Chemometric Tools for Beer Quality and Stability

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Achieving beer quality and stability remains the main challenge for the brewing industry. Despite all the technologies available, to obtain a high-quality product, it is important to know and control every step of the beer production process. Since the process has an impact on the quality and stability of the final product, it is important to create mechanisms that help manage and monitor the beer production and aging processes. Multivariate statistical techniques (chemometrics) can be a very useful tool for this purpose, as they facilitate the extraction and interpretation of information from brewing datasets by managing the connections between different types of data with multiple variables. In addition, chemometrics could help to better understand the process and the quality of the product during its shelf life.

sensory quality brewing process multivariate analysis

1. Brief Introduction for Beer Production Process

The beer production process can be divided into five steps: malting, mashing, boiling, fermentation, and maturation. It starts with malting the barley via controlled steeping, germination and kilning, transforming it into a product that is much more friable, with active and increased enzyme levels and with different chemical and physical properties [1].

The next step is to grind the malted barley for mashing. The grinding characteristics (coarse or fine) will depend on the type of equipment used by the brewer. Mashu tuns or lauter tuns use a coarser grind produced by roll mills, but mash filters can use much finer grist produced by a hammer mill [1]. During mashing, the ground malt—and cereal adjuncts, if used—are mixed with hot water and the enzymes present in the cereal will degrade the proteins in small molecules and the starch into small sugars such as maltose and glucose, producing a soluble malt extract, wort. Before being sent to boiling, the wort is separated from the solid part. For the classic infusion mashing, this separation takes place in the mash tun. For the other separation, such as decoction mashing and Double mashing the mash separation is usually carried out using other equipment such as a lauter tun or a mash filter [1]. This filtration process aims to brighten the wort and obtain the maximum amount of extract from the solid residue.

After filtration, the wort is boiled, and the hops are added to the kettle. The main objectives of this step are wort sterilization, extraction of bitter and aroma compounds from hops, coagulation of the excess of proteins and tannins to form trub that can be removed later, color and flavor formation, removal of undesirable volatiles via evaporation, and concentration of the wort by evaporation of water [2][3].

Before sending the wort to the fermentation vessel, it must be aerated and cooled until it reaches the appropriate fermentation temperature, which is typically 8 to 15 °C for lager and 14 to 25 °C for ale beers. During fermentation, yeast is mainly responsible for converting sugars into alcohol and carbon dioxide, but many aroma compounds are also generated. A great fermentation performance demands control of many key variables, such as yeast amount and viability, oxygen input, nutritional wort ability, pH, temperature, and agitation [3][4]. The key to fermentation efficiency and, for definition, of many sensory characteristics that contribute to the quality of the final beer, is the yeast.

At this point in the brewing process, the wort has already been transformed into beer, known as '*green beer*', and most of the yeast is removed from the vessel, giving a path to maturation. The function of the remaining yeast during maturation is to produce higher carbon dioxide amounts and chemical removal of undesirable compounds. The main objective of this step is to initiate beer clarification and prevent oxidation of the product during the storage by maintaining beer in a reduced state [3]. To contribute to the microbiological stability and help with the clarification process, some types of beer are filtered.

Despite the importance of each step in the beer production process, the focus is on the main three of them: malting, boiling, and fermentation. These steps have important contributions to the quality and stability of the final product, such as improvement of color, transparency, bitterness, and foam properties; wort sterilization and protection; and alcohol, carbon dioxide, and desirable flavors formation.

2. Analysis of Beer Quality and Stability Using Chemometric Tools

2.1. Exploratory Analysis

The chemometric techniques applied to monitor and control the quality of beer production and aging processes cover a wide range of applications, which include exploratory data analysis (EDA), pattern recognition (or classification), and multivariate calibration. The EDA techniques, also known as unsupervised pattern recognition, are usually the first approach used to visualize the data, as they reduce the dimensionality of complex datasets and produce a graphical representation that is easy to understand and interpret. The procedure consists of grouping data based on their similarities, with no prior assumptions about class membership. In food science, the most popular EDA techniques used are principal component analysis (PCA) and hierarchical cluster analysis (HCA) [5].

