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## Comparison of Headspace Sampling and Stir Bar Sorptive Extraction in the Detection of Whiskey Adulteration with a Mass-Spectrometry Based Chemical Sensor

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### KEYWORDS

Headspace sampling, stir bar sorptive extraction (SBSE), adulteration, chemical sensor, multivariate analysis

### ABSTRACT

Verification of authenticity is a crucial aspect of food quality control. In particular, alcoholic beverages have been targets of numerous adulteration schemes. Addition of caramel coloring, water and lesser value whiskies to aged straight bourbons will be addressed in the present study.

Unadulterated and adulterated bourbons were analyzed using a mass spectrometry based chemical sensor. Sample introduction to the sensor using headspace (HS) and stir bar sorptive extraction (SBSE) was used to detect the adulteration. Multivariate analysis techniques such as soft-independent-modeling-class-analogy (SIMCA) and principal component regression (PCR) successfully identified the adulterated bourbons.

Adulterants such as inexpensive bourbons add trace levels of new components to the bourbon. Classification models for both sample introduction techniques indicate the successful discrimination of adulterated bourbons. Detection

of the adulterants using SBSE revealed the presence of ions not detected with headspace sampling. The ability to detect compounds using SBSE that were not detected in the headspace may be advantageous in discriminating some types of samples. Identification of adulterated bourbons using HS and SBSE techniques demonstrates the usefulness of the MS chemical sensor to differentiate samples with close chemical composition quickly and accurately.

## INTRODUCTION

Bourbons are distilled spirits produced from fermenting a mix of grains that must contain at least 51% corn. They also must be aged for at least two years in new white oak barrels that have been charred. The aging process naturally affects odor, flavor and color of the bourbon. Additives that change these physical properties should not be introduced during bottling [1]. It is known that phenolic acids from the wood are extracted into whiskeys when they are in contact [2]. The longer the whiskey is in contact with the oak wood the higher their phenolic acid content. UV/vis methods have been used to estimate the age of the whiskeys by measuring the content of phenolic compounds [3].

Bourbon whiskeys can also be aged longer than two years. If there is no indication of age on the label on the bottle, the bourbon probably has been aged 4 years. Older bourbons are normally sold at premium prices, depending on how long the aging process is allowed to occur. This price difference opens the door to fraud by different means, including adulteration.

The possible adulteration of whiskeys is a well-known issue [4, 5]. For example, bourbons that have been aged for only 4 years could be made to appear older by adding caramel coloring. Other possibilities for adulteration include diluting the bourbons with water or lesser value bourbons. A robust technique that quickly identifies adulteration is therefore needed.

In this study, a mass spectrometry based chemical sensor is used to detect adulteration of bourbons. The mass spectra of bourbons aged in the same environmental conditions are used to train the sensor with acceptable profiles for different years. The mass spectrum becomes like a fingerprint to which adulterated bourbons are compared.

The comparison of multiple mass spectra is facilitated with the use of multivariate statistical techniques. The use of chemometric tools to visualize natural grouping is essential when comparing large numbers of mass spectra. For example, principal component

analysis (PCA) reduces the dimensionality of the data set by constructing a new set of coordinates. PCA searches for correlations among all ion abundances simultaneously and extracts linear combinations of highly correlated ion's abundances (principal components, or PCs) that describe the greatest amount of sample variability. If enough variance is captured within the first few PCs then meaningful plots can be used to visualize grouping of samples.

## EXPERIMENTAL

*Materials.* Bourbons aged in the same environmental conditions were used to train the chemical sensor. These bourbons were aged 10, 6, 3, and 1 year. A lesser value bourbon (B) aged 4 years with 40% ALC/VOL and a more expensive bourbon (A) were purchased at a local store.

*Instrumentation.* The chemsensor used was a Gerstel ChemSensor 4440 (Figure 1) that includes a headspace unit (7694, Agilent Technologies) with a mass selective detector (5973, Agilent Technologies). This instrument integrates chemometric software from Infometrix (Pirouette 3.02 and Instep 2.0). The instrument was used in the scan mode from 48 to 170 amu with a 1.00 min run. The Gerstel ChemSensor 4440 introduces the sample with a 30:1 split that is achieved using an open split interface.



**Figure 1.** Gerstel ChemSensor 4440.

Stir bar sorptive extraction (Twister, Gerstel) was used to extract bourbon samples and was compared to the results obtained with HS sampling on the Gerstel ChemSensor 4440. These analyses were performed on a GC (6890, Agilent Technologies) with a MSD (5973, Agilent Technologies) equipped with a thermal desorption unit with autosampler (TDS2 and TDSA, Gerstel). The split ratio was set a 30:1 at the programmed temperature vaporizer (PTV) inlet.

*Sample preparation - Headspace sampling.* 5 ml aliquots of each of the 6 bourbons were placed into 10 mL vials which were crimped and equilibrated for 20 minutes at 75 °C before headspace sampling. Since the Gerstel 4440A ChemSensor does not use a column for a separation prior to the mass selective detector (MSD), the entire headspace of each sample is introduced into the MSD.

*Sample preparation - Twister extraction.* Samples were diluted 10 fold in water and extracted for one hour with

stirring at room temperature. The twister was removed, rinsed with water, dried and placed directly in a conditioned thermal desorption tube for analysis.

*Adulteration.* The lesser value bourbon (B) was used to spike the expensive one (A) using a simplex centroid experimental design, as described in Table 1. Caramel coloring from D&D Williamson was added to the inexpensive bourbon (B) to make it visually resemble the 10-year-old. Water was added at the 2 –50% w/w levels to the 10-yr old bourbon.

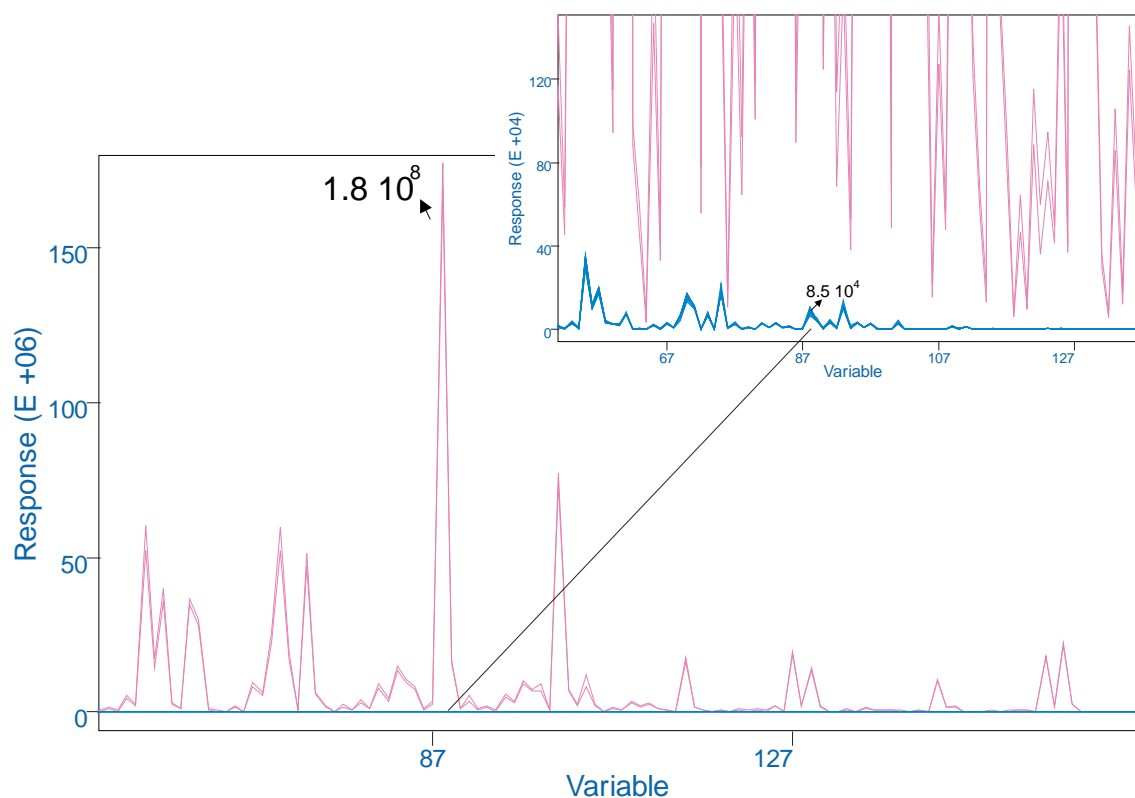
**Table 1.** Composition of adulterated mixtures using a simplex centroid design.

Bourbon	Solutions and volumes of components [mL]									
	1	2	3	4	5	6	7	8	9	10
Lesser value (B)	2.50	5.00	3.75	1.25	2.50	5.00	1.25	0	3.75	0
Expensive (A)	2.50	0	1.25	3.75	2.50	0	3.75	5.00	1.25	5.00

## RESULTS AND DISCUSSION

The mass spectra of the bourbons obtained with Twister and static headspace (HS) were significantly different. The relative abundance obtained with Twister introduction was about four orders of magnitude higher on

certain ions. Figure 2 illustrates these results in which mass spectra of the expensive bourbon (A) is compared. The intensity of ion 88 was four orders of magnitude higher using Twister introduction than HS.

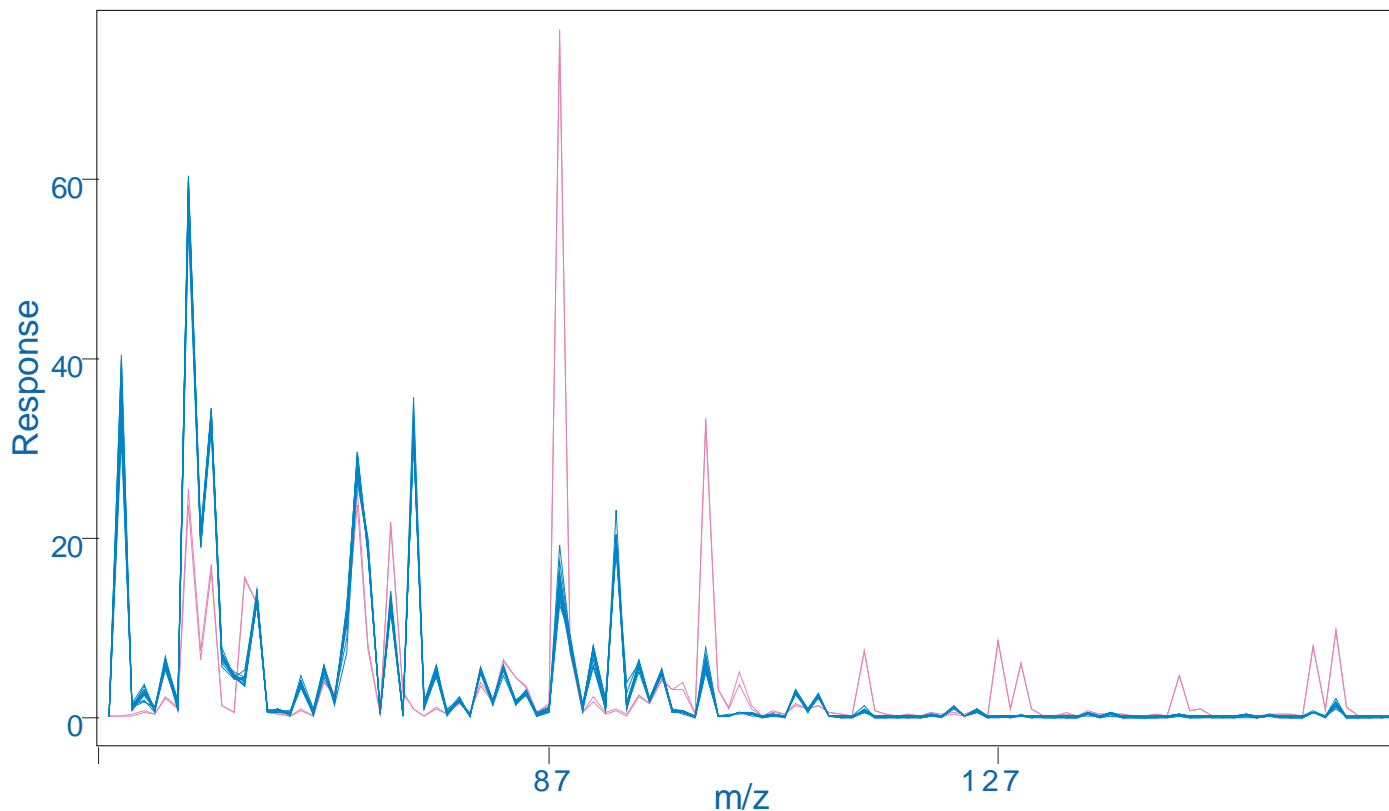


**Figure 2.** Mass spectra of Bourbon A obtained using Twister (magenta trace) and static headspace (blue trace).

The bourbon samples were normalized to remove sample to sample variability. Normalization of the data set puts all the samples in the same scale by removing systematic variation due to sample size.

After normalization, the spectra obtained with Twister and HS introduction can be easily compared. Figure 3 shows the data set from Figure 2 after norma-

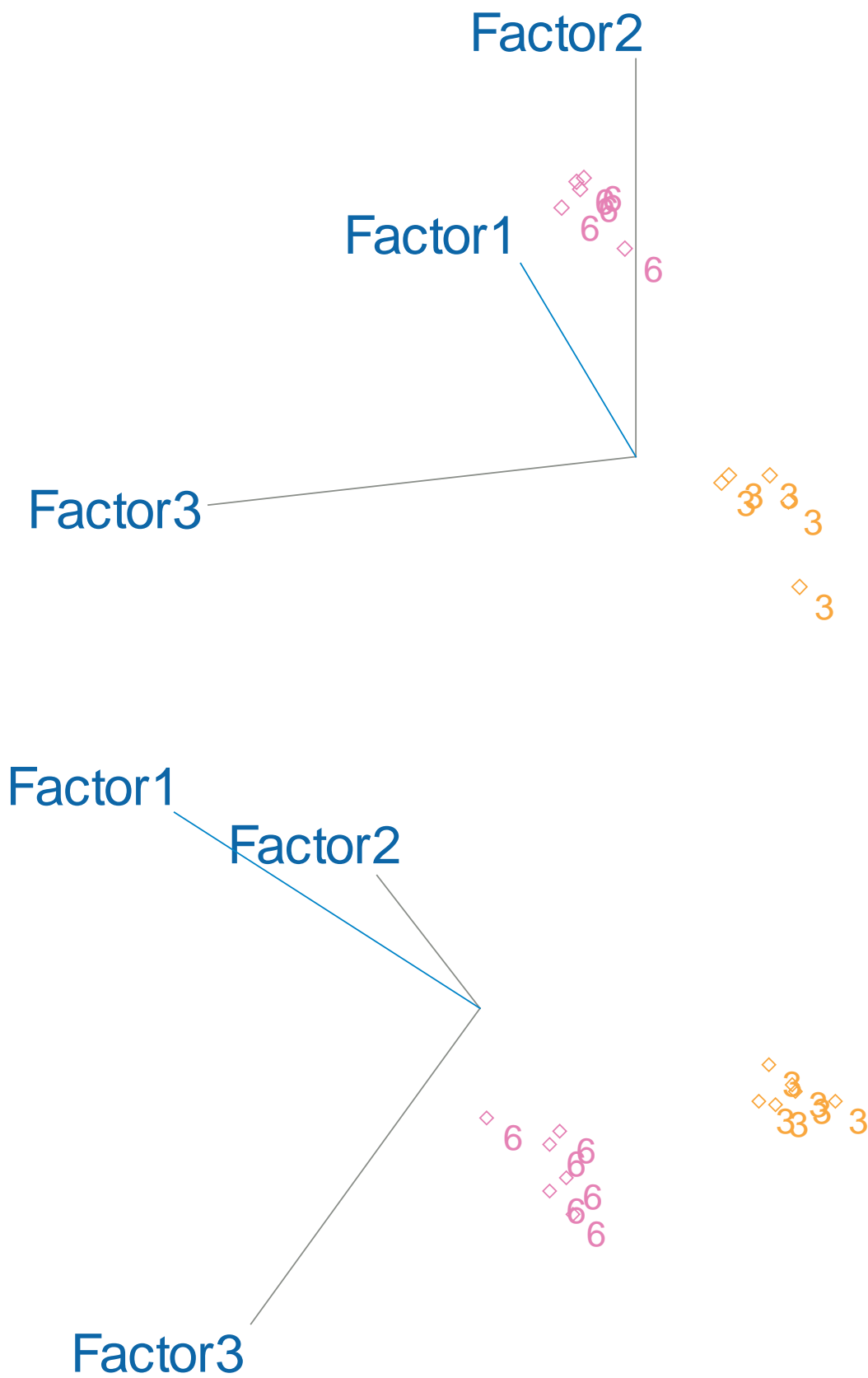
lization. The presence of higher molecular ions ( $m/z = 100$  to  $170$ ) is evident using Twister but not detected using static HS. These results are in agreement with previous studies in which higher sensitivities have been achieved using Twister as sample introduction instead of static HS [6, 7].



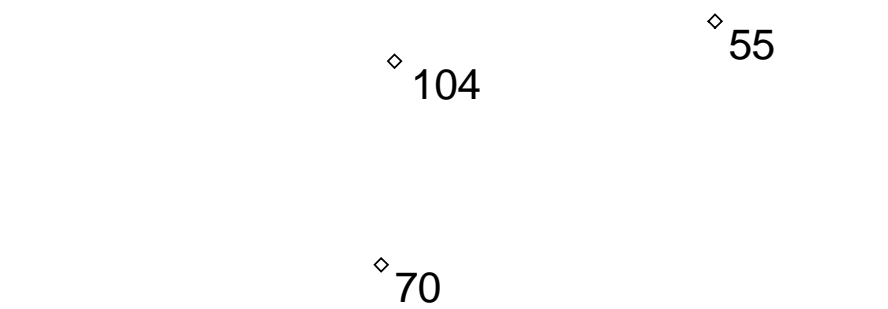
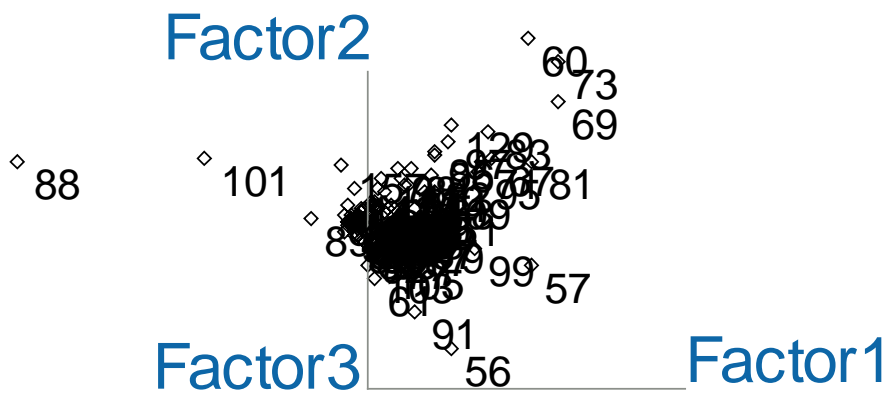
**Figure 3.** Normalized mass spectra of bourbon A.

Projection of the mass spectra of two bourbons (aged 3, and 6 years) into the space of the first three principal components (Figure 4A and 4B) shows good clustering between replicas for both models. The variance captured within the first 3 PCs was 87.3% for the HS

model and 99.5% for the Twister one. An inspection of the loadings (Figure 5A) indicates the presence of ions 104 and 157 used in the Twister model that are not detected with HS (Figure 5B).



**Figure 4.** Projection of the mass spectra of two bourbons (aged 3, and 6 years) into the space of the first three principal components. 4A obtained using Twister and 4B using static headspace.



**Figure 5.** Loadings plot for models of Figure 4. 5A, using Twister and 5B using static HS.

Two soft-independent-modeling-of-class-analogy (SIMCA) classification models were created. Model 1 was obtained using static headspace, model 2 using Twister introduction. Table 2 contains the interclass distances for both models. The interclass distances are used to assess the quality of the SIMCA model.

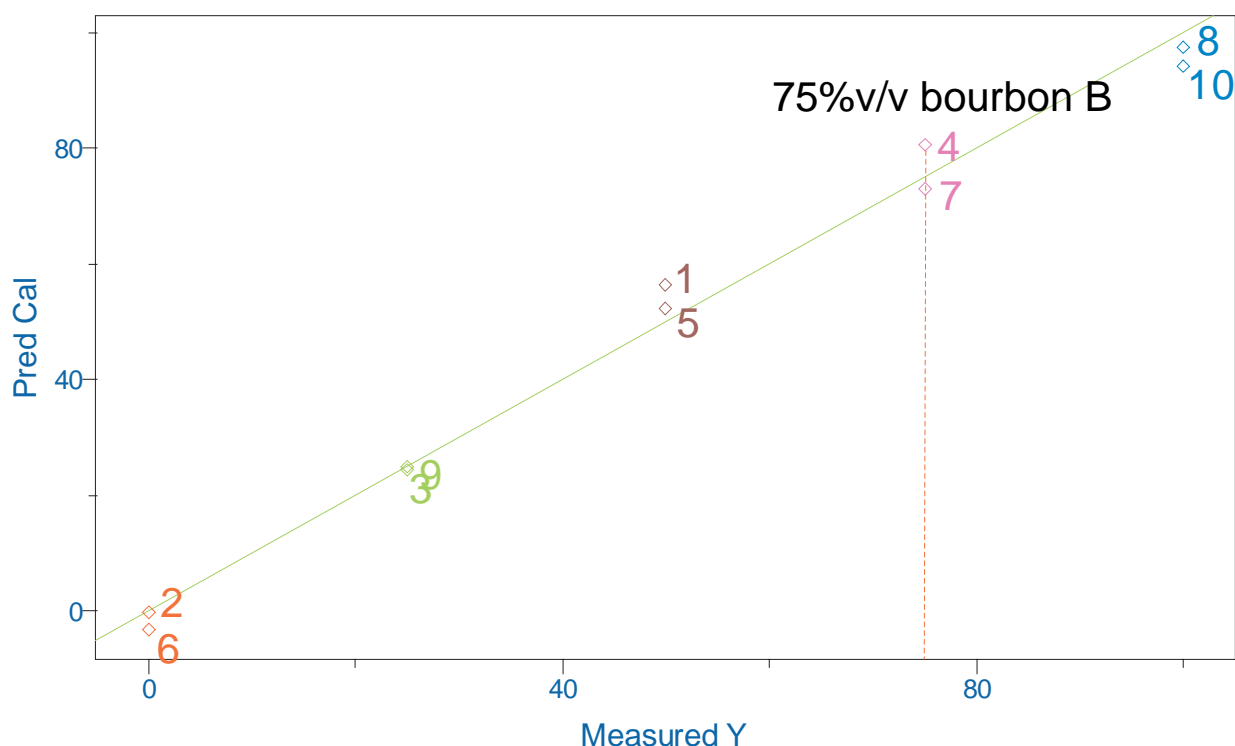
Large distances between classes suggest well-separated classes. As a rule of thumb, classes are considered well-separated if the class distance is greater than 3. Overall, higher interclass distances were obtained using Twister.

**Table 2.** Interclass distances for Twister and headspace introduction.

	Twister			Headspace		
	Year 1	Year 3	Year 6	Year 1	Year 3	Year 6
Year 1	0,00	5,89	5,83	0,00	3,98	1,47
Year 3	5,89	0,00	3,60	3,98	0,00	2,95
Year 6	5,83	3,60	0,00	1,47	2,95	0,00

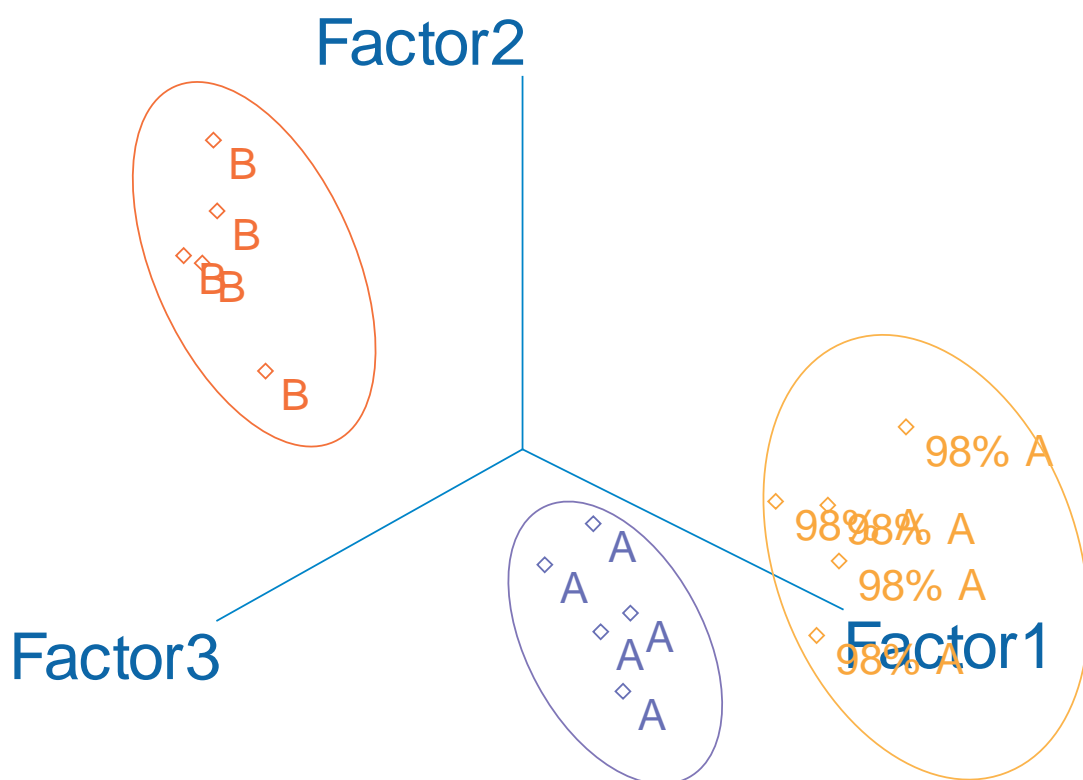
Identification of adulterated bourbons was achieved using SIMCA and principal component regression (PCR). Projections of the higher-value-bourbon spiked with the lesser-value-one (Table 1) into the SIMCA model resulted in no classification of the adulterated samples. This implies that the headspace of the adulterated samples does not match the mass spectrum fingerprint of the pure higher-value bourbon. As seen

in Figure 6A, the adulterated bourbons were easily detected at the 25% level spike with static headspace. We decided to test the model against a smaller spike. Figure 6B shows the projection of the mass spectra of 98% v/v bourbon A with 2% v/v of bourbon B obtained using HS sampling. Twister analysis (not shown) were also capable detecting spiked as low as 2%.



**Figure 6A.** Predicted versus actual concentration obtained with experimental design of Table 1.

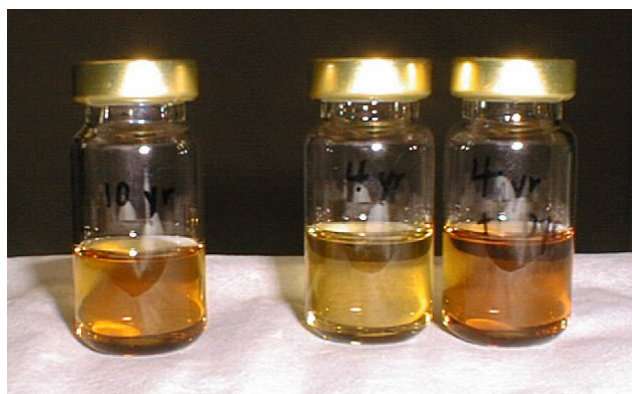
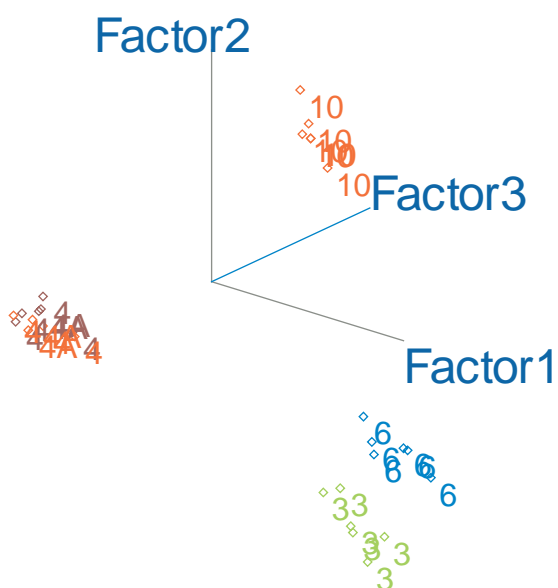




**Figure 6B.** Projection of 98% bourbon A with 2% bourbon B in the space of the first three principal components.

Figure 7 shows the mass spectra's projection of the bourbon adulterated with coloring into the space of the PCA model. Even though the appearance resembles bourbon aged for 10 years (see picture), the chemometric model correctly classifies it as a 4 yr. old. Both sample introduction techniques (HS, Twister) were capable of detecting this type of adulteration. It

appears that the headspace composition of the adulterated bourbon does not change significantly when the coloring is added. Further studies using typical GC/MS (not shown) confirmed this finding. The coloring used in the adulteration contains very little volatiles that do not change significantly the overall headspace composition of the bourbon.

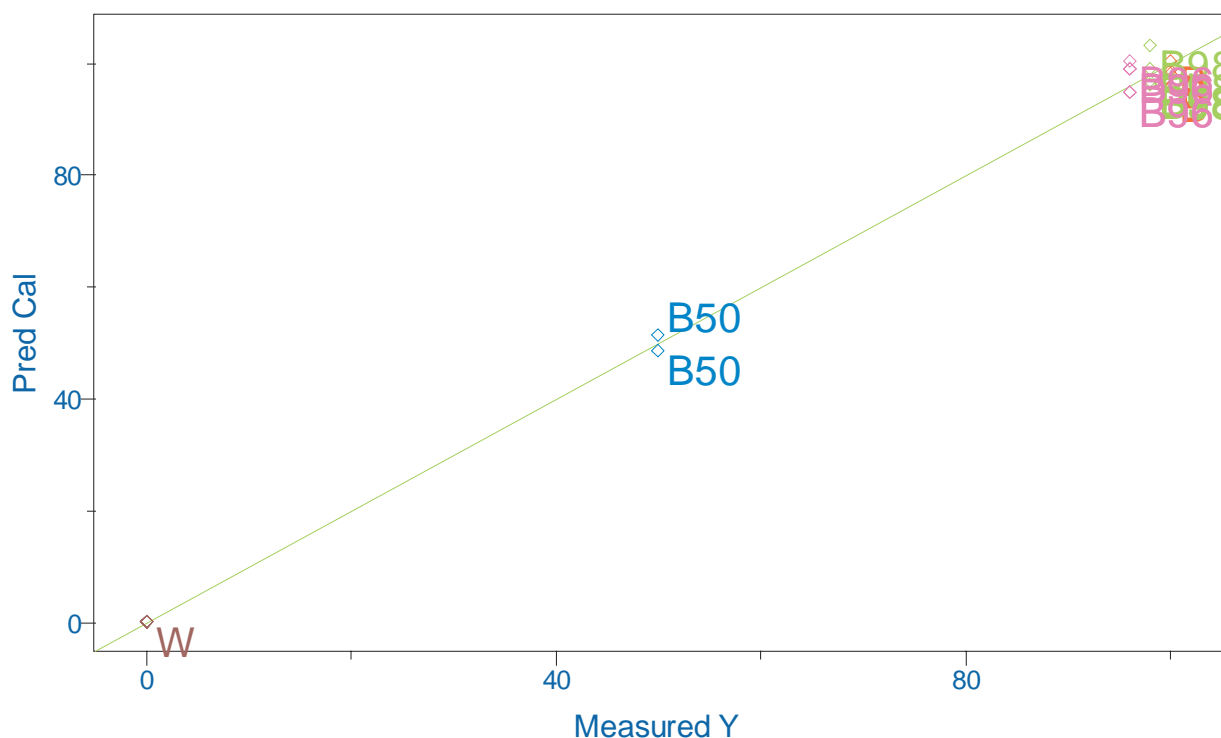


**Figure 7.** Projection of a four-year-old bourbon adulterated with coloring into the space of the first three principal components.



The dilution of bourbon with water was detected using multivariate calibration models. Principal component regression is a factor-based regression that models continuous properties, such as concentration. Figure

8 shows a predicted versus known concentration plot obtained with the HS instrument. Dilution of bourbons with as little as 10% v/v water was also detected with Twister.



**Figure 8.** Predicted versus actual percentage for diluted bourbons with water.

## CONCLUSIONS

The identification of adulterated bourbons was possible using chemometric models built with mass spectra. PCA models using Twister as sample introduction captured higher variance within the first three principal components than models built with static headspace. Higher interclass distances in the SIMCA model were obtained using Twister introduction.

Adulteration of a four-year-aged bourbon with coloring affected the visual appearance but not the overall headspace composition. The SIMCA model classified this bourbon as a four-year-old. Spikes with lesser value bourbon were detected in the low percentage levels. Dilution of bourbons with water was also detected in the low percentage range. Although, both Twister and HS chemometric models performed well for the bourbons, other type of samples could benefit from the higher sensitivity of Twister of higher molecular ions.

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