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Simple and Accessible Food Testing with RADIAN™ ASAP

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INTRODUCTION

Robust food quality systems require surveillance throughout the food supply chain to ensure safety and quality of food products. High profile food scandals such as the illegal substitution of beef with horsemeat,[1] and addition of melamine into infant milk powder[2] underscores the importance of analytical testing during food manufacturing and throughout the food supply chain to support traceability systems. As such, rapid screening analytical approaches that require minimal sample preparation have been gaining popularity to quickly profile food products. Compared to other rapid screening analytical approaches, mass spectrometry offers high selectivity and sensitivity.[3] In addition, the fast speed of acquisition, and the depth of chemical information acquired makes direct mass spectrometry techniques attractive for the purpose of rapid screening of food products.

The RADIAN ASAP is a novel, dedicated direct analysis system specifically designed for rapid, easy and low-cost analysis of liquids and solids. The system has been engineered using proven and robust single quadrupole mass spectrometry technology, the ACQUITY™ QDa,™ combined with dedicated ambient ionziation Atmospheric Pressure Solids Analysis Probe (ASAP). The highly compact footprint of the mass spectrometer allows the instrument to be placed in many laboratory environments, and the optimized front-loading source design improves sampling throughput, minimizes sample contamination and improves ionization of sample. Data is acquired rapidly through the spotting of samples on a clean disposable glass capillary, which is then inserted horizontally into the instrument whereby samples are volatilized, ionized and analyzed by the mass spectrometer (Figure 1). The entire process of loading sample, inserting capillary into the instrument, and acquiring data takes less than a minute which improves productivity.
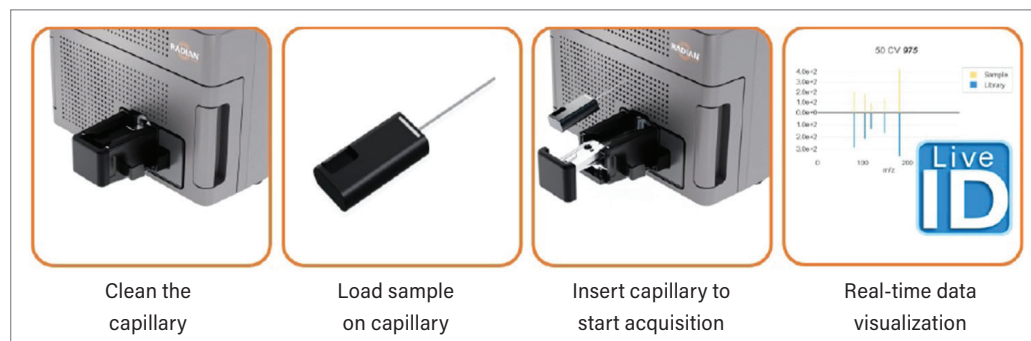

| Clean the capillary | Load sample on capillary | Insert capillary to start acquisition | Real-time data visualization |

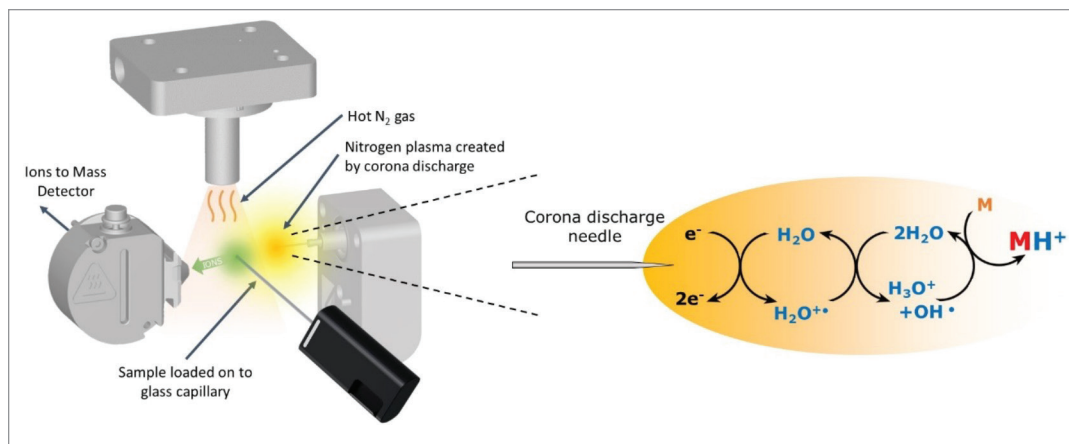*Figure 1. Steps to analyze samples using the RADIAN ASAP.*

Figure 2. Mechanism of sample volatilization and ionization.