An example of the application of EDA in the beer production process is related to the characterization of raw materials. Inui et al. [6] used PCA to characterize the volatile compounds responsible for the differences in hop aroma characteristics in beer. PCA was able to indicate the positional relationship among six hop aroma characteristics and five hopped beers. Furthermore, it could be concluded that understanding the relationship between instrumental data and organoleptic evaluation using PCA is effective and reliable for determining the key aroma compounds from numerous unknown components. Dong et al. [7], applied PCA to evaluate the variability of volatile aroma compounds among barley cultivars. The results obtained via PCA analysis showed that the aroma characteristics of brewing barley cultivars from different countries were quite different, while those from the same country were similar, especially the Chinese domestic barley cultivars. In another work, Bettenhausen et al. [8] used PCA to study if there were sensorial differences among six different beers produced from six different malt sources. A descriptive sensory analysis was performed on 45 attributes at 0, 4, and 8 weeks of storage, revealing flavor differences at 8 weeks and thus showing the importance of the malt source in beer flavor stability.

Another example using both EDA techniques, PCA and HCA, was shown by Rendall et al. [9], where the evolution of the volatile fraction of Portuguese beers over a period of one year was analyzed under standard shelf storage conditions using gas chromatography coupled with mass spectrometry (GC-MS). A total of 39 lager beers from the same production batch, kept at room temperature for 12 months, were analyzed. The chemometric analysis conducted focused on detecting the early onset of meaningful changes in chemical composition, and then on the analysis and characterization of the evolution of groups of compounds. The chemometric analysis revealed that the chemical composition of the beer presented a statistically significant deviation after 7 months, although the deviation trend had its onset during the sixth month. Furthermore, it was concluded that there is no single resulting compound that can be identified as a unique aging marker, but rather two sets of compounds acting in a synergistic or antagonist way to produce significant changes in fresh beer flavor.

To propose a methodology aiming at fast non-destructive metabolomic characterization of a beer, exploring its compositional profile, and highlighting potential trends or peculiar samples, Cavallini et al. [10], combined NMR spectroscopy and chemometrics. One hundred pale beers from different brands were analyzed. PCA was used for exploratory purposes, on both the full spectrum and features datasets and Multivariate Curve Resolution (MCR) was used for extracting the chemical features from the NMR spectra, which allowed a reduced dataset of resolved relative concentrations to be obtained. This approach using NMR spectroscopy and chemometrics offered clear information about beer composition, providing valuable information about beer characterization that proved to be very useful to the producers in terms of both quality control and innovation.

Coelho et al. [11] studied the beer aging process in wood barrels previously used to age Port wines. The volatile GC-MS fingerprints of unaged beers and beers aged in different times and conditions were analyzed using PCA. Samples showed groups depending on the aging time, which in turn was positively correlated with the presence of a higher number of volatile compounds. Differences in volatile composition were also found between the barrel aged beers and the unaged beer, thus showing that reutilized barrels may have an impact on aged beer production.

2.2. Classification Techniques

Pattern recognition methods or classification techniques are supervised methods that aim to recognize patterns in the data and classify observations by assigning a new sample into a category or class [5]. There are two different approaches in classification: discrimination and class modeling. When a discriminant approach is used, the focus is put on the difference between classes, and a sample is always assigned to a given class. For a two-class problem (classes A and B), a sample will be always assigned to A or to B. Examples of discriminant techniques are *k*-

