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Workflow for food classification and authenticity using yerba mate and high-resolution GC/Q-TOF

Sofia Nieto, Melissa Churley

Agilent Technologies, Santa Clara, CA

Introduction

Food fraud is a highly profitable business and includes activities such as misbranding, mislabeling, dilution, counterfeiting and adulteration. Among foods and food ingredients most frequently found adulterated, there are olive oil, seafood, milk, honey, fruit juices, spices, coffee and tea. In order to streamline the characterization of foods, a novel workflow using high-resolution GC/Q-TOF and Classifier software has been developed. The workflow was evaluated using yerba mate, a traditional South American caffeinated tea. The model was able to easily distinguish between different brands of commercially available yerba mate. In addition, compounds that are characteristic to yerba mate and contribute to its unique flavor are discussed, as well as the presence of contaminating polycyclic aromatic hydrocarbons (PAHs).

Experimental

Yerba mate samples, purchased at a supermarket in Buenos Aires, Argentina, were extracted using a standard QuEChERS protocol. The samples were analyzed using a 7890 GC with and the 7250 high-resolution Q-TOF MS in full acquisition mode. The retention indices were calculated based on the alkane ladder to ensure compound identification. The GC/Q-TOF data were processed using the Unknowns Analysis tool of MassHunter Quantitative Analysis Software 10.1, Mass Profiler Professional (MPP) 15.1 and Classifier 1.1. The parameters are described in detail in Table 1.

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	Splitless
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.

Results and Discussion

Classification Workflow

To build the classification model, six replicates of each type of yerba mate from three different brands were extracted and analyzed using a high-resolution GC/Q-TOF (Figure 1).

The general workflow is outlined in Figure 2. First, a classification model is built and validated in MPP and Classifier following the feature finding step in Unknowns Analysis tool (Figure 2a). After the classification model is created and exported, unknown samples can be characterized directly using Unknowns Analysis and Classifier, bypassing MPP (Figure 2b).

A

B

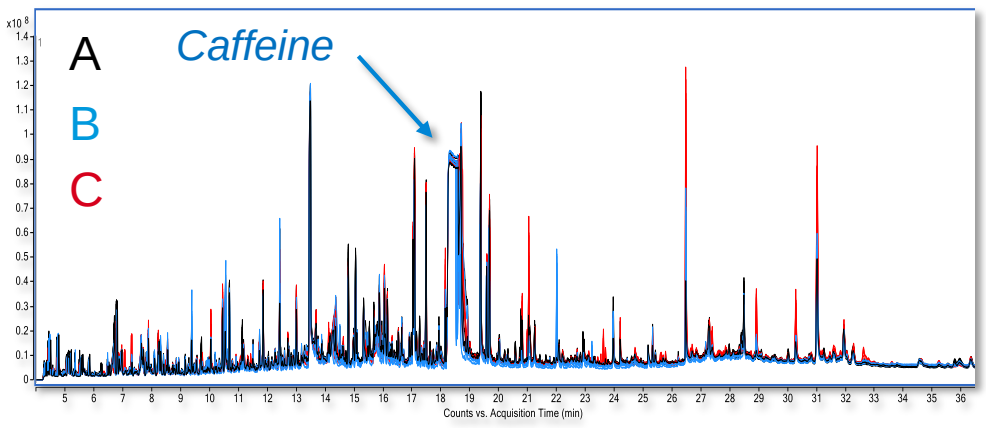

Figure 1. A) Yerba mate and mate gourds B) Overlaid chromatograms from the extracts of the three brands of yerba mate labeled A, B and C. Arrow points to caffeine.

Feature finding was performed in Unknowns Analysis using SureMass deconvolution followed by NIST17.L library search (Figure 3). Identity of the compounds was confirmed with Retention Indices (RI) as well accurate mass (facilitated by ExactMass feature of Unknowns Analysis).