In the source region, a stream of hot nitrogen gas is focused onto the sampling capillary which provides the heat for sample volatilization and acts as the medium for charge transfer during the ionization of the sample. Ionization is similar to that of atmospheric pressure chemical ionization (APCI) and occurs predominantly through proton transfer due to the open-source design and the abundance of water molecules in the atmosphere (Figure 2).[4,5] As such, it allows the analysis of a wide range of analytes including low to high polarity analytes, and volatile and semi-volatile solids and liquids.[6]

LiveID™ is an informatics solution for direct analysis mass spectrometry and has been developed with easy-to-follow workflows for the purposes of chemometric model building and spectral library matching. The chemometric model building workflow allows users to build multivariate statistical models for the purposes of sample correlation, class differentiation, and outlier detection. LiveID 2.0 includes an additional spectral library matching workflow which enables the identification of unknown samples from precursor and fragment spectra. A unique feature of LiveID 2.0 is 'live recognition' for both workflows, in which sample classes and analytes are identified on-the-fly during sample acquisition. The workflows are designed in an easy-to-use sequential manner which guides the user through each step. As such, RADIAN ASAP coupled with LiveID 2.0 allows users new to mass spectrometry to learn it quickly which minimizes start-up time and enhances productivity.

In this white paper, we consider possible experimental designs, analytical workflows, and case studies from key collaborators on the use of RADIAN ASAP for the purpose of rapid analysis of a variety of food products.

EXPERIMENTAL DESIGN

SAMPLES
Food samples vary widely in compositional complexity, physical properties and homogeneity. They can be simple such as vitamin C food additives, or very complex such as meat or spices. They can take the form of solids, liquids or even gelatinous forms. Homogenized solid and liquid samples can be analyzed directly, however to avoid overloading, sample preparation through dilution or extraction can be used. A summary of sample preparation strategies has been compiled and categorized in Table 1. to provide a starting point for the analysis of different food matrices depending on the properties of the sample, and the objective of the study.

DIRECT ANALYSIS
Direct analysis of samples is best suited for low complexity matrices that provide discernable mass spectral information to meet the needs of the study objective. For example, direct analysis of Chinese alcoholic beverages by Yu et al[8] allowed rapid discrimination of Chinese Baijiu by flavor type and real-time identification and authentication of Maotai, a high value Baijiu that is often counterfeited. A general rule of thumb for direct sampling is to start with the smallest amount of homogenized sample possible before increasing the amount to prevent saturation of the instrument and contamination of the source.

Approaches	Food Matrices	Properties	Examples	References
Direct	Dried herbs	Low moisture and high fiber content	Tea leaves	Tan et al[7]
	Alcoholic beverage	Watery	Baijiu, maotai, whisky and beer	Yu et al[8]
	Oils and fats	Solid or highly viscous	Cocoa butter	Chan et al[9]
Dilution and extraction	Dried herbs	Low moisture and high fiber content	Tea, oregano, hops	Tan et al[7]; Damiani et al[10] and Stead et al[11]
	Oils and fats	Solid or highly viscous	Extra virgin coconut oil	Li et al[12] and Ng[13]
	Honey	High sugar, highly viscous	Syrup and honey	Lee and Ng[14]; Loh et al[15]

Table 1. Summary of the sample preparation approaches for different food matrices.

