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Authentication of Trappist Beers by LC-MS Fingerprints and Multivariate Data Analysis

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The aim of this study was to asses the applicability of LC-MS profiling to authenticate a selected Trappist beer as part of a program on traceability funded by the European Commission. A total of 232 beers were fingerprinted and classified through multivariate data analysis. The selected beer was clearly distinguished from beers of different brands, while only 3 samples (3.5% of the test set) were wrongly classified when compared with other types of beer of the same Trappist brewery. The fingerprints were further analyzed to extract the most discriminating variables, which proved to be sufficient for classification, even using a simplified unsupervised model. This reduced fingerprint allowed us to study the influence of batch-to-batch variability on the classification model. Our results can easily be applied to different matrices and they confirmed the effectiveness of LC-MS profiling in combination with multivariate data analysis for the characterization of food products.

KEYWORDS: Food authentication; LC-MS profiling; O2PLS; Rochefort beer; Trappist beer; peak picking

INTRODUCTION

The control of food products is an essential part of consumer protection and represents an ongoing challenge for analytical laboratories. It is also in the interest of the producers to defend their products against low quality imitations or substitutes that may cause unfair competition with the original brand. In this context, the European Commission founded the project TRACE (http://www.trace.eu.org). One of the objectives of TRACE was to develop integrated traceability systems based on molecular fingerprints obtained through different spectroscopic techniques. Unlike conventional analytical approaches, fingerprinting methods do not rely on the detection of a limited number of known compounds but use a large number of chemical variables to generate a molecular signature used for classification purposes. Historically, trace elements and the natural abundance of stable isotopes were used to control the geographical origin of food products (*1*−*3*). More recently, the untargeted detection of as many metabolites as possible (i.e., metabolic profiles in the range from 100 to 1000 Da, usually referred to as small molecule profiles) has become a powerful tool for fingerprinting in food science (*4*). A variety of techniques can be used to collect metabolic profiles: for years, Nuclear Magnetic Resonance (NMR) was considered the leading technique because of its capacity to obtain the simultaneous quantification of a number of compounds and the simple requirements for sample preparation (*5*). In recent years, ultrahigh pressure liquid chromatography (UPLC) coupled with mass spectrometry (MS) has gained importance as a technique for collecting metabolic profiles, showing a higher sensitivity than NMR (*6*). The analysis of the large data sets generated by LC-MS requires data processing tools such as those based on multivariate data analysis. These techniques are robust to noise and missing data and enable one to deal with correlated variables (*7*, *8*).

One of the aims of TRACE was to assess the applicability of metabolic fingerprinting and multivariate data analysis to guarantee the authenticity of the brand name of different food products, among which is the Trappist beer, namely, Rochefort8.

Since medieval times, Trappist beers have been produced by Cistercians monks, who used to sell the surplus production to cover the subsistence fees of their abbeys. Even nowadays, Trappist beers are produced for noncommercial purposes and are considered hand crafted products brewed according to the traditional manufacturing process. However, the mass-production of commercial, standardized beers, known as Abbey beers, has emerged in the last decades. The difference between these Abbey beers and the Trappist beers became less evident to the consumer since commercial breweries had licensed the name of existing abbeys or used advertizing suggesting a monastic origin (*9*). To counter this competitive threat, the appellation of origin "Authentic Trappist Product" was created in 1997 (http://www.trappist.com). In the beginning, only six abbeys could use this label. These were Orval, Chimay, Rochefort, Westmalle, Westvleteren (in Belgium), and La Trappe (in The Netherlands). Achel (Belgium) was added in 1999. The Rochefort brewery is located inside the Abbey of Notre-Dame de Saint-Remy and produces three types of beer, called simply as 6, 8, and 10. These beers should be brewed following the same recipe, the only difference being the fermentation period which results in different alcohol

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contents for the three different beers. The brewery remains closed to the public, and the manufacturing process is kept secret and is strictly hand crafted. The only thing known about the process is that the water is drawn from a well located within the monastery and that unmalted sugar is added (http://www.abbaye-rochefort.be).

Only few studies have been carried out using fingerprinting techniques for the characterization of beers. NMR and principal component analysis (PCA) were used to uncover batch-to-batch variability between samples of a single commercial brand (*10*) or to group samples according to some major characteristics (e.g., the use of barley or wheat, ales or lager color, brewing sites, or deterioration) (*11*, *12*). The results obtained encouraged the use of PCA of NMR fingerprints for quality control issues. The use of MS for untargeted fingerprinting of beer is more limited, and only one study on this argument has been reported (*13*). In that study, 25 beer samples and 4 samples of an artificially sweetened Brazilian beer were analyzed by direct injection in a Quadrupole-Time of Flight (Q-TOF) analyzer. PCA showed clustering of samples in 3 major groups: pale colored, dark colored, and malt beers. The most intense and indicative signals were identified and arose from ionization of simple sugars, oligosaccharides, and iso-α-acids. Another attempt to use MS for the characterization of beer was made by the group of Obruca and co-workers who tried to assess the authenticity of Czech beer by analyzing a subset of phenolics (*14*). In that preliminary study, 7 different beers were investigated, and they found that some individual phenolics are qualitatively present/absent in Czech beer, suggesting the use of these compounds as markers of authenticity.