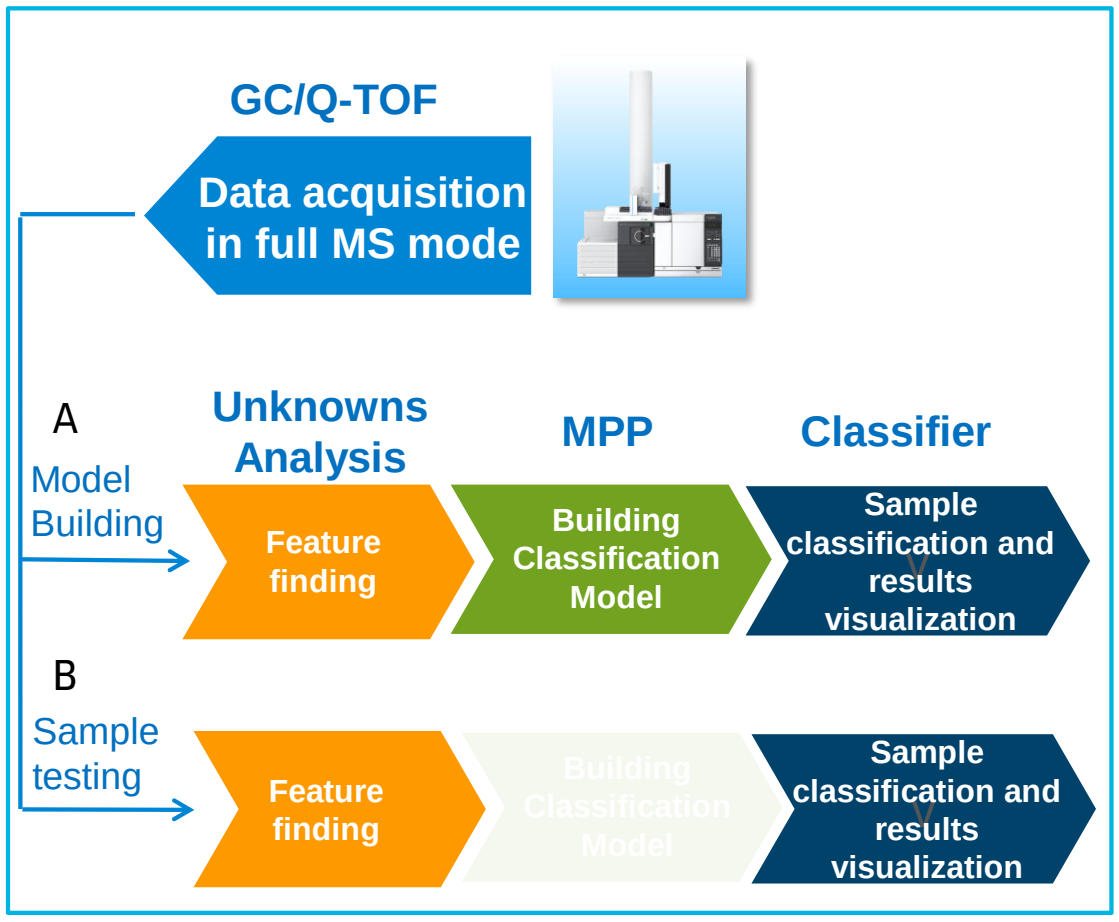

Figure 2. Workflow for sample classification. A) Model building and validation. B) Unknown samples classification.

