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Chemometric analysis of the volatile fraction evolution of Portuguese beer under shelf storage conditions

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ABSTRACT

In this work we present a multivariate statistical analysis of the evolution of the volatile fraction of Portuguese beer over an extended period of 1 year under standard shelf storage conditions, using gas chromatography coupled with mass spectrometry (GC–MS). A systematic methodology is proposed for detecting the onset of meaningful changes in chemical composition during shelf storage and to monitor its evolution along time. We also put forward and discuss chemometric procedures for analyzing the contributions of different chemical components in the definition of dynamic ageing trends. In summary, the chemometric analysis reveals that the chemical composition of beer presents a statistically meaningful deviation from the reference scenario after a period of 7 months, although the deviation trend has its onset during the 6th month. The analysis performed also underlines the limitations of current variable contribution methods, and an alternative procedure was proposed based on the analysis in the original domain which finally led to a consistent and interpretable clustering structure of the volatile fraction compounds. Esters and higher alcohol compounds stand up on a cluster arrangement suggesting that their strict control can effectively point out meaningful changes on beer aroma. Organic acids, namely caprylic, capric and acetic acids can also be very helpful in that sense.

1. Introduction

Brewery industry has been well-succeeded in dealing with a wide variety of relevant quality issues in its products. Quality aspects related with beer appearance and safety, namely colloidal stability, haze formation or microbiological spoilage, are now considered to be completely under control. Currently, the most important quality parameter and the shelf-life-limiting quality aspect is beer flavor [1–4] and efforts have been conducted towards maximizing and predicting beer shelf life taking into account flavor information.

Several compounds have been pointed out as key agents of beer sensory changes and suggested as deterioration markers during ageing, in particular diacetyl [5], acetaldehyde and ethyl acetate [6], (E)-2-nonenal [7], Iso-α-acids [8], riboflavin and amino acids [9] and 5-hydroxymethylfurfural [10]. However, the evaluation of beer shelf life should be more comprehensive and take into account the heterogeneity of chemical volatile groups presented in beer, which can either individually or simultaneously, influence the beer stale flavor in a synergistic or antagonistic sense [11]. Furthermore, beer aroma is also an important aspect in customer preferences and loyalty, since the typical beer consumer, once he/she has approved and selected a given brand, expects the same "degree of excellence" (i.e., quality) from the product on future experiences [6,12–15].

Aware of the importance of aroma evolution during ageing, brewers and the scientific community have been developing approaches to evaluate the overall beer aroma quality as well as strategies to verify the accomplishment of expected attributes in the final product. Electronic sensors have deserved special attention in that sense. A number of papers have proposed the use of electronic tongue devices for quality control, in particular regarding the prediction of beer sensory attributes frequently evaluated according to the brewers' panel list [16–19]. Results achieved in these works suggest that e-tongue can be used as a fast screening tool to check quality control parameters in a routine way. Electronic nose systems have also been tested to evaluate aroma fingerprint changes in beer during the ageing process. M. Ghasemi-Varnamkhasti et al. reviewed the last applications of e-nose in brewery applied to quality assessment [20], reporting applications for hop's quality assessment [21], on-line process control [22], monitoring the odor and taste of active compounds [23] and as a brand authenticity checking tool [24,25]. All works reported in this review take advantage

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of the use of multivariate techniques for data analysis, being reported a plethora of techniques used in exploratory data analysis, calibration and pattern recognition tasks. The information of spectroscopy techniques (FTIR, NIR, RMN) combined with chemometric modeling has also generated good results on monitoring the fermentation process [26,27] and on assessing quality control parameters [28]. Other works explored the information of beer aroma obtained from chromatography techniques to discriminate products by brands, categories and production areas [17,29–31]. Chemical composition underlying beer deterioration has also been analyzed. Most of the work has been conducted under ageing acceleration conditions, namely by submitting beer samples to high temperatures [32–35]. In these cases, the classification models typically achieve good discrimination performances concerning the differentiation of fresh and matured beer. Multivariate tools applied to classification and calibration models also elucidate the flavor characteristics responsible for the differences identified during ageing. However, and notwithstanding the value of the information reported in these studies, to the best of our knowledge, no strategy or methodology is yet available for specifically addressing the key issue of beer shelf life. This is the focus of the presented work, where a methodology is proposed for determining the period of time after which beer fresh features start to change in a statistically significant way under standard shelf storage conditions and, accordingly, all degradation phenomena begins to accelerate. In this study, bottles were maintained at normal ageing conditions and the entire analysis carried out during one year. This is another differentiating feature of the present study, with the purpose to make the whole assessment as realistic as possible, mitigating the risks in the transfer of results to the industrial practice.