DILUTION AND EXTRACTION

Some compounds, when present in the sample in high abundance, can impact the analysis. Therefore, sample preparation may be required to dilute concentrated samples, or to reduce the complexity of samples. In the studies by Li et al[12] and Ng,[13] oils and fats were dissolved in suitable solvents and analyzed on the RADIAN ASAP for the identification of oil adulterations and quantification of oil mixtures respectively. For the analysis of complex samples such as honey, it has been shown by An et al[16] that the presence of the sugars glucose and fructose suppress the ionization of minor constituents in honey such as organic acids. As such, biphasic extraction methods have been used to minimize the amount of sugars and enrich the minor components in honey that could be used as unique chemical fingerprints for the authentication of honey.[14,15] Figure 3 shows the mass spectra obtained from the use of different solvent extraction methods to prepare honey and syrup samples. Extraction using a suitable solvent mixture has also been used for the analysis of oolong tea and oregano.[7,10,11] One additional advantage of such solvent extraction procedure is that the same extracts can be submitted for complimentary analysis, for example by liquid-chromatography-mass spectrometry (LC-MS).
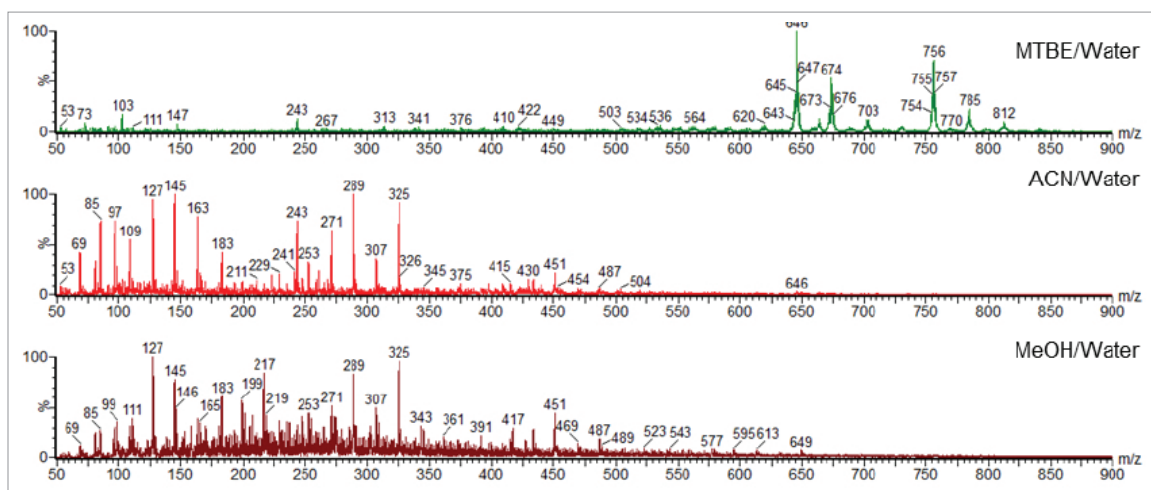

Figure 3. Mass spectra of honey extract obtained from different sample extraction methods.

SAMPLE INTRODUCTION

Capillaries used for sample introduction are melting point glass capillaries of length 100 mm and diameter 1.9 mm. There are several variations of these capillaries such as open-ended, single open-ended and closed-ended capillaries. In general, closed-ended capillaries have been used for a broad range of applications and sample types, whereas single-ended capillaries have been used on non-viscous and volatile samples to draw up a small volume of sample for analysis. Before use, capillaries are inserted into the instrument and cleaned using the 'Bakeout' function in the RADIAN ASAP detector console. Nitrogen gas at 600 °C is sprayed onto the capillary tip, causing surface contaminants of the glass capillary to pyrolyze thereby reducing contamination interference and improving reproducibility of results. The entire bakeout process can be monitored by observing the Total Ion Chromatogram (TIC) trace which typically shows a peak that plateaus at a minimum when pyrolysis is complete (Figure 4).
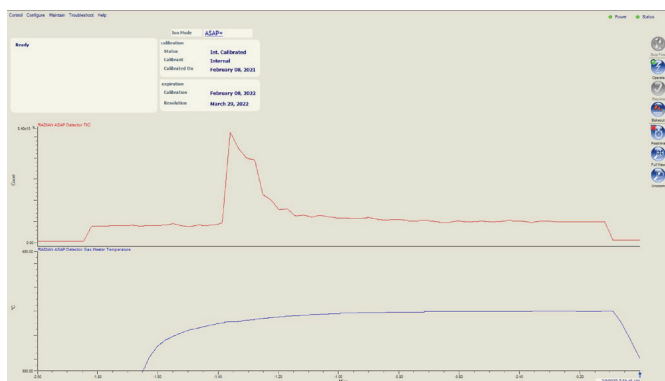

Figure 4. Instrument console showing the progress of capillary bakeout.