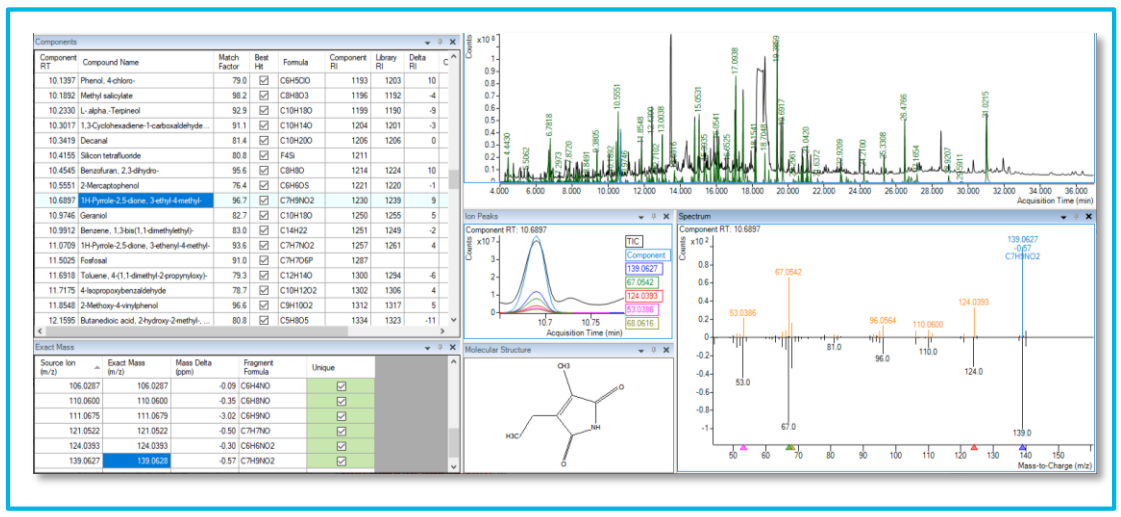

Figure 3. Feature finding in Unknowns Analysis. Yerba mate sample A. RI calibration supports compound ID. ExactMass feature provides additional ID confirmation using accurate mass.

Then, classification models using two different algorithms, PLSDA (Partial Least Square Discrimination) and SIMCA (Soft Independent Modeling of Class Analogy), were built in Mass Profiler Professional (MPP) using CEF files imported from Unknowns Analysis.

Once the data are imported into MPP, sample grouping, alignment, normalization, filtering, QC using Principle Component Analysis (PCA, Figure 4), statistical analysis and Fold Change analysis were performed.

The models were exported from MPP directly to the Classifier software.

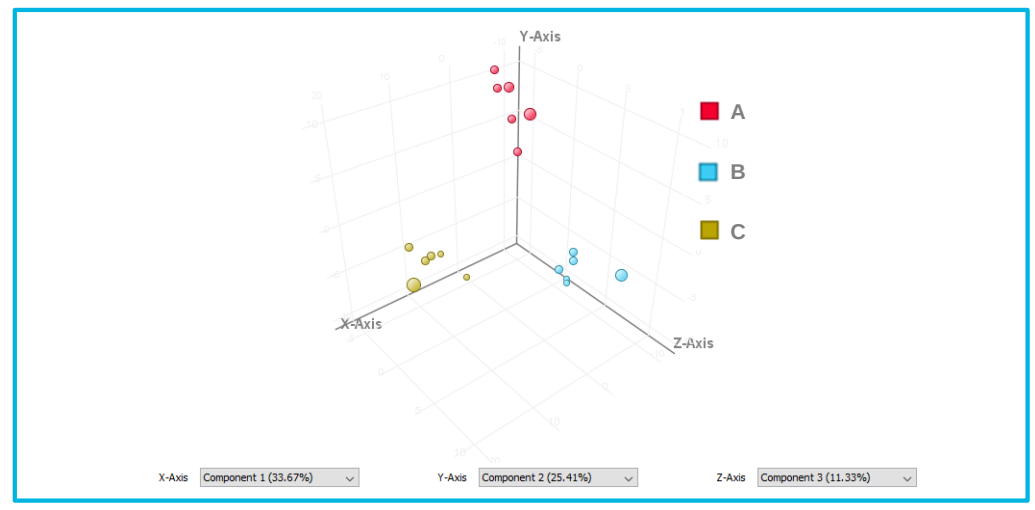

Figure 4. All the three samples groups can be easily separated on PCA plot.

To validate the classification model, both positive and negative controls were prepared using pure and mixed with various proportions of yerba samples.

Results of Differential Analysis, Flavors and Contaminants Screening

Characteristic volatile compounds that predominantly occur in one of the yerba mate brands tested, including those associated with flavor and aroma, have been identified.

Selected results from the Fold Change Analysis performed in MPP are shown on the Volcano plot (Figure 5) comparing extracts from brands A *vs* C. Compounds highlighted in red are those that are present in significantly higher levels in A as compared to C, and those labeled in blue accumulated in sample C vs A.