These studies indicated that metabolic profiles of beer should contain enough information for the classification of samples, at least according to some major features, such as the color or the geographical origin. Liquid chromatography and ion spray ionization allow the analysis of the nonvolatile fraction of small organic compounds (up to 1000 Da) of beer. This fraction is mainly constituted by metabolites produced during fermentation and by the slight portion of soluble additives possibly introduced during manufacturing. Volatile flavors represent another class of compounds useful for beer fingerprinting. Recently, headspace solid-phase microextraction coupled to gas chromatography mass spectrometry has been successfully applied to analyze beer volatiles for classification purposes (*15*).

In the present article, as part of the TRACE project, we tested the possibility of using multivariate data analysis of metabolic profiles collected by LC-MS to authenticate a specific type/brand of Trappist beer and to produce an efficient classifier capable of discriminating the selected beer from similar samples of other Trappist and Abbey brands.

MATERIALS AND METHODS

Samples, Sample Preparation, and Chemicals. A total of 232 commercial bottles of beer, representative of the Rochefort, Trappist, and Abbey productions, were sampled and purchased by the Walloon Agricultural Research Centre (CRA-W, Gembloux, Belgique) in two different sessions more than one year apart. Beer samples were labeled as follows: R8 (Rochefort8 samples), NR8 (samples among Rochefort6 and Rochefort10), and NR (Non-Rochefort samples). Overall, the NR group included 15 different types of non-Rochefort Trappist beers, as well as 37 types of Abbey beers. Both the batches contained bottles of each of the three classes of beers (i.e., R8, NR8, and NR); the two batches of bottles were independently collected, dispatched, and analyzed. The first batch contained different samples of Rochefort beers (i.e., R8 + NR8 samples) sampled simultaneously in the same period of the year; thus, they are likely to belong to the same production. The second batch enclosed Rochefort samples collected in different periods of the year (18 and 7 sampling sessions for the R8 and NR8, respectively), thus more representative of the batch-to-batch variability of this kind of hand crafted beers.

Table 1. Batch Composition in Terms of the Number of Bottles per Class

class	batch I	batch II
R8	16	30
NR8	11	17
NR	63	95
total	**90**	**142**

The composition of each batch is given in **Table 1**. Sulfadimetoxine and [5-leucine] enkefaline analytical standard grade were provided by Fluka Chemie (Buchs, Switzerland).

For each beer, an aliquot of approximately 3 mL was transferred in a PTFE vial and stored open overnight at a temperature of 4 °C for initial degassing. The day after, vials were sonicated for 1 min at maximum intensity (FALC Ultrasonic water bath sonicator) for a complete degassing, then 2 mL of each sample was filtered using an ISOLUTE 20 μm polyethylene filtration plate (Biotage, Uppsala, Sweden) and immediately analyzed.

Sample Analysis and Experimental Design. For each sample, 4 μL was injected on a Waters Acquity UPLC system (Waters Corporation, Milford, MA) using an Acquity HSS T3 column (100 mm × 2.1 mm, 1.8 μm) maintained at 60 °C. The chromatographic flow rate was 0.6 mL/min, finally split 1:10 before being injected into the spectrometer. The mobile phases were 0.1% formic acid in water (solvent A) and 0.1% formic acid in methanol (solvent B). The total run time was 9.5 min. The starting conditions were 98% of solvent A maintained for 1 min. Then, solvent B was gradually increased from 2% to 25% over the first 3.5 min, from 25% to 60% between 3.5 to 6.75 min, and from 60% to 95% between 6.75 and 7 min. Isocratic conditions with 95% of solvent B were maintained from 7.01 to 8 min, then the starting conditions were restored and maintained from 8.01 to 9.5 min.

The chromatographic system was coupled to a Micromass Q-TOF Premier mass spectrometer (Waters), equipped with an electrospray source and a lockspray interface. The instrument was tuned as follows: capillary, 3.5 Kv; sampling cone, 40; extraction cone, 3; ion guide, 3; source temperature, 100 °C; desolvatation temperature, 200 °C; cone gas flow, 35 L/h; desolvatation gas flow, 400 L/h; and collision gas flow, 0.3 mL/min. Mass spectra were acquired over the mass range 50−1000 m/z in centroid, W positive ionization mode. A solution of [5-Leucine] enkephaline 0.1 ng/μL infused at 50 μL/min was used as the reference mass for accurate mass measurements. The period over which the signals acquired by the detector were summed to give a complete spectrum (scan time) was increased from 0.15 to 0.3 s from the first to the second batch of analysis. At the same time, the number of scanned functions was decreased from two (with collision energies of 5 and 30 eV) to one (with a collision energy of 5 eV), with an interscan delay of 0.02 s for both the analytical sessions.

For each batch, three independent sample lists containing all the beer samples in a randomized order and quality controls at regular intervals were analyzed. Quality control consisted of two test samples: a solution 98/2 of water/acetonitrile containing 2 ng/μL of sulfadimetoxine and a mixture of 9 samples representative of the three classes of beer. These controls were used to validate the mass accuracy and retention times, as well as to best tune the peak picking parameters.

Peak Picking Method and Data Pretreatment. For each of the two batches of analysis, raw LC-MS data of the three injections were pooled together and analyzed through MarkerLynx XS v4.1 SCN639 (Waters). This software allowed for the so-called peak picking process, e.g., the creation of a list of variables extracted from the raw chromatograms. Variables must be present in at least two samples to be collected and are characterized by retention time, m/z, and intensity. For both the batches of analysis, only function 1 (e.g., the function with a collision energy of 5 eV) was considered. Peak picking parameters were settled as follows: