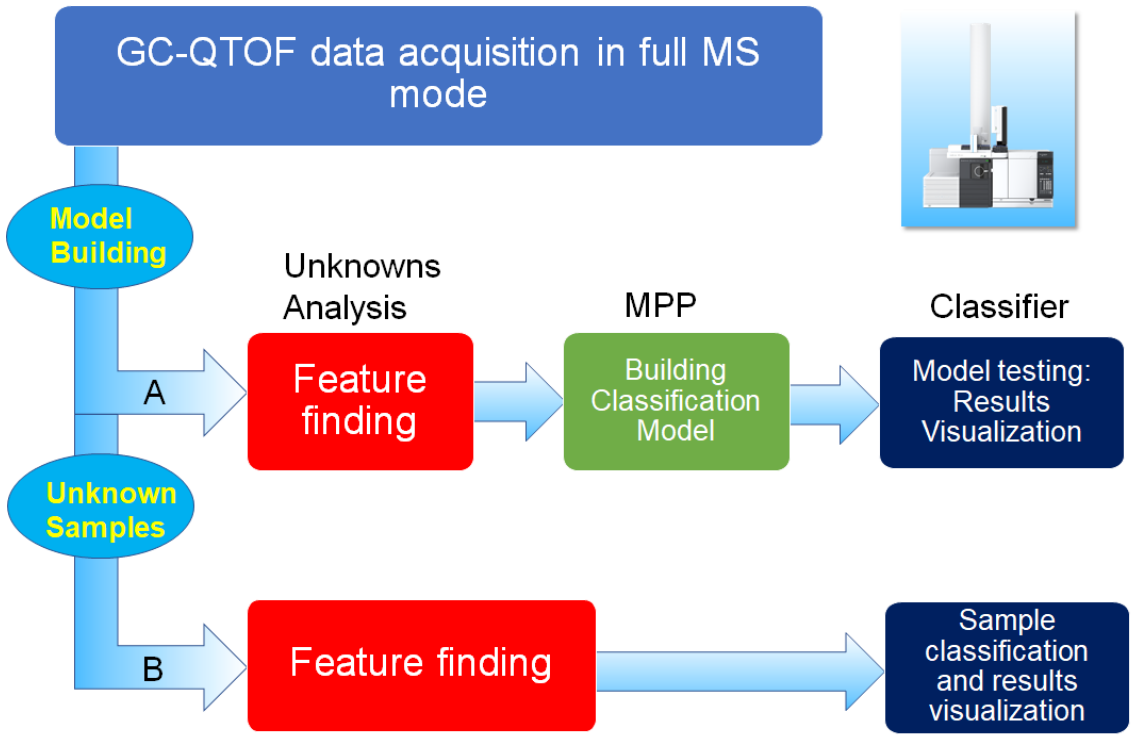


Figure 2. Workflow overview for a classification study using GC/Q-TOF. A) Building classification model. B) Analysis of unknown samples.

MPP is then used to build, test and validate the classification model. An example of such workflow in MPP in outlined in Figure 4. Then, the data are exported to Classifier that allows for easy results visualization and reporting that facilitate model testing. After the model is finalized, the unknown samples are processed in Unknowns Analysis and Classifier, bypassing data processing in MPP (Figure 2B).

The classification models were built using two different algorithms: PLSDA (Partial Least Square Discrimination) and SIMCA (Soft Independent Modeling of Class Analogy).

