Rapid recognition of beer specimen based on multivariate analysis of non-volatile fingerprint

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Introduction

Growing interest in food quality and traceability by regulators as well as consumers demands advances in more rapid, versatile and cost-effective analytical methods. Ambient mass spectrometry (AMS) technologies such as desorption electrospray ionization (DESI), direct analysis in real time (DART), atmospheric pressure solid analysis probe (ASAP), and Rapid Evaporative Ionization Mass Spectrometry (REIMS) have emerged as tools for rapid and on-site testing of foodstuffs. Yet, two caveats of these technologies may include the potential contamination of the mass spectrometer inlet after a few injections (<100) and the fact that the sample must be in close proximity with the mass spectrometer.

Sample preparation technologies directly interface to-MS have surged as a means to improve the performance of either existing AMS by allowing remote sampling, sample clean-up, and retaining relevant chemical information that facilitates its classification via chemometric tool. Coated Blade Spray (CBS) is a sample prep technology that integrates the collection of analytes of interest from a sample and the direct interface to mass spectrometry systems as a substrate spray ionization. The device comprises a thin-flat sheet with a pointed tip and it is manufactured of a conductive substrate such as stainless steel (see **Figure 1**). As a SPME device, the substrate is partially coated with an extraction phase comprised of polymeric particles and a binder. The function of the polymeric particles is to enrich the analytes of interest from the sample matrix. As a direct to MS device, the device requires a pre-wetting of the extraction material so to elute the analytes collected on it. Subsequently, a differential potential is applied between the non-coated area of the substrate and the inlet of the MS system generating an electrospray at the tip of the CBS device. Herein, we demonstrate, as a proof-of-concept, how CBS coupled to Nominal Resolution Mass Spectrometry (MS) enables rapid discrimination of aqueous food matrices (i.e. beer samples).

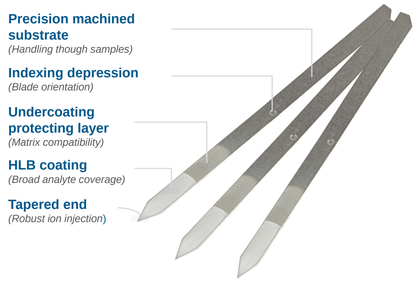


Figure 1 Anatomy of a Coated Blade Spray. CB-HLB (Hydrophilic-Lipophilic Balance), Catalog # 23248.

Materials & Methods

CBS devices coated with Hydrophobic-Lipophilic Balance (HLB) particles were manufactured by Restek Corporation (Bellefonte, PA, USA). The coating length was 10 mm and coating thickness 30 µm. The sampling workflow comprise sonicating beer samples for 5 min. Then, 300µL of each sample was dispense into a 96-well plate and, using a Sample Handling Unit (SHU, see **Figure 2**) developed by Restek corporation, blades were immersed on the samples for 5 minutes. Finally, the blades were immersed on a well plate containing 300µL of 95:5 Water:Methanol for 10 seconds in order to clean the coating surface of matrix components that may cause significant suppression or MS contamination.

Data collection was conducted on an SCIEX 4500 triple quadrupole (Redwood, CA) operated in Q3 mode. A fully automated interface for CBS compatible with SCIEX instruments was developed by Restek Corporation. The interface has complete control over the I/O communication with the MS, accurate positioning of the CBS in regards to the MS inlet; application of the elution solvent; timing of the elution step and control and timing of the high-voltage application (i.e. electrospray time). In addition, an inlet cleaning system was included between CBS injections so to prevent chances of false positives or instrument contamination. Electrospray was generated by adding 5 µL of the elution solvent (i.e. 87.5:7.5:5.0 MeOH:IPA:Water) on the coated area and, after 2 seconds, applying a positive voltage of 3.25 kV. Spray time was set to 5 seconds per blade and the total instrument time was under 23 seconds per sample. Average mass spectra data was extracted using Analyst™. Chemometric analyses of each data set was performed with Mass Mountaineer™ software for mass spectra collected over the m/z range between 100-1000. Electrospray background signal collected with clean blades was subtracted from each sample.