To analyze solid samples, a clean glass capillary is gently dabbed against the sample to transfer a small amount of sample onto the surface of the capillary. The solid sample should be just about visible to the naked eye. To analyze liquid samples, a clean glass capillary is dipped into the sample and swirled to coat the surface with the sample, or a controlled amount of sample (1 to 2 μL) is pipetted onto the capillary. For the analysis of samples in negative polarity, it is preferable for liquid samples to be dried on the capillary before analysis for best results.

INSTRUMENT PARAMETER OPTIMIZATION

Prior to starting data acquisition of samples, some instrument parameters may need to be optimized. The parameters shown in Table 2. work well with most applications and require minimal optimization.

The default cone voltage of 15 V can be used for most applications. Higher cone voltage leads to more fragment ions, which will be needed during the library matching workflow with LiveID 2.0 to produce precursor and fragment ions for the comparison against spectral libraries.

Two options are available for the gas heater temperature, the isothermal heater method, and the step heater method. In the isothermal heater method, the gas heater temperature is stabilized at the set temperature prior to acquisition and is maintained during the duration of the acquisition. In the step heater method, the user defines the length of time and the temperature of each step, and the sample timeout. When the capillary is inserted into the instrument during the sample timeout period, the temperature step method is initiated. As the temperature of the heater gas is stepped up, groups of analytes are sequentially volatilized by virtue of their boiling points. Therefore, the temperature step method is useful during method optimization to identify the optimal gas heater temperature or to reduce the complexity of the spectral profile by the stepwise volatilization of analytes.[17]

Parameter	Positive Polarity	Negative Polarity
Corona current	3 μA	2.5 μA
Cone voltage	15 V	15 V
Scan speed	1–2 Hz	1–2 Hz
Detector gain	1x	10x

Table 2. Instrument parameters.

ANALYSIS WORKFLOWS

MULTIVARIATE CHEMOMETRICS ANALYSIS

Chemometrics utilizes mathematical and statistical modelling to recognize patterns and relationships within highly complex data and translate them into useable analytical parameters. Using RADIAN ASAP and LiveID 2.0, the multivariate property of the peak intensity values of the mass spectrum are analyzed using principal component analysis (PCA), linear discriminant analysis (LDA) or a combination of the two (PCA-LDA) to reduce the dimensionality of the data, while providing a graphical representation of the trends within the dataset. During class recognition, a validated training model can be used to predict the classification of unknown samples by transforming the spectra into the model space and calculating the probability of the unknowns to be related to defined classes in the model. In the authenticity context, a typical requirement is to classify samples as either "authentic" or "non-authentic" using one-class models or to a particular group using multiclass models.

An extension to multivariate analysis of discrete variables or classes is multivariate calibration, in which mathematical regression methods are used to draw the relationship between the multivariate property, and a continuous variable for the purpose of quantitation.[13] The model describing the relationship allows predictions to be made of an unknown variable when the mass spectra is compared against the model.

The quality of mathematical models requires careful consideration of experimental design, samples for training set and validation, and quality of mass spectra acquired. It is vital that the training set is built using good quality analytical data from well-characterized, authentic samples that are representative of the study objective. To further understand the concepts and applications of chemometric model building for the purposes of food analysis, one can refer to the following excellent tutorials and reviews.[18–20]

An example of chemometric model building workflow with RADIAN ASAP and LiveID 2.0 with the aim of honey classification is presented here:
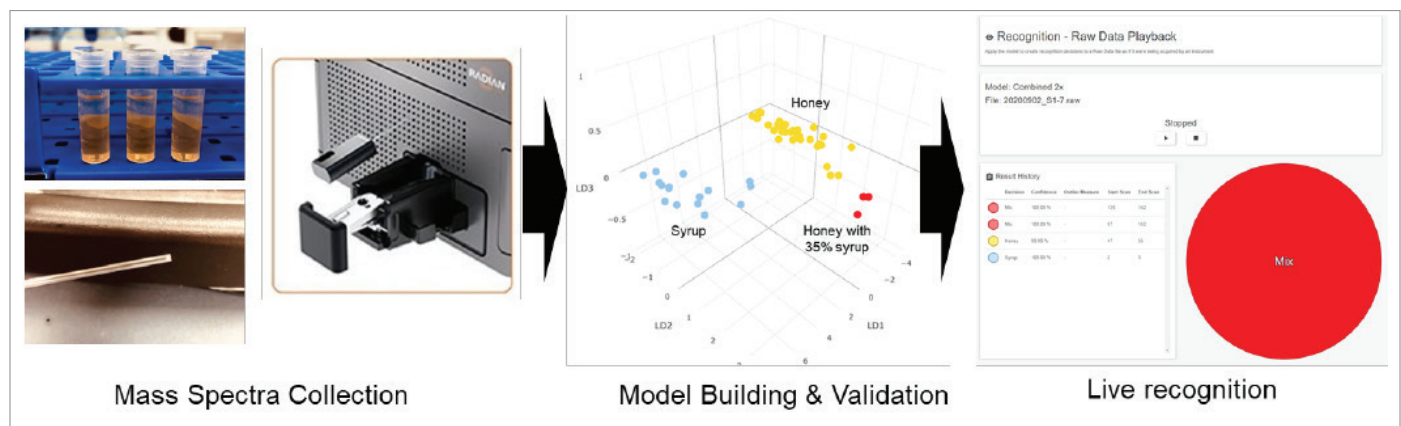

