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Advanced method optimization for volatile aroma profiling of beer using two-dimensional gas chromatography time-of-flight mass spectrometry

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ABSTRACT

The complex mixture of volatile organic compounds (VOCs) present in the headspace of Trappist and craft beers was studied to illustrate the efficiency of thermal desorption (TD) comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry (GC × GC-TOFMS) for highlighting subtle differences between highly complex mixtures of VOCs. Headspace solid-phase microextraction (HS-SPME), multiple (and classical) stir bar sorptive extraction (mSBSE), static headspace (SHS), and dynamic headspace (DHS) were compared for the extraction of a set of 21 representative flavor compounds of beer aroma. A Box-Behnken surface response methodology experimental design optimization (DOE) was used for convex hull calculation (Delaunay's triangulation algorithms) of peak dispersion in the chromatographic space. The predicted value of 0.5 for the ratio between the convex hull and the available space was 10% higher than the experimental value, demonstrating the usefulness of the approach to improve optimization of the GC × GC separation. Chemical variations amongst aligned chromatograms were studied by means of Fisher Ratio (FR) determination and F-distribution threshold filtration at different significance levels ($\alpha = 0.05$ and 0.01) and based on *z*-score normalized area for data reduction. Statistically significant compounds were highlighted following principal component analysis (PCA) and hierarchical cluster analysis (HCA). The dendrogram structure not only provided clear visual information about similarities between products but also permitted direct identification of the chemicals and their relative weight in clustering. The effective coupling of DHS-TD-GC × GC-TOFMS with PCA and HCA was able to highlight the differences and common typical VOC patterns among 24 samples of different Trappist and selected Canadian craft beers.

1. Introduction

Beer is one of the oldest beverages created by humans. Archeologists discovered traces of fermented grain-based mixtures that are 10,000 years old. Even if current technology has improved the quality control placed on the process of brewing, the idea of fermenting vegetal base to produce alcohol remains identical to this primitive resource [1]. According to the United States Brewers' Association, the beer market is quite stable, with a growth of 0.5% during 2015. However, within the beer market, the behavior of beer drinkers is evolving. Consumers have begun to purchase lower amounts of industrial beer and higher amounts of abbey and craft beers in a quest for taste authenticity [2]. The aroma of beer is known to derive mainly from malted barley [3] (due to the barley itself, as well as the thermal treatment during malting), hops [4], yeast [5,6], and the development of the beverage during maturation and aging [7,8]. Despite the fact that these ingredients are common to all beers, some traditionally produced beers exhibit particular aromas and flavors, and appear particularly suited to aging. However, little is known regarding the chemistry of beer aroma. This is espe-

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cially true in terms of the composition of the volatile components of the aroma that can greatly vary between different traditional brewing processes. Several approaches towards the characterization of complex volatile organic compound (VOC) signatures of beer headspace currently exist but could be improved in order to obtain more exhaustive chemical information.

When conducting volatile aroma profiling, several approaches are possible for sampling the headspace of beer or other matrices. Among them, solid-phase microextraction (SPME) has previously been demonstrated to be capable of isolating a limited number of volatile organic compounds (VOCs) in beer [9–14]. However, possible limitations in trapping efficiency have not been estimated, especially when compared to other robust techniques such as *multiple* stir bar sorptive extraction (*m*SBSE) [15,16], static headspace (SHS) [17], and dynamic headspace (DHS) [18]. When considering these instrumental headspace extraction techniques, thermal desorption (TD) is required for sample introduction into the gas chromatography (GC) system for the separation step. Even when coupled with mass spectrometry (MS) that offers powerful deconvolution algorithms, a classical one-dimensional (1D) GC approach only provides partial information for aroma analysis. This is largely due to the fact that the number of components that must be separated within the VOC mixture can easily reach several hundred, rapidly surpassing both the peak capacity and the analytical resolution of a 1D GC–MS approach [11].

Comprehensive two-dimensional gas chromatography (GC × GC) coupled to time-of-flight MS (TOFMS) has been reported to be very effective for VOC measurements in the headspace of complex samples [19]. GC × GC-TOFMS combines the advantages of a semi-orthogonal two-dimensional chromatographic separation to the analytical power of signal deconvolution of non-skewed mass spectral data. Although this provides several obvious advantages over 1D GC–MS, an underlying concern of GC × GC-TOFMS is the treatment of data sets that are inherently large and complex in nature. Additionally, due to the complexity of signals generated from a mixture of compounds spanning dynamic ranges of several orders of magnitude, most available peak finding tools report large quantities of artifact signals as ‘peaks’ that have to be removed from raw peak tables prior to any further data processing. Most GC × GC-TOFMS data produced from replicate VOC measurements in complex biological matrices also require statistical treatments to extract the characteristics of an analytical sample population and properly differentiate between samples types while biological variation is minimized. A major challenge in the selection of appropriate statistical tools is that very often, if not always, the number of replicate (n) is much lower than the number of features (p) that are produced by GC × GC-TOFMS measurements. This high-dimensionality situation is of concern, as most of the classical statistical approaches are based on low-dimensional situations where n is much greater than p (i.e. $n \geq 3p$) [20]. In practice, a typical GC × GC-TOFMS data set will exhibit several hundred of features, p, while the number of replicates n rarely exceeds triplicates. This is an extreme case of high-dimensionality. Processing such data using unsuited statistical tools has the potential to lead to data overfitting issues or misinterpretation of results [20].

Classical multivariate statistical methods, such as Principal Component Analysis (PCA), are often used to visualize GC × GC-TOFMS data without consideration of this potential issue. Since it is often not possible in practice to increase the number of replicates to match or exceed the number of features, other solutions of various complexities have been proposed for data reduction. An emerging approach is to calculate a Fisher ratio (FR) for each component of the multidimensional data set to highlight chemical differences between classes of samples [21,22]. Such a supervised approach was demonstrated to be efficient in treating sets of biological samples prior to further statistical treatment [21–24].

In order to investigate the specificity of Trappist and craft beer aroma, the VOC profile of this family of beers was investigated using combinations of available headspace extraction techniques and a specific approach for GC × GC-TOFMS data processing. SPME, *m*SBSE, SHS, and DHS were compared in terms of their extraction efficiency for beer VOCs prior to TD and GC–MS. As the true peak capacity and separation efficiency rely on the number of peaks that can practically be separated in the chromatographic space, the occupation of the two-dimensional space was optimized using experimental design and a convex hull (or convex envelope) approach [25]. For GC × GC-TOFMS data processing, a modified FR approach was used by applying an F-distribution threshold strategy prior to further processing. This reduced data set was then processed using different supervised and non-supervised statistical approaches such as Fisher Ratio discrimination, PCA, and hierarchical cluster analysis (HCA). In this approach, a theoretical F-distribution was applied based on an F-critical (F_{crit}) value derived from the number of degrees of freedom, the number of classes, and a significance level ($\alpha = 0.05$ and 0.01). The large data sets produced from statistically optimized TD-GC × GC-TOFMS of beer analysis were used to evaluate the different parameters affecting this simple but valuable alternative FR method.

2. Experimental section

2.1. Sampling

All Trappist beers were bought on the same day in one local beer shop in Liège, Belgium. All Trappist beers were bought in 330 mL (33 cL) glass bottles. Each beer was opened and an aliquot of 100 mL was transferred into a 1 L glass beaker. The craft beers came from the Rorschach Brewery (Toronto, Canada). They were shipped in 50 mL sealed vials. Samples were degassed by sonication for 5 min. Each beer was sampled in a minimum of triplicates of 10 mL, which were transferred into a 20 mL headspace vial containing 2 g of NaCl (Merck KGaA, Darmstadt, Germany). A list of all beers is given in Table S-1. Sample blanks were also prepared by adding 2 g of NaCl in a 20 mL headspace vial containing 10 mL of type 1 deionized water. The optimization of sampling was conducted on a Japanese Pilsner and the optimization of the chromatographic separation was conducted on a batch of Chimay Bleu purchased in Belgium. Several VOC sampling methods were compared in order to determine the best approach for beer aroma analysis. For SBSE, a PDMS coated stir bar (10 mm length, 1 mm film thickness) was used (Gerstel®, Tokyo, Japan). For *m*SBSE, a combination of PDMS and EG-Silicone stir bars (10 mm length, 32 μL phase volume) were tested (Gerstel®). For SPME, an 85 μm Carboxen/PDMS fiber was used (Supelco®, Bellefonte, PA, USA). For SHS and DHS, a TD tube filled with Tenax TA was used (Gerstel®). The optimized method parameters are given in Table 1. Based on method comparisons, the sampling was performed using dynamic headspace (DHS) using a fully automated Gerstel® MPS System (Gerstel®). First, samples were incubated at 80 °C for 15 min with agitation. Second, each vial was transferred to the DHS module for headspace trapping. The trapping was performed using a glass tube filled with Tenax TA (Gerstel®). The purge volume was 60 mL with a flow of 20 mL/min (3 min sampling). Due to the high water content, a drying step was conducted with a drying volume of 900 mL and a flow of 50 mL at 40 °C (18 min total). The drying volume required to evaporate the water was calculated using the Maestro software 1.4.26.11 (Gerstel®).