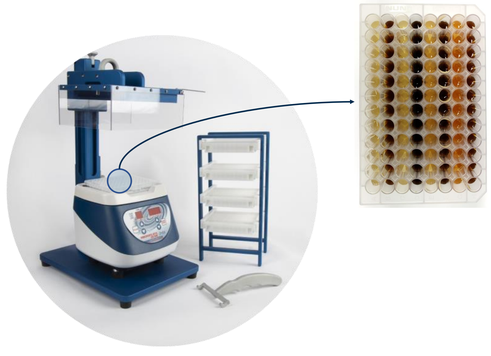
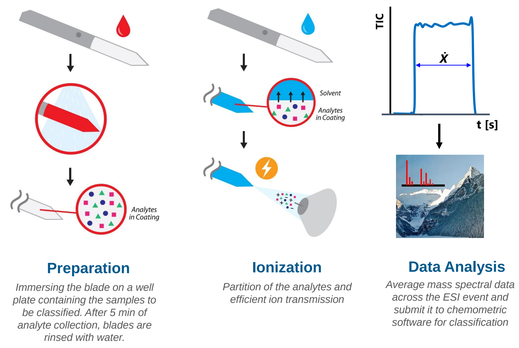


Figure 2 Sample Handling Unit (SHU) used for simultaneous analyte collection with 96-CBS blades.



Schematic 1 Representation of the sample preparation, ionization and data processing steps carried our using CBS technology for food safety applications.

Results

As can be seen in **Figure 3**, the discriminant analysis of principal components (DAPC) in combination with Principal Component Analysis (KPCA) allowed for adequate classification of each of the beer classes under evaluation. Further, when using 60 principal components (PC), the leave-one-out cross validation (LOOCV) and the Support Vector Machine (SVM) unequivocally identified each of the samples. Similar results were obtained when the model was used to classify different beer brands (see **Figure 4**). Aiming to further evaluate the robustness of the model, it was used to classify beers of the same beer class (IPA). As presented in **Figure 5**, the statistical model was capable of unambiguously ascertaining each beer brand between this class.

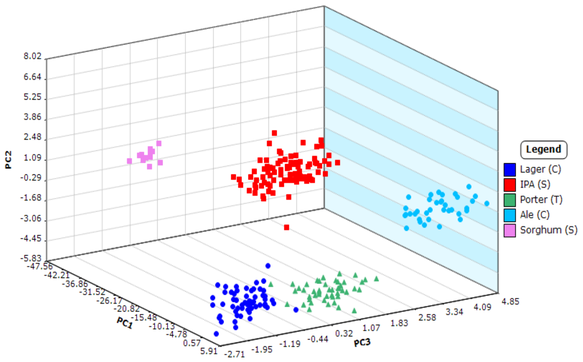


Figure 3 PCA-DAPC plot obtained with Mass Mountaineer™ from the MS profile data of sample sets of five different beer classes.

Results

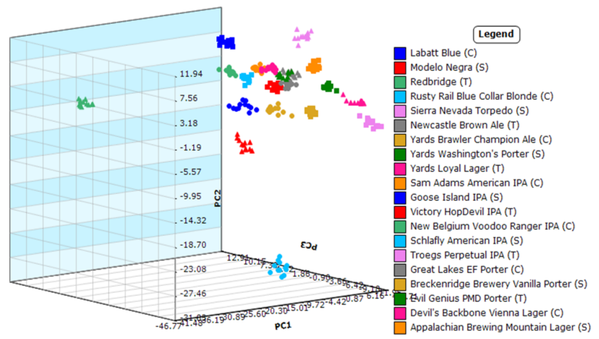


Figure 4 PCA plot obtained with Mass Mountaineer™ from the MS profile data of sample sets of twenty different beer samples.

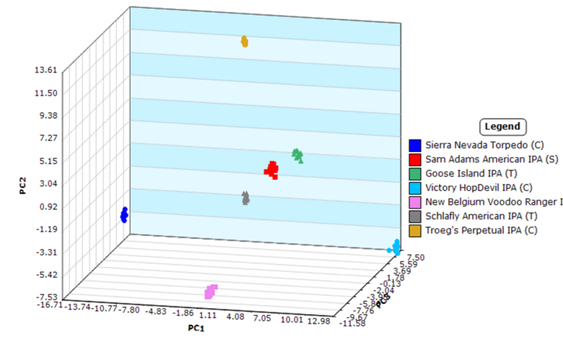


Figure 5 PCA plot obtained with Mass Mountaineer™ from the MS profile data of sample sets of seven different Indian Pale Ale (IPA) beer samples.

Remarks

1. Herein, we showed how CBS can be used to collect and store chemical the non-volatile fingerprint from beer samples of different classes and brands. When in combination with adequate statistical tools, it can be used for rapid recognition of beers. Certainly, this proof of concept has further potential to be explored in other food matrices. Consequently, the authors foresee further development of CBS-based methods in Food Safety applications.
2. Since the chemical information of the sample is stored in the coated material, it is invisible to the bare eye and encrypted to the trained eye; thus, making CBS an ideal tool, not only for fingerprinting, but also from a chain of custody perspective.