Figure 5. Chemometric model building workflow with RADIAN ASAP and LiveID 2.0.

Honey samples are extracted and the organic phase is sampled with a clean glass capillary for collection of mass spectra. Raw data is imported into LiveID 2.0 for further processing.

1. **Peak selection**
   Peaks are selected automatically above an adjustable TIC threshold
   and imported into a mass spectral library.

2. **Building multivariate statistical model**
   Mass spectra from groups of known sample types are assigned to model classes,
   and model parameters are selected to build the multivariate statistical model.

3. **Model Training and Validation**
   The model is validated either by leave 20% out cross-validation,
   or through the use of an independent validation set.

4. **Class recognition**
   A validated training model can be used to predict the classes
   of unknown samples either in real time or offline.

LIBRARY MATCHING

Library matching using RADIAN ASAP and LiveID 2.0 allows the identification of unknown compounds based on the comparison of the precursor and fragment spectra from a library against that of an unknown analyte. A typical library matching workflow involves defining the type of matrix being tested and the compounds expected to be identified. A spectral library is built with the precursor and fragment spectra of each of the expected compounds using a sequence of different cone voltage, for example 15, 25, 35, and 50 V. The spectral library is reverse matched against the spectra of an unknown compound and a match score between 0 and 999 is generated from the contributions of the spectra at the different cone voltages. A higher score indicates a better identification match.

An example of a library matching workflow with RADIAN ASAP and LiveID 2.0 with the aim to identify an industrial dye in food matrices, based on a suspect compound list is presented here:
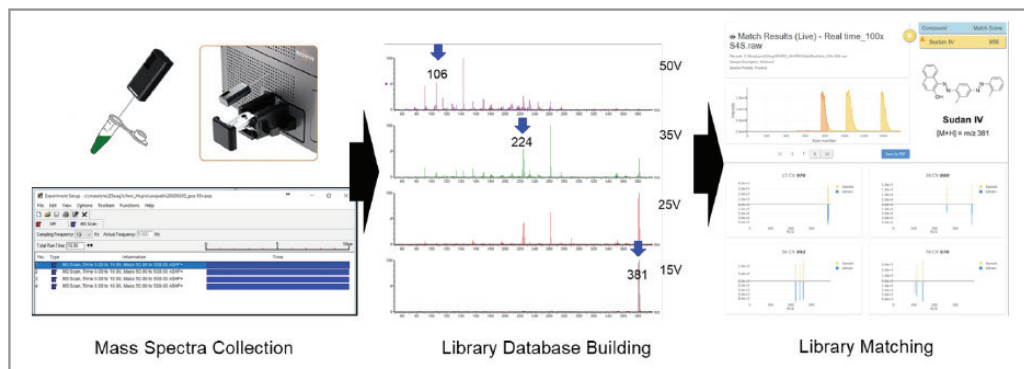

Figure 6. Library matching workflow with RADIAN ASAP and LiveID 2.0.

1. **Mass spectra acquisition**
   Mass spectra of pure standards at multiple cone voltages are collected.

2. **Library database building**
   Raw data is centered and imported into MSP Librarian for library database building.

3. **Library matching**
   The library is matched against the mass spectrum of unknown samples either in real time or offline,
   and a match score is given for identified compounds to indicate the confidence of the match.