nearest neighbors (*k*NN), Artificial Neural Networks (ANN), Linear Discriminant Analysis (LDA), or Partial Least Squares Discriminant Analysis (PLS-DA). On the other hand, class-modeling methods focus on the similarities among samples from the same class rather than the differences between classes. In class modeling, classes are modeled individually and independently, and a sample can be assigned to a given class, to more than one class, or to none of the classes. For a two-class problem (A and B), a sample could be assigned to A, to B, to A and B, or to neither A nor B. A typical example of a class-modeling technique is SIMCA (Soft Independent Modeling of Class Analogies). In beer science, classification methods are extensively applied to discriminate between geographical origins, to assess brand authenticity or beer style, to assess raw materials, and also to discriminate between fresh and aged beer [5][12][13]. The choice of a particular technique will depend on the nature of the problem at hand; that is, whether it is discriminant or class modeling (i.e., PLS-DA vs. SIMCA), whether it is linear or non-linear (i.e., LDA vs. ANN), or depending on the number and degree of correlation of the measured variables (i.e., LDA vs. PLS-DA).

Classification techniques can be applied in the beer production process in order to verify the authenticity of the product based on the raw material or the production process. Silva et al. [14] built PLS-DA models to distinguish lager beers based on the type of raw materials employed in the brewing process. NMR spectroscopy was used combined with chemometrics to discriminate lager beer samples according to their style and the raw material information provided on the label. It was concluded that the approach adopted could be very useful when applied to a suitable set of samples. The models obtained had a prediction power higher than 90%, considering the raw material employed in the brewing processes. Vivian et al. [3] used PLS-DA to characterize markers of key production stages of the brewing process of a Brazilian craft brewery using electrospray ionization (ESI) high-resolution mass spectrometry (HRMS). It was concluded that their approach allows a quick assessment of the process status before it is finished without the subjectiveness of sensorial analysis, thus preventing higher production costs, ensuring quality, and helping with the control of desirable features, such as flavor, foam stability, and drinkability. Gianetti et al. [15] evaluated the flavor profile from craft beers (unfiltered and unpasteurized) and industrial beers. It was to characterize craft beers to differentiate them from industrial mass-produced beers. PLS-DA was used to classify beers according to their different production methods. The results showed a good classification, both in calibration (96.2%) and cross-validation (94.2%), enabling a good separation between beer categories with high prediction accuracy (96.23%).

Several studies involving pattern recognition methods to monitor the aging process have been carried out. Linear techniques such as PCA and LDA were applied by Ghasemi-Varnamkhasti et al. [16] to characterize the change of aroma of alcoholic and non-alcoholic beers from the same brand during the aging process by using metal oxide semiconductor-based electronic nose. The results did not reveal clear discrimination among alcoholic aged beers, showing more stability of such types of beer compared with non-alcoholic aged beers. Rodrigues et al. [17] applied PCA and PLS-DA to NMR spectra to monitor the chemical changes occurring in a lager beer exposed to forced aging. Inspection of PLS-DA loadings and peak integration enabled the changing compounds to be identified and revealed the importance of well-known aging markers, as well as other relevant compounds.

Understanding and controlling the aging process of a beer remains a hard task for brewers. By Ghasemi-Varnamkhasti et al. [18], the potential of NIR spectroscopy for the qualitative analysis of different types of beer during the aging process was measured. PCA, KNN, LDA, Stepwise LDA, Genetic Algorithms (GA) and Gram–Schmidt supervised orthogonalization (SELECT) were employed to characterize the aging phases as well as beer types. The results demonstrated that the computational tools were capable of discriminating and classifying the aged beers, showing high classification accuracies for all aging treatments.

Classification techniques have also been applied for authentication purposes. Tan et al. [19] discriminated Chinese lager beers produced by different manufacturers, with good accuracy. They used a data fusion approach by combining fluorescence, UV, and visible spectroscopies. LDA and PCA-LDA (LDA applied on the scores of a previous PCA on the data) were applied, showing a much better classification accuracy (79–87%) when compared with the classification models on the individual instrumental techniques (42–70%). Gordon et al. [20] used Mid-infrared (MIR) spectroscopy coupled with attenuated total reflectance (ATR) to classify different beer types (ale vs. lager and commercial vs. craft beer). PLS-DA was used to analyze and to discriminate the beer samples based on their infrared spectra. Correct classification rates of 100% were achieved in all cases, showing the capability of MIR spectroscopy combined with PLS-DA to classify beer samples according to style and production. Furthermore, dissolved gases in the beer products were shown not to interfere as overlapping artefacts in the analysis. The benefits of using MIR-ATR for rapid and detailed analysis coupled with multivariate analysis can be considered a valuable tool for researchers and brewers interested in quality control, traceability, and food adulteration.