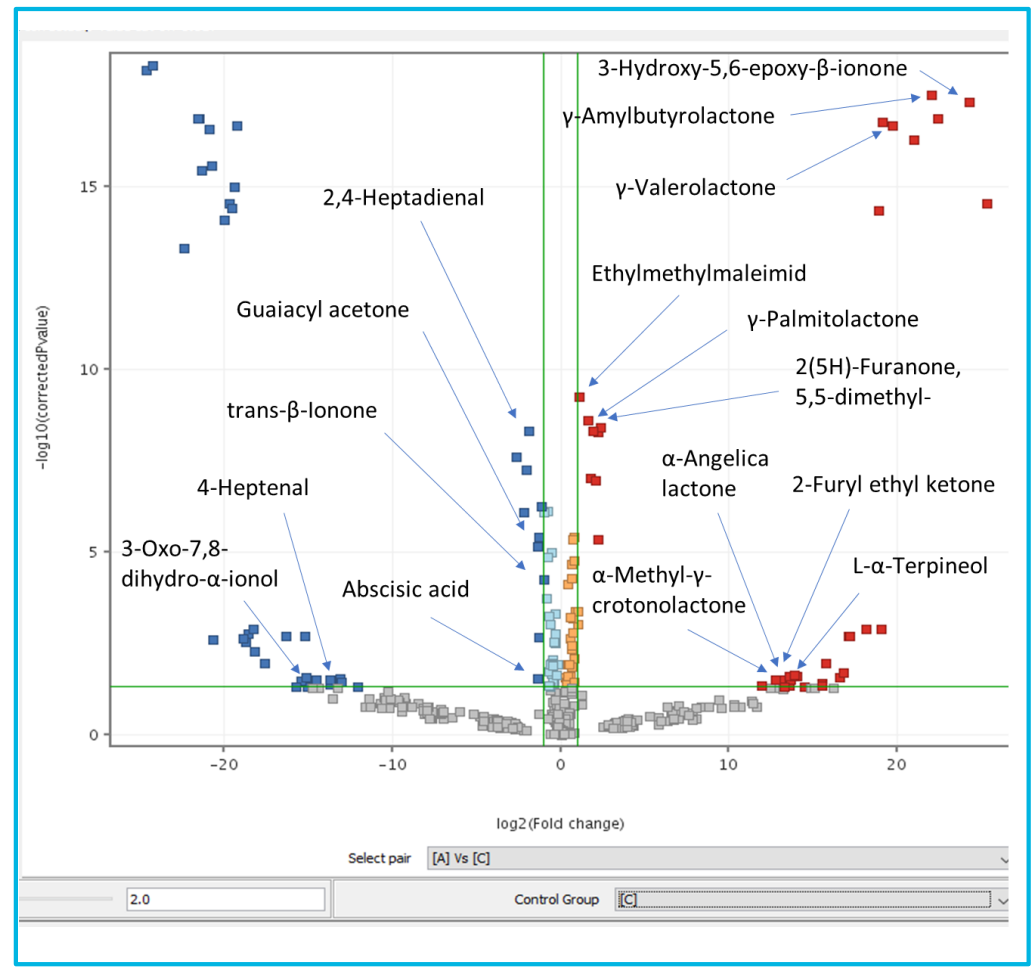

Figure 5. Volcano plot and Fold Change analysis.

Results and Discussion

Volatile compounds are labeled. Further details for these potential compounds of interest are shown in Table 2. 3-Hydroxy-5,6-epoxy-β-ionone showed one of the most significant Fold Change among identified flavor compounds (with high p-Value) between the two groups, thus potentially contributing to a significant difference in flavor between these brands of yerba mate.

RT	Compound	Mass Error*	p	Regulation	Log FC	Alias	Flavor
4.96	2(3H)-Furanone, 5-methyl-	0.8	0.006450	up	14.0	α-Angelica lactone	Sweet, solvent-like, oily, coconut, nutty with coumarin, tobacco nuances[1]
5.51	4-Heptenal, (Z)-	1.0	0.013103	down	-13.7		oily, dairy, creamy[1]
6.25	2(5H)-Furanone, 5,5-dimethyl-	0.5	2.557E-10	up	2.4	4,4-Dimethyl-2-buten-4-olide	Aroma component of hop extract, and of lavender, sagebrush, narcissus and salmon oils[2]
6.29	2(3H)-Furanone, dihydro-5-methyl-	0.4	5.834E-19	up	19.7	γ-Valerolactone	milky, fatty [1]
6.64	2(5H)-Furanone, 3-methyl-	0.4	0.009483	up	13.1	α-Methyl-γ-crotonolactone	sweet, tobacco -like odor[3]
7.22	1-Propanone, 1-(2-furanyl)-	0.5	0.006653	up	13.5	2-Furyl ethyl ketone	Fuity taste, sweet and caramelic odor[4]
7.3	2,4-Heptadienal, (E,E)-	0.3	3.468E-10	down	-1.9		fatty, oily, cinnamon[1]
10.23	L-α-Terpineol	0.6	0.017277	up	14.4		citrus, tropical fruits, apple, tomato and coffee flavors[1]
10.69	1H-Pyrrole-2,5-dione, 3-ethyl-4-methyl-	0.3	3.401E-11	up	1.1	Ethylmethylmaleimide	sweet, adds body, flue-cured note[5]
14.12	trans-β-Ionone	1.1	0.000007	down	-1.0		Cedar woods, violets[2]
14.63	2-Propanone, 1-(4-hydroxy-3-methoxyphenyl)-	0.4	3.949E-07	down	-1.3	Guaiacylacetone	vanilla, wood origin[6]
16.48	3-Buten-2-one, 4-(4-hydroxy-2,2,6-trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)-	0.6	5.053E-20	up	24.3	3-Hydroxy-5,6-epoxy-β-ionone	fruity, sweet, berry, woody, violet, orris, powdery[1]
16.72	2-Cyclohexen-1-one, 4-(3-hydroxybutyl)-3,5,5-trimethyl-	0.9	0.000446	down	-18.9	3-Oxo-7,8-dihydro-α-ionone	unknown
21.23	Abscisic acid	0.6	0.008350	down	-1.4		plant hormone