CASE STUDIES

CASE STUDY 1: AUTHENTICATION OF CHINESE OOLONG TEAS WITH RADIAN ASAP

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According to the US National Centre for Food Protection and Defense, tea is among the top 18 food ingredient categories with high rates of economically motivated adulteration. The aim of this study was to develop a rapid and direct method to authenticate Chinese oolong tea using RADIAN ASAP. The two sample preparation methods experimented with were grinding of tea leaves and solvent extraction of tea leaves using 70% v/v methanol/water (30 mg tea to 1.2 mL solvent). Both the ground tea leaves (coarse and fine) and extract (10 times diluted) were analyzed. Reproducible spectra across technical replicates were obtained for all sample preparation approaches. This finding highlights the versatility of RADIAN ASAP, where either direct analysis of ground tea leaves without an additional solvent extraction step or analysis of liquid extract is possible. Three main oolong tea varieties Guangdong Dancong, Taiwan Dongding, and Anxi Tieguanyin were successfully classified using methanolic extract analyzed on RADIAN ASAP.
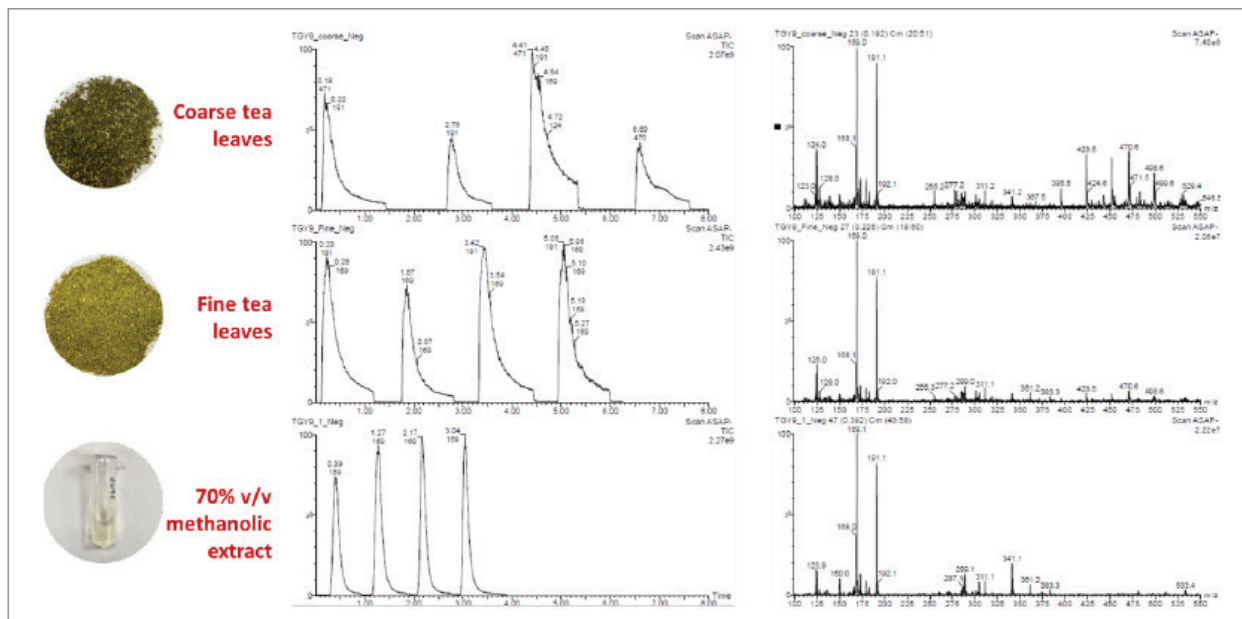

Figure 7. Comparison of the total ion chromatograms and mass spectra from tea samples prepared with different methods.