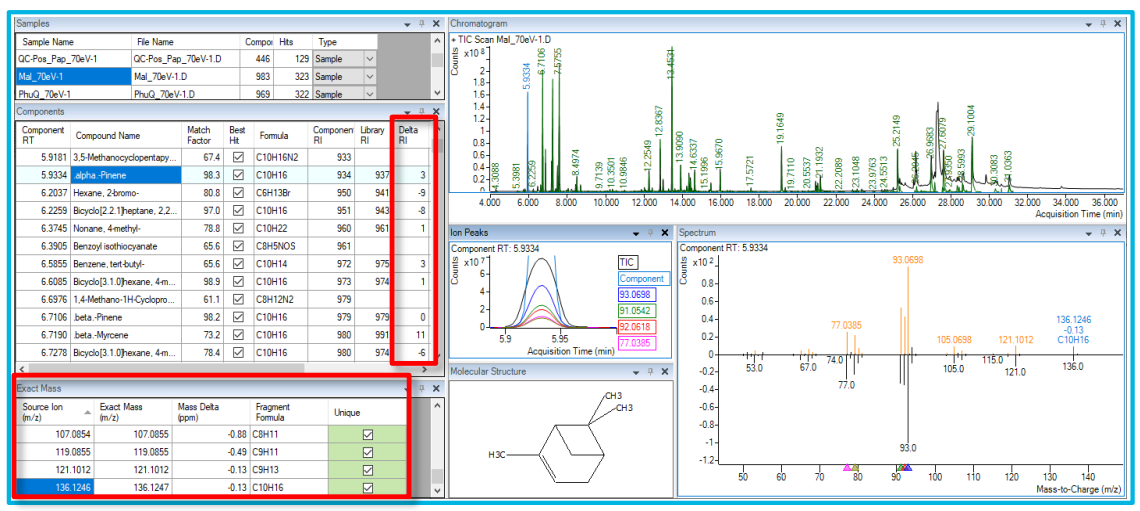


Figure 3. Feature finding in Unknowns Analysis. Highlighted in red boxes: RI calibration function helps confirm compound ID. ExactMass feature provides additional confirmation of compound identification using accurate mass spectral information.

### Example of MPP workflow for Building a Classification Model

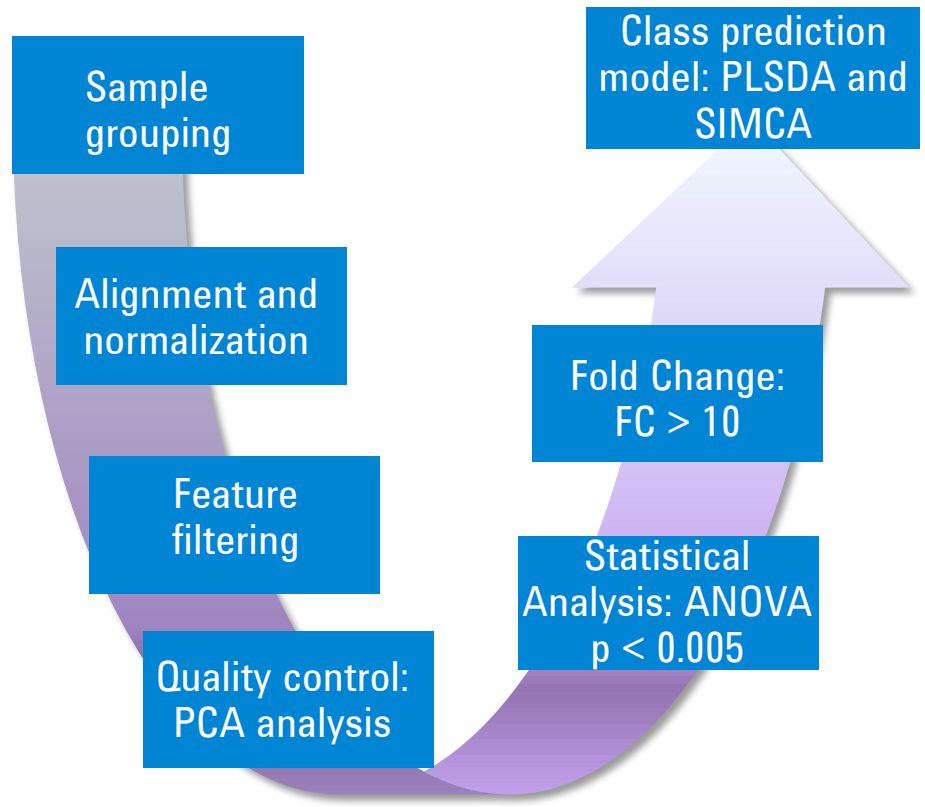


Figure 4. MPP workflow summary.