*Mass error shown for quant ion

[1]The Good Scents Company

[2]PubChem

[3]Perfume and Flavor Chemicals (Aroma Chemicals) Vol.1, By Steffen Arctander, Lulu.com, May 10, 2019

[4]Coffee Flavor Chemistry. Ivon Flament. 2002

[5]Tobacco Flavoring for Smoking Products. John C. Leffingwell, Harvey J. Young & Edward Bernasek. 1972

[6]Red Wine Technology. Antonio Morata. 2019

Table 2. Results of Fold Change analysis for selected volatile compounds. Note, some of these compounds were not necessarily included in the final classification model.

Several PAHs and other environmental contaminants have also been identified, and typically predominated in one brand versus another (Figure 6).

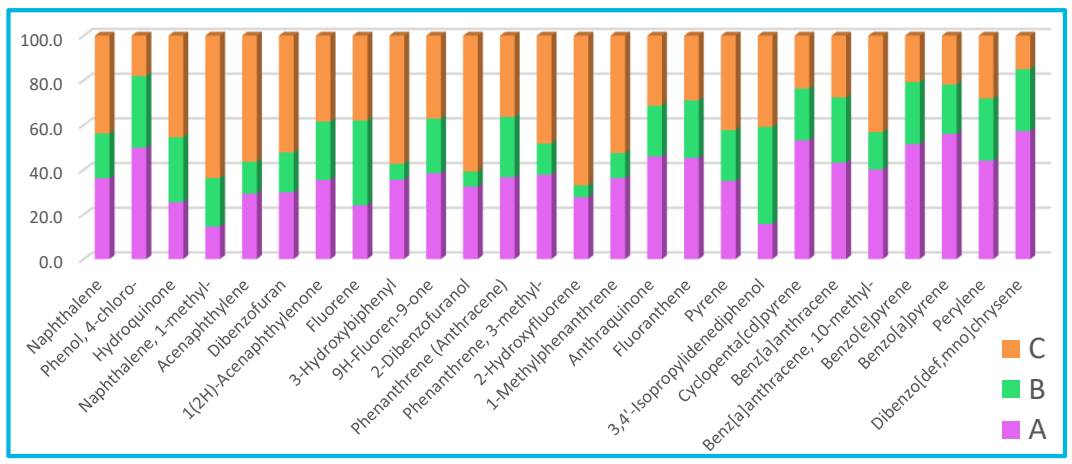

Figure 6. PAH and other environmental contaminants identified in yerba mate extracts

Classification results

The classification models were evaluated using the "adulterated" yerba mate samples created by mixing 5-80% of one of the brands (C) into the other one (A). Both PLSDA and SIMCA models were tested. SIMCA model showed a better distinction between the sample groups. The visualization examples for SIMCA are shown in Figure 7. Note that for a positive control for the extract A most of the model compounds are in the model range (highlighted in green, Figure 7a). For a sample A adulterated with 5% C, a few compounds are out of the model range (Figure 7b).