CASE STUDY 2: CLASSIFICATION OF HOPS BY CULTIVAR WITH RADIAN ASAP

Jessica Prenni[c]

[c]Department of Horticulture and Landscape Architecture, Colorado State University

Hops are one of the four primary raw ingredients used to make beer and they are employed by brewers to impart specific flavor and aroma profiles, particularly in craft beer styles. These unique sensory attributes are a result of chemical compounds in hops such as alpha acids (non-volatile) and terpenes (volatile) which can vary significantly between hop cultivars and can also be influenced by the growing environment in which the hops were produced. Thus, while it is clear that understanding the chemical profile of hops is critical for beer quality (and the development of new, unique beers), the optimal analytical tools are often out of reach for the craft brewer due to cost, time, and required expertise. Instead, hop producers and brewers primarily rely on sensory evaluation for hop characterization which requires a large group of trained panelists, is often subjective, and is time consuming and fatiguing – limiting throughout. The use RADIAN ASAP coupled with predictive computational tools provides an alternative "fit for purpose" approach for hop characterization based on detection of flavor and aroma (volatile and non-volatile) relevant compounds. Using a simple 80% ethanol extraction followed by analysis with RADIAN ASAP and chemometric modelling using LiveID, hop cultivar Strata, Cent, and Cas were differentiated from each other (Figure 8).
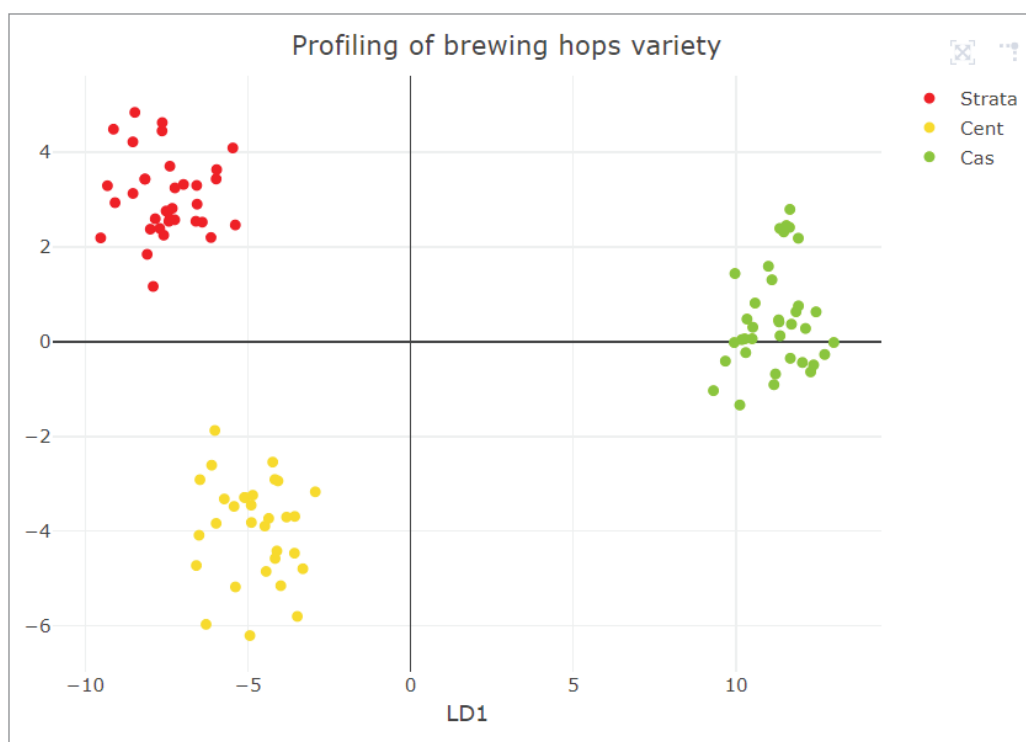

Figure 8. PCA-LDA score plot of hop cultivar Strata, Cent, Cas, and QC samples.

CASE STUDY 3: EARLY DETECTION OF ADULTERATION SCENARIOS
IN ORIGANUM VULGARE L

Tito Damiani[d], Giuseppe Sammarco[d], Chiara Dall'Asta[d], Michele Suman[d]