## Closer Look at Model Compounds

| | | Malabar | Phu Quoc | Szechuan | Papaya seeds |
|---|---|---|---|---|---|
| | RT | % base peak | | | |
| α-Pinene | 5.93 | 21.7 | 16.9 | 8.4 | |
| Sabinene | 6.61 | | | 16.7 | |
| β-Pinene | 6.71 | 37.0 | 31.3 | | |
| β-Myrcene | 6.86 | 1.3 | 2.0 | 19.4 | |
| α-Phellandrene | 7.18 | 4.5 | 10.6 | | |
| 3-Carene | 7.23 | 64.5 | 86.6 | | |
| o-Cymene | 7.49 | 2.1 | 1.7 | | |
| D-Limonene | 7.58 | 23.9 | 21.4 | 22.1 | |
| Eucalyptol | 7.64 | 23.7 | 1.6 | 100 | |
| β-cis-Ocimene | 7.82 | | | 3.2 | |
| γ-Terpinene | 8.04 | | | 4.5 | |
| 4-Thujanol | 8.25 | | | 4.0 | |
| Terpinolene | 8.49 | | 1.0 | | |
| Linalool | 8.69 | | | 2.1 | |
| Benzyl nitrile | 9.30 | | | | 2.8 |
| L-4-terpineneol | 10.01 | | | 2.8 | |
| L-α-Terpineol | 10.22 | | | 2.5 | |
| δ-Elemene | 12.26 | | 1.1 | | |
| α-Terpinyl acetate | 12.37 | | | 10.3 | |
| Benzyl isothiocyanate | 12.61 | | | | 80.7 |
| Copaene | 12.84 | 4.1 | | | |
| β-Cubebene | 12.99 | | 1.4 | | |
| Caryophyllene | 13.45 | 24.9 | 59.6 | | |
| α-Guaiene | 13.61 | | 1.6 | | |
| Humulene | 13.91 | 1.1 | 3.4 | | |
| β-Eudesmene | 14.33 | | 4.4 | | |
| α-Selinene | 14.40 | | 3.3 | | |
| β-Bisabolene | 14.47 | | 1.5 | | |
| δ-Cadinene | 14.64 | | | 2.2 | |
| Caryophyllene oxide | 15.48 | | 1.7 | | |
| Pellitorine | 19.16 | 5.4 | 4.8 | | |
| 9,12-Octadecadienoic acid (Z,Z)- | 21.02 | | | 5.3 | |
| Kalecide | 21.17 | | 1.1 | | |
| Hydroxy-sanshool 2 | 22.55 | | | 4.3 | |
| Hexadecanoic acid, octyl ester | 24.71 | | | | |
| Piperanine | 25.23 | 18.6 | 3.7 | | |
| Piperlonguminine | 25.29 | | 3.8 | | |
| (2E,4E)-N-Isobutyloctadeca-2,4-dienamide | 26.22 | | 2.9 | | |
| Squalene | 26.48 | | | | 2.4 |
| Piperyline | 26.96 | 4.4 | 3.7 | | |
| Piperine | 27.21 | 100 | 100 | | |
| Pipersintenamide | 27.61 | 2.2 | 11.0 | | |
| Kusunokinin | 28.64 | | 2.3 | | |
| Piperoleine B | 29.01 | 8.3 | 5.1 | | |
| γ-Sitosterol | 31.03 | 1.6 | 1.5 | 1.6 | 100 |
| Stigmastanol | 31.22 | | | | 8.1 |
| 4-Campestene-3-one | 31.54 | | | | 2.9 |
| Sitostenone (Stigmast-4-en-3-one) | 32.79 | | | | 7.8 |

Table 2. Major compounds included in PLSDA model. Relative amounts in each of the extracts (% base peak on the chromatogram) are shown. Percent of base peak was calculated after averaging across all the replicates from each group (<1% if not indicated).

## Results and Discussion

Fragment formula annotated compound spectra for two predominant, unique compounds identified in Szechuan pepper and papaya seeds extracts are shown in Figure 5. Note, that Hydroxy-sanshool does not have a spectrum in NIST17. However, a tentative ID can still be assigned based on published information [2].

2. Yue Ji, Shiming Li, Chi-Tang Ho. *Food Science and Human Wellness.* 8 (2019) 115–125

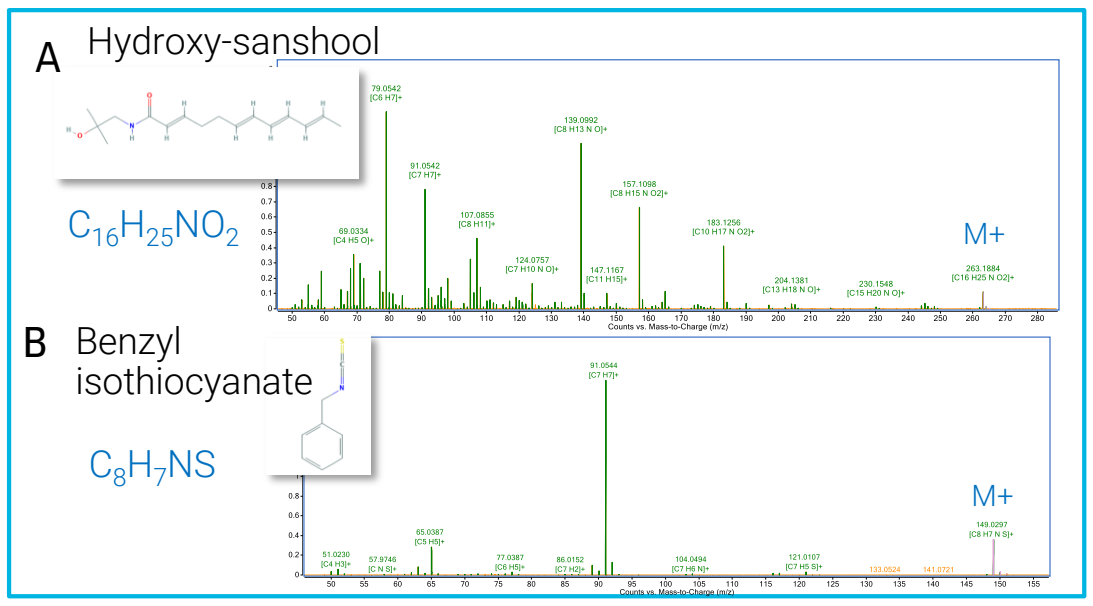


Figure 5. Example spectra for two unique and predominant compounds identified in "adulterants": A) in Szechuan pepper and B) in Papaya seeds. Structures were exported from PubChem database.

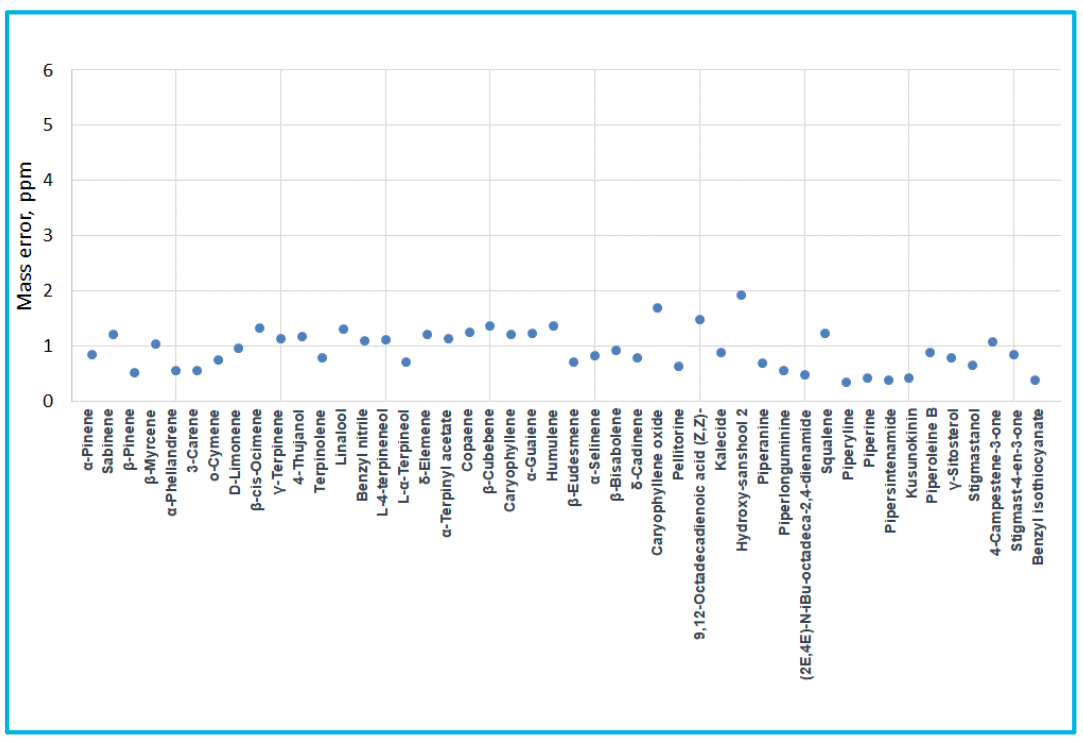


Figure 6. Mass accuracy of the major model compounds.

## Analysis of "Unknown" Samples in Classifier

The models were exported to the Classifier software for further validation using both positive and negative controls. Finally, the models were evaluated using the samples "adulterated" with 5-50% of either papaya seeds or Szechuan pepper (Figure 7).

For the PLSDA model to effectively determine adulteration with papaya seeds, papaya seeds samples needed to be included in the model. The SIMCA model was able to distinguish as low as to 5% dilution (adulteration) with Szechuan and papaya seeds even when papaya seeds were not included in the classification model. (Figure 8).

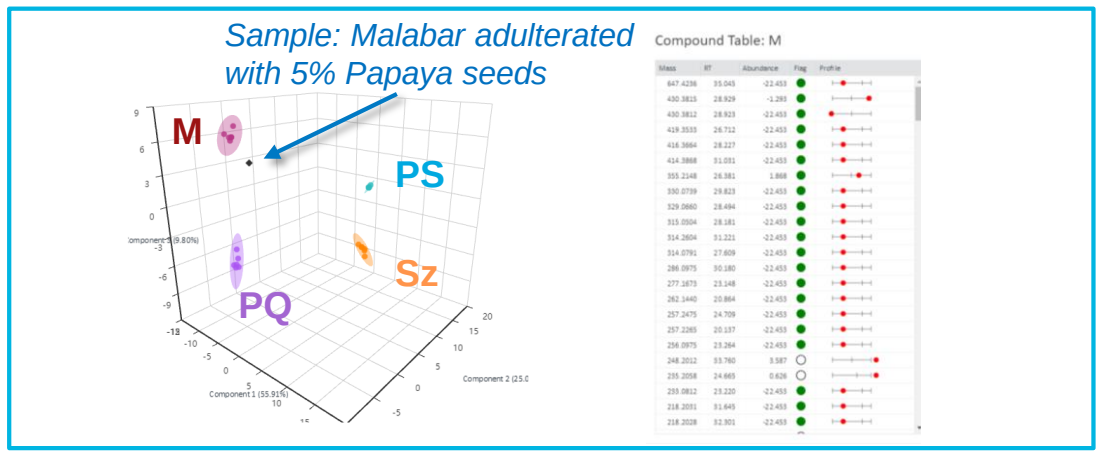


Figure 7. Results visualization in Classifier.

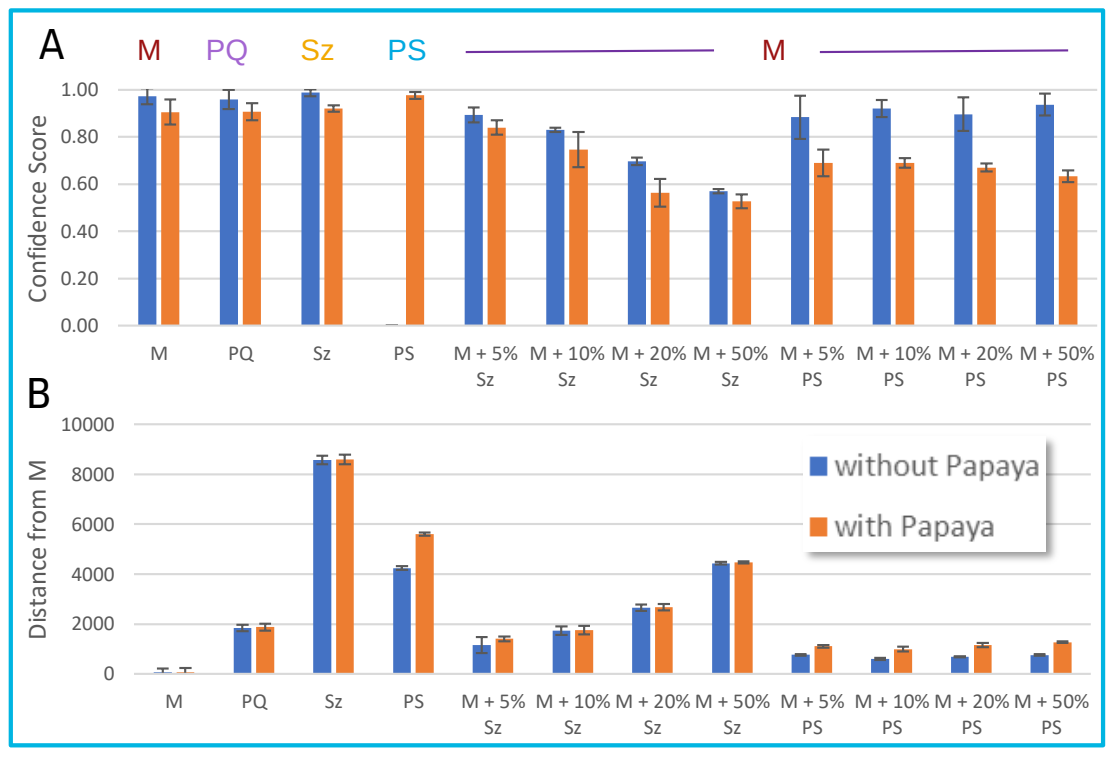


Figure 8. Results of PLSDA *vs* SIMCA model comparison, both with and w/o papaya seeds extracts included in the model. A) PLSDA, classification category for the confidence score given on the y-axis is annotated above the bars. B) SIMCA

## Conclusions

- Novel classification workflow for black pepper authenticity using high-resolution GC/Q-TOF and Classifier software has been demonstrated.
- The model was able to better distinguish between pure and adulterated black pepper when adulterant was included in a model in which case, as low as 5% adulteration with both papaya seeds or Szechuan pepper can be detected.


Published in USA, June 1, 2020

Agilent
Trusted Answers