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NMR and Chemometric methods: A powerful combination for characterization of Balsamic and Traditional Balsamic Vinegar of Modena

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ABSTRACT

This work presents the capability of NMR spectroscopy combined with Chemometrics in predicting the ageing of Balsamic and Traditional Balsamic Vinegar of Modena. The need of an analytical method is an important requirement for both research oriented and commercial evaluation of these very valuable products. ^{1}H NMR spectroscopy, based on the advantage of rapid sample analysis without any manipulation or derivatization, is here proposed as a valid tool to describe Balsamic and Traditional Balsamic Vinegar of Modena. For this purpose, 72 reliable samples, were divided into three different groups according to their ageing process: young (<12 years), old (>12 and <25 years) and extra old (>25 years). Hierarchical Projection to Latent Structures Discriminant Analysis (PLS-DA) allowed us to characterize the ageing process. Variables showing the largest VIP (Variable Importance in the Projection) were extracted from PLS-DA model, thus shedding lights onto the role played by specific compounds in this complex ageing process. Two robust classification models, were built by PLS-DA and Naïve Bayes classifier and compared to prove the accuracy of the representation on both training and test sets. The predictions obtained for 41 "unknown" vinegar samples with these both methods gave more than 80% agreement among them.

1. Introduction

Balsamic and Traditional Balsamic Vinegar of Modena are very well known and largely appreciated Italian products. Traditional Balsamic Vinegar of Modena (TBVM) is an original and PDO product (Reg. CEE no. 813/2000 of April 17, 2000) whose consumption saw a large increase in the last years all over the world. It can be sold only after having experienced an ageing process of at least 12 years in a set of wooden casks of decreasing volume; cooked must and "traditional procedures" are the allowed ingredients and no additional products can be added. Detailed set rules (G.U. no. 124, May 30, 2000) regulates the correct production and the producers, joined in the "Consortium", are supervised for the complete process up to the product commercialization. This product, unique from every point of view, is subjected to a traditional production process named topping up procedure. Taste may be quite different from each producer. Different analytical studies investigated

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TBVM [1–5]: some were focused on authentication of natural vinegar [6,7] using isotopic ratio determination while others characterized the effect of acetic bacteria [8,9]. Balsamic Vinegar of Modena (BVM) is a different product: it is made from wine vinegar with the addition of caramel and a small quantity of aged wine vinegar, as described in the set rules (D.M., December 3, 1965) for the preparation procedure. BVM samples can experience a further ageing process, thus reaching higher quality parameters, but always clearly differentiated from TBVM quality. Both BVM and TBVM certifications are nowadays obtained by means of sensorial analysis and by very simple chemical–physical property determinations, like total acidity, density and dry residual.

Most of the analytical techniques nowadays intensively used (HPLC, GC, etc.) involve some kind of sample pretreatment, to concentrate or to derivate the desired class of compounds to be investigated. Moreover, the choice of methods and protocols often depends on the type of compound under investigation, making the overall characterization process very time consuming and not completely reproducible. In this respect, NMR method gives the full picture of soluble compounds, in a quantitative fashion with very high reproducibility in a single experiment. Analysis of food products should require the use of non-invasive techniques because sensorial and safety properties are related to the structural and compositional complexity and heterogeneity of the matrix, which must be preserved as much as possible in its original state. NMR has recently achieved general acceptance as a powerful tool for food quality determination: its characteristics of being non-destructive, non-selective (observes all protonated compounds in NMR suited concentration with no discrimination) and the improved sensitivity have proven its analytical power in a wide range of applications. Furthermore NMR displays the best combination of both very fast data acquisition and predictive capability: other techniques, like HPLC method, for instance, due to their high instrumental instability, could not be used for predictions. In the last years, several papers appeared in the literature concerning the quantification of specific metabolites in vinegars as possible indicators for the ageing process, traditional procedures and adulterations. In this context, amino acids [4,10–12], substances derived from sugar degradation [13] or carboxylic acids [14] were investigated with different analytical techniques.

Food, and in particular TBVM and BVM, displays an enormous range of compounds that are associated with certain quality levels. NMR can provide information on chemical composition, concentration of soluble metabolites and their structure.

Several examples reported in the literature demonstrate the power of NMR spectroscopy for quality assessment on multiple food materials, like beer, extravergin olive oil, juices, wine and vinegar [15–20]. In a previous study we proposed ^{1}H NMR spectroscopy applied in combination with statistical methods as a possible tool for the determination of ageing in TBVM [5]. In the case of BVM, commercial requirement [21] refers to 2 age ranges (below or above 3 years) rather than absolute age determination.

Nowadays, for both these products quality certifications are obtained by means of sensorial analysis (D.M., February 9, 1987 no. 63 and D.M., March 3, 1987 no. 191) and by very simple chemical–physical property determinations, like total acidity, density and dry residual. No objective analytical techniques were employed.

Here we present a characterization of both BVM and TVBM using high-resolution ^{1}H NMR spectroscopy combined with multivariate statistical data analysis mainly aiming at the characterization of the ageing process. Hierarchical Projection to Latent Structures Discriminant Analysis (PLS-DA) approach applied to complete ^{1}H NMR spectrum allowed us to correlate spectral data and sample ageing in an unbiased way recognizing the compounds playing the main role in the ageing process. A reduced PLS-DA model obtained by selecting the variables showing the largest VIP (Variable Importance in the Projection) clarified the role of different metabolites. The robust classification model built by PLS-DA with two classes approach was compared with three classes Naïve Bayes classifier to prove the accuracy of the representation on both training and test sets. These models were also compared on the basis of the predictions obtained for the “unknown” vinegar samples.

2. Experimental

2.1. *NMR samples*

113 samples of both BVM and TBVM from different producers have been used (Table 1). Among them, 72 were of known age, while 41 were unknown. Samples of known age were divided into three different groups: (a) less than 12 years (24 samples) named “young”, (b) between 12 and 25 years (17 samples) named “old”, (c) older than 25 years (31 samples) named “extra old”. The unknown samples were declared from the Consortium older than 12 years.

^{1}H NMR spectra have been recorded on a Bruker AV600 spectrometer operating at 14.09 T and equipped with a 5-mm inverse probe with z-gradient. Samples were prepared by dissolving 75 ± 5 mg of each sample into 540 μL of DMSO-D$_6$. pH was checked to be 7 ± 0.5 for all samples, as required for commercial distribution. Spectra were acquired in a completely automated procedure, consisting of “sample charger” and “automatic shimming procedure”, with the use of T2 filtered experiment (with standard CPMG sequence, with 64 loops of 2×300 μs delay, relaxation delay of 4 s). Spectra acquired with T2 filter allowed removal of broad peaks and large humps, characterized by a relatively short T2 relaxation, most likely due to slow exchanging signals, and used for statistical analysis. These problems were instead present in single pulse experiments, whose signal intensities were severely altered, thus resulting in useless data. NMR spectra were processed by applying an exponential line broadening of 1 Hz before Fourier transformation, phased, baseline corrected and referenced by using the solvent signal at 2.5 ppm as chemical shift standard (Bruker BioSpin Software applied: TOPSPIN2.0®, AMIX® 3.7). A selective TOCSY spectrum on sample 74 has been acquired with 5 mm Cryoprobe and z-gradient using a standard Bruker pulse sequence.

The NMR spectra were divided into small intervals of 0.04 ppm each (buckets) in the range of 10–0 ppm, with the exclusion of the solvent region from 2.58 to 2.46 ppm. Buckets