The remaining parts of this article are organized as follows: Section 2 describes the experimental protocol and the analytic and chemometric methods. Section 3 presents the analysis workflow and the main results obtained. In Section 4, we discuss relevant aspects of the analysis and some of its implications. Finally, in Section 5, we summarize the main findings in this work and address future work worth undertaking in the sequence of the results reported in this article.

2. Materials and methods

2.1. Beer samples

A total of 39 lager beers were analyzed in this study. Samples were kept at room temperature, within 12 months on amber glass bottles of 330 mL. The analysis of volatile compounds was done monthly, for three different bottles randomly selected. All samples were kindly supplied by the *Empresa de Cervejas da Madeira, Lda*, and collected from the same production batch to minimize inter-batch variability.

Throughout the text, samples will be denoted by the month in which each bottle is open and analysis carried out and, when necessary, a letter (A, B and C) will be used to distinguish among the replicates analyzed for the same ageing time.

2.2. Analytical procedures

The analysis of volatile compounds was carried out on a TRACE GC Ultra gas chromatograph equipped with the ISQ single quadrupole (electron impact mode) and the TriPlus autosampler (liquid mode) from Thermo Scientific (Hudson, NH). The extract injected was obtained through solid phase micro-extraction as described elsewhere [36]. A total of 70 volatile compounds were identified and quantified in terms of the relative area with respect to the internal standard (3-octanol). The identification of compounds was made by comparison of the mass spectra obtained with those present in NIST08 and Wiley 6.0 MS library databases, and comparing the obtained Kovats indices with those stated in the NIST Chemistry WebBook [37].

2.3. Chemometric methods

Chemometrics encompass a wide range of methodologies and approaches for extracting useful information from complex data sets originated in analytical instrumentation and industrial processes. There is no standard and universal protocol to conduct a chemometric analysis, and the specific path to follow is always dependent on the problem in hand, goals to achieve and the data structure originated by the analytical procedure adopted. In fact, chemometrics can be seen as an integral part of the scientific method when it comes to analyzing chemical information with modern instrumentation.

For the current problem, the collected data is structured in a two-way table, where one mode stands for the different components quantified with resource to gas chromatography coupled with mass spectrometry, GC–MS, (columns' mode) and the other mode regards time information relative to the moments where bottles from a homogeneous group were randomly opened and analyzed (rows' mode). The existence of such two meaningful modes in the data structure implies that one should select and adopt data analysis methodologies with the potential to extract and explore the fundamental pieces of information relative to both the composition and time modes, and eventually their interaction, if relevant. In this section we present the main frameworks employed in the chemometric analysis, which will integrate the workflow presented in Section 3.

2.3.1. Principal component analysis (PCA)

Principal component analysis is a well-known methodology to extract the linear correlation structure shared by multiple variables [38–40]. It essentially aims at explaining most of data variability using as few linear combinations of all variables (called variates) as possible. The linear combinations of the variables constitute new transformed variables, the principal components, PC_a, whose values are the scores, stored in $(n \times 1)$ column vectors t_a, (where n stands for the number of observations in the data set — in this work it is the total number of beer samples collected after storage in shelf conditions and analyzed in the laboratory). The coefficients of the linear combination are called loadings, which for each PC can be stored in the $(m \times 1)$ column vector l_j (where m is the number of variables — in the present work it corresponds to chemical compounds). As a scale-dependent technique, PCA is usually applied after proper pre-processing of the original $(n \times m)$ raw data matrix, **X**. The resulting pre-processed matrix with the same size, **Z**, presents better properties to achieve the purposes of analysis. In this work we applied autoscaling (centering to zero mean followed by scaling to unit variance) in order to provide all components with the same a priori weight in the PCA procedure.

In mathematical terms, the scores and loading vectors are gathered in matrices, called the score matrix, **T**, and the loading matrix, **L**, leading to the following decomposition of the pre-processed data matrix:

$$\mathbf{Z} = \mathbf{T} \cdot \mathbf{L}^T + \mathbf{E} \quad (1)$$

where **T** and **L** are $(n \times a)$ and $(m \times a)$ matrices, respectively, a is the number of retained components, also known as the pseudorank ($a << m$) and **E** is a $(n \times m)$ residual matrix, with information that is orthogonal to that described by the retained principal components. In practice, an analyst looks for information regarding the variable mode in PC loadings (such as groups of correlated variables) and concerning the observation mode in the scores (such as trends and clusters of observations).

By construction, PCA is entirely focused on the variable mode and has no built-in capacity to describe or incorporate any part of the observation structure in its implementation. It tacitly assumes samples to be generated from an independent and identically distributed multivariate distribution (the usual *iid* assumption). Even its evolution to cope with the time-related mode, dynamic principal component analysis (DPCA), consists of using the trick of adding artificial variables corresponding to