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Instrumental methods for measuring flavor changes over the shelf life of beer

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Abstract

The chemical disequilibrium that exists in fresh beer results in numerous chemical transformations during storage that have vast implications for the flavor of the product. Quantifying and understanding the nature of these changes has presented a challenge to brewing chemists for decades. Several new tools are becoming increasingly available that show promise for gauging age related changes in beer. Aroma Extraction Dilution Analysis (AEDA), and Multivariate Analysis (MVA) can all contribute to the analysis of aged beer. AEDA is a technique where serial dilutions of an aroma extract of a sample are analyzed by Gas Chromatography-Mass Spectrometry/ Olfactometry (GC-MS/O). The compounds that persist by olfactometry at the highest dilution levels have more impact on the overall aroma of the sample than those that are only present in lower dilutions. Performing this analysis on beer at different time points can supplement instrumental data and focus analysis on compounds that are more important to perception rather than those that simply have the most dramatic change. MVA is a statistical analysis technique that can be used for non-targeted analysis of raw spectroscopic data, thereby allowing a much more holistic and thorough approach than the quantification of individual compounds traditionally considered to participate in the aged flavor of beer. These techniques used individually and in combination show a great deal of promise to further knowledge on the nature of the chemical changes to the aroma of beer during storage. This poster will present the combination of AEDA and MVA to study how the aroma of a commercial ale changes over time when packaged with and without yeast. Additional applications of AEDA, and MVA to better understand chemical changes in beer will also be discussed.

Introduction

As beer ages, its flavor changes, often dramatically. The nature of these changes is understood only in a relatively broad sense and varies in beer based on style, raw material usage, and processing factors. In general, as beer ages, the aroma stales as bound aldehydes are released (Baert, De Clippeleer et al. 2012) and maillard and strecker degradation reactions occur (Vanderhaegen, Neven et al. 2006). Compounds responsible for key attributes when the beer is fresh, such as esters and hop derived terpenes also tend react chemically over time, reducing the masking of less desireable attributes in the beer aroma.

Many of the compounds that change in concentration as a beer ages have an extremely low flavor threshold, which makes them difficult to identify and analyze. Additionally, there are many volatiles present in beer that have no impact on the perception of the aroma, while still others have a disproportionately large impact. As a result, many readily available techniques used for analyzing volatile compounds, such as GC-MS or GC-FID, have limited utility in correlating specific compounds to the impact they have on overall beer aroma. To better understand the impact of compounds on the sensory attribute of foods, olfactometry was first proposed in 1964 (Grosch 2007). In Olfactometry, the eluent from the chromatographic column is evaluated by a trained operator. The result is an aromagram, which, when overlaid on the Total Ion Chromatogram (TIC) from a GC-MS, can direct analytical effort.

While a trained panelist is the detector whose signal is the most pertinent to the analysis of beer volatiles, there are obvious limitations to its use as an analytical instrument. Anosmias to certain compounds as well as biases introduced by the isolation and chromatographic separation of aroma compounds yield a single GC-O run of limited utility. Thus, several aroma dilution techniques were developed, notably AEDA (Grosch 2007, Schieberle 1995).

In this study, AEDA was adapted for use with Solid Phase Microextraction (SPME) as the aroma isolation technique. With SPME, the dilution of the samples was carried out at the split/splitless injector of the GC. This method was used to measure the impact of the addition of yeast on the aroma of an amber ale over a 6 month period.

Materials and Methods

A commercially available amber ale was used for this study. Beer from the same production batch was packaged in bottles without yeast and cans with yeast. The beer was stored at 20°C for 1 month and then 8°C for the remainder of the study. The beer was run on a sensory panel at one month intervals in addition to analytical testing.

AEDA: 5mL of beer was placed in a 20mL amber glass headspace vial (Restek) and extracted using an MPS sampler (Gerstel) with a carboxen/polydimethyl siloxane/divinylbenzene SPME fiber (Supelco) in the headspace at 60°C for 20 min. The fiber was desorbed at 250°C for 3min. and subjected to GC separation on a 7890A GC (Agilent) with a SOLGEL-WAX capillary column (60m x 0.32mm, 0.25µm film thickness, SGE Analytical). The oven profile was as follows:

An open split interface with a nitrogen sweep carried a portion of the column eluent through an olfactory port (Microanalytics) heated to 220 °C. Olfactory data was collected using a touchscreen interface and AromaTrax 9.1 software (Microanalytics). MS data was collected with a 5975C single-quad MSD (Agilent).

Olfactory data from serial dilutions of the beer was compiled using the AEDA feature of the AromaTrax software. The AEDA from each month and package type was aligned by hand. PCA was performed using Pirouette data analysis software (Infometrix).

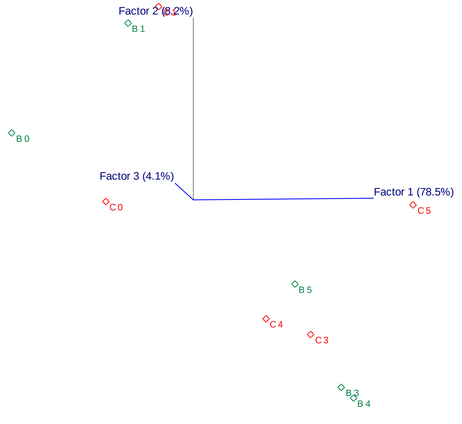

Fig. 3 Scores. B denotes beer without yeast and C beer packaged with yeast and the number the months after packaging. The change observed is initially described by factor 2 and subsequently by factor 1. The separation between treatments initially decreases after one month of warm storage. Subsequent months see the separation increase, particularly at month 5. Month two was omitted as an outlier.

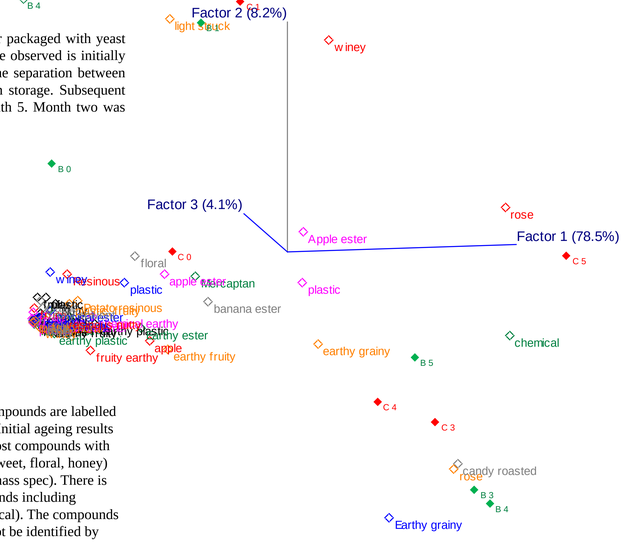

Fig. 4 Loadings (scores overlaid, solid markers). The compounds are labelled with the main descriptors from the olfactory evaluation. Initial ageing results in an overall decrease in flavor dilution (FD) value of most compounds with an increase in phenylethyl acetate (described as winey, sweet, floral, honey) and light struck (likely 3-MBT, cannot be identified by mass spec). There is then a decrease and an increase in FD of several compounds including phenylethyl alcohol (rose) and ethyl dodecanoate (chemical). The compounds described as earthy grainy, candy roasted, and rose cannot be identified by mass spec.

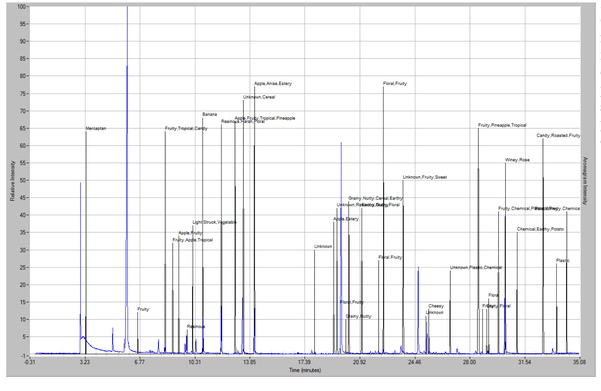

Fig. 1 An aromagram overlaid on a Total Ion Chromatogram of a 1:2 split of beer aged 2 months. Many compounds differ in analytical intensity and perception at the aroma port. An example of this is ethanol (RT ~5min.), which presents no aroma at the olfactory port. In contrast, several compounds that have no signal on the TIC present strong fruity and estery aromas at the olfactory port.

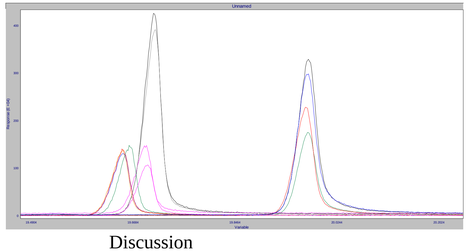

Fig. 2 An overlay of chromatograms showing Ethyl Octanoate, a common beer volatile. Different signal intensities and retention time shift during the 6 months required to collect the data for this study make principal component analysis of the raw data impossible.

Discussion

Over the course of this study, a number of challenges to work of this nature became apparent. The stability and consistency of the chromatographic technique used posed a unique challenge. During the course of the study, routine maintenance on the GC was performed resulting in a substantial retention time shift. There was also large varability in overall signal intensity (Fig. 2). Such shifts can be accounted for using internal standards but introduce complexity to the analysis.

While the use of AEDA in this study accounted for some of the chromatographic difficulties, there were other limitations imposed by the technique. It is very time consuming, requiring 6 hours of operator time at the olfactory port for each aroma dilution set. Hence, scheduling the time to run both package types was difficult. Operator fatigue is also an issue, so the runs must be carried out over two days. One solution that would allow for analysis to be carried out at the same time, thus limiting variation in the measurement technique is to produce a stable liquid extract of the volatiles using a technique such as Solvent Assisted Flavor Evaporation (SAFE). This is a gentle vacuum distillation method that is performed at low temperature to limit chemical transformation of labile compounds (Engel, Bahr et al. 1999). The extract produced would be much more stable than the beer, especially when stored in a deep freeze. Future studies will utilize SAFE for sample preparation and all the analyses of all samples will be performed as close together as possible.

Data analysis required manual alignment of the different time points. Principal Component Analysis (PCA) was used to analyze the data since it gives a holistic visualization of the data. PCA was run on the flavor dilution values from the AEDA. The scores (Fig. 3) show that the data is grouped meaningfully, with the fresh points grouping on the left side near the origin. As the beer ages, the packages diverge, with the beer with yeast moving up and to the right and the beer without yeast moving down. Month 2 is an outlier in the data, with the divergence of the treatments occuring most at months 4 and 5. This finding is consistent with the data produced by the sensory panel wich found the difference between the treatments to only be significant at month 5.

The Loadings (Fig. 4) show the descriptors of the compounds most responsible for the variation between the samples. It is further possible to identify the compounds most correlated with aging of the beer from this plot and focus analysis on those that are responsible for the divergence of package types. Knowledge of the specific compounds whose concentration changes as the beer ages can also help to determine the source and mechanism of the chemicals that cause the aged aroma of the beer.

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