[d]University of Parma

This study deals with the undeclared blending of dried Mediterranean oregano (*Origanum vulgare L.*) with cheaper non-aromatic plants having similar silvery grey color. The aim of the study was to assess the feasibility of the RADIAN ASAP for the detection of gross addition of bulking materials to dried herbs. Specifically, the adulteration of Mediterranean oregano was taken as a case study and a non-targeted fingerprinting approach was adopted. A range of adulterants was sourced from commercial producers or collected from growing plants of known species. Olive leaves (*Olea europaea*), cistus (*Cistus incanus* and *Cistus cyprius*), rhododendron (*Rhododendron indicum*), thyme (*Thymus serpyllum*), and two types of marjoram (*Origanum majorana*), were used to prepare illicit admixtures at five different adulteration levels (5, 10, 20, 30, and 50% w/w). Samples were extracted by adding 10 mL of methanol + 0.1% of formic acid to 0.2 g of finely ground plant material and further diluted with the extraction solvent (1:5 dilution) to reduce ionization suppression as well as potential carry-over into the sources. After data pre-processing, the whole mass spectra were used for the subsequent multivariate data analysis. Soft Independent Modelling of Class Analogy (SIMCA) was used as the classification algorithm. The SIMCA model was trained on the authentic dried oregano samples (n=75) and then challenged with both new genuine samples and in-house prepared blends (n=40). Overall, the lowest detectable level was found to be between 5–20% for all admixtures, except for *Origanum majorana*. These results can be considered commercially useful due to herbs adulteration is expected to occur at gross levels (over 70% w/w substitution).
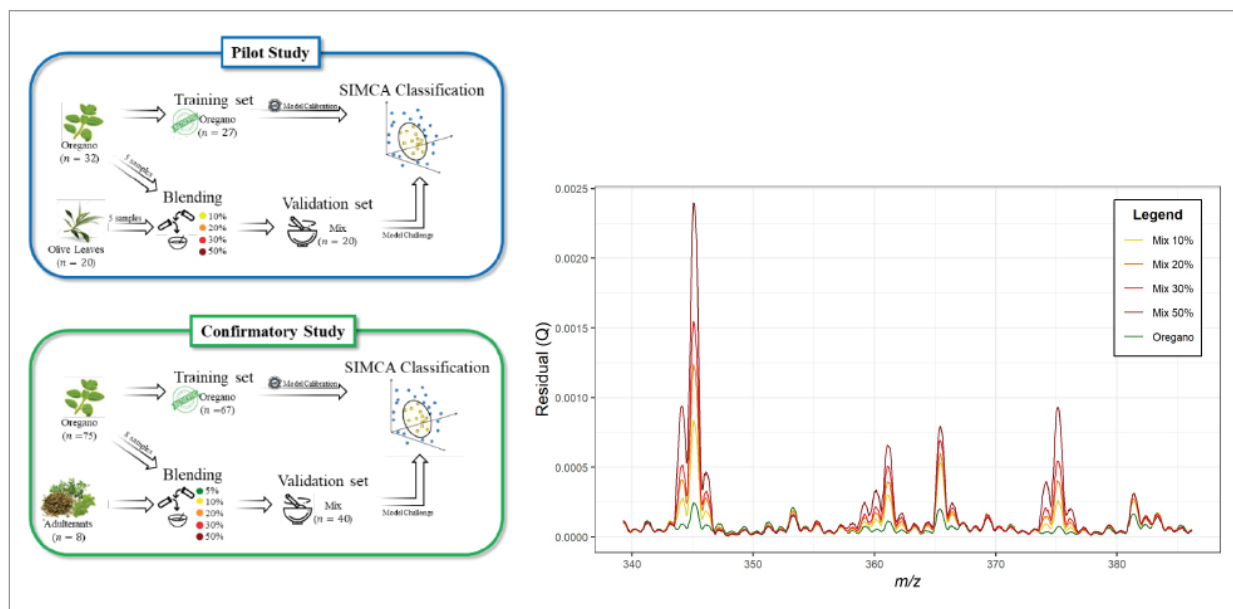

Figure 9. Authentication workflows of oregano herb and the residual plots of oregano at different mix percentage of adulterants.

CONCLUSION

The frequent occurrence of food fraud has driven the need for food surveillance throughout the food supply chain in support of new regulations and due diligence within the food industry. However current analytical tools are complicated or time consuming for routine use at different nodes of the supply chain. The RADIAN ASAP along with LiveID 2.0 informatics solution makes food testing simple and accessible.

The mass spectrometer is easy to use, easy to learn and requires minimal parameter optimization. In addition, intuitive workflows within LiveID 2.0 allows users to quickly build chemometric models or match unknown compounds against a library. The 'Live Recognition' feature within LiveID 2.0 means that results can be obtained near-instantaneously. Therefore, food samples can be characterized quickly which facilitates faster decision making on the quality and safety of food samples.

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