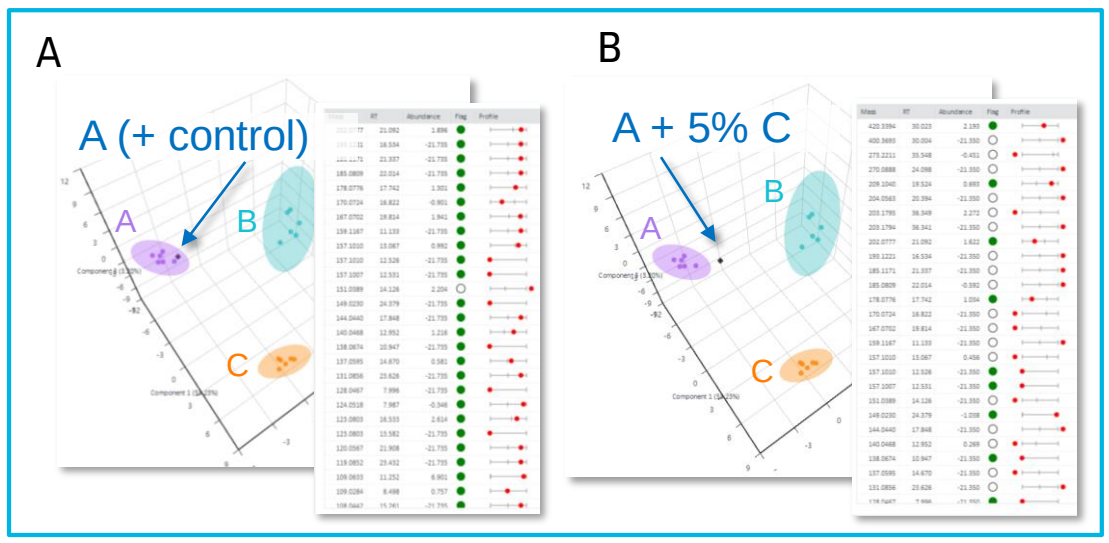

Figure 7. Results visualization in Classifier

SIMCA model was able to successfully distinguish pure samples A from other brands, including one not considered in the model (D), as well as yerba A adulterated with various levels of yerba C (Figure 8).

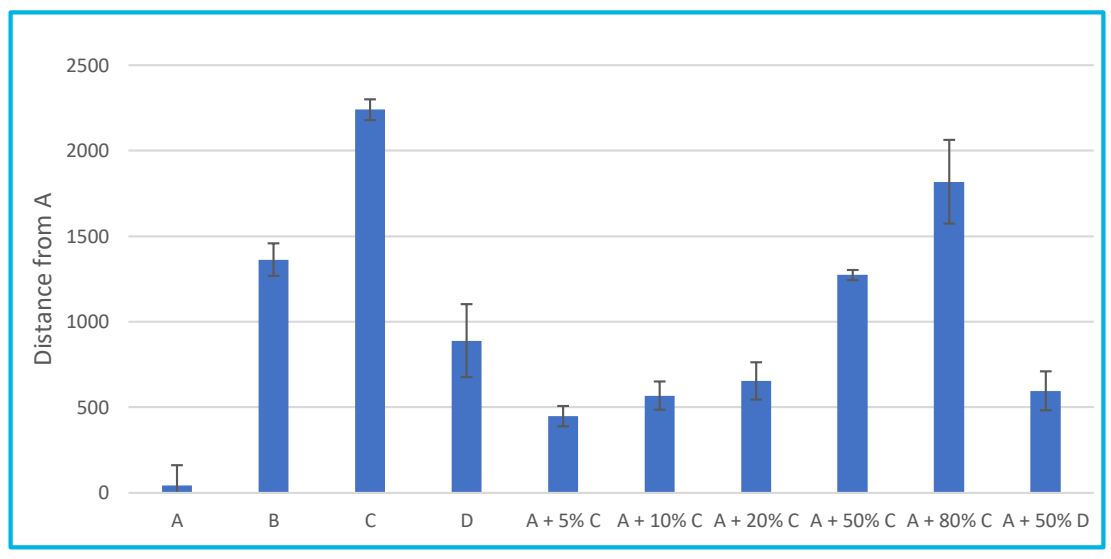

Figure 8. Classification results using SIMCA model. The distance from sample A is displayed

Conclusions

- Novel classification workflow for yerba mate authenticity using high-resolution GC/Q-TOF and Classifier software has been demonstrated.
- A classification model was able to distinguish between different brands of yerba mate as well as "adulterated" yerba mate samples
- Several PAHs have been identified in yerba mate extracts.
- A variety of flavor compounds were identified predominantly in brand A

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