2.3. Multivariate Calibration

Multivariate calibration aims to create a mathematical model to predict properties of interest from instrumental measurements. Modeling can help control and optimize process performances, which are very useful to the beer production process. Moreover, to better understand the aging process, multivariate calibration can link chemical and sensory data [12]. The most commonly used multivariate calibration techniques are multiple linear regression (MLR), principal component regression (PCR), and partial least squares (PLS) regression. These calibration models need a thorough validation before they can be used to reliably predict system or product properties [5].

An example of the use of multivariate calibration was shown in Sturm et al. [21]. The dynamics was investigated in the drying behavior and quality development of hops using visual and environmental sensors combined with chemometrics. To better understand the dynamics of the drying process, a full array of visual sensors was integrated into a pilot scale drying system to investigate the color changes, Hop Storage Index, α acids and β acids, product and air temperature, and air humidity, throughout the drying process with different bulk weights and drying temperatures. PLSR was applied in combination with spectroscopy and hyperspectral imagining. The results showed that, besides bulk weight and temperature, harvesting conditions and specific air mass flow have a significant influence on both drying time and color changes of hops during drying in identical conditions.

Gagula et al. [22] created mathematical models using two partial least squares regression methods: polynomial regression (PLSR-PR) and response surface method (PLSR-RSM) to describe changes in beer properties during storage based on three measured properties: color, bitterness, and haze values. The samples used were lager beers packed in glass bottles and polyethylene terephthalate (PET) bottles and samples of malt beer in glass bottles. It was concluded that PLSR-RSM models were more accurate when describing property changes for the lager and malt beer in glass bottles, while PLSR-PR was better for the lager beer in PET bottles. By comparing the samples, the models showed that beer packaging in PET bottles showed larger changes than lager beer in a glass bottle during the storage period. In contrast, both lager beer and malt beer showed great changes in different periods of storage.

Partial Least Squares (PLS) regression was used by Krebs et al. [23] to create a model to predict the palate fulness intensity in beers. It was reported that a chemometric analysis of 41 lager beers based on the evaluation of the analytical data of beer composition, palate fullness, and mouthfeel. Ethanol, original gravity, dynamic viscosity, nitrogen and β-glucan were analyzed. The macromolecular profile of the samples was analyzed and a sensory characterization was performed by certified panelists. It was concluded that palate fullness and mouthfeel are key factors that determine the quality of lager beer and consumers' acceptance, and that the prediction model can be used for a targeted design of palate fullness by weighting the influence factors. Calibration models can also be used to predict the organoleptic quality of the beer during the aging process. Hemp et al. [24] used an optical oxygen sensor to assess the level of residual oxygen in the headspace of bottled beers by monitoring the product over time before and after pasteurization. A sensory panel was also used to determine the effect of the residual oxygen on the sensory quality of the product. PLS-R was used to process the sensory data obtained by the 26 panelists. The results showed that the higher the oxygen level prior to pasteurization, the more negative the attributes associated with the sensory quality of the beer, especially those related to beer staling.

Another example of multivariate calibration was used to create a method for the retrospective determination of temperature based on the determination of carbonyl compounds determined by GC-MS. Čejka et al. [25] used three approaches: regression graph, multiple linear regression (MLR), and neural networks, to calculate the storage temperature of samples. 11 samples from the eight major Czech breweries were stored for 6 months at 0, 8, 20, and 30 °C. The MLR calculation used only 2-furfural as representative indicator of aging. The exponential dependency of 2-furfuralwith storage was converted to a linear dependency using a logarithmic transformation and a regression equation was created, with months and ln $_c$(furfural) as the input variables and storage temperature as the output variable. The uncertainty of the final predictions was 5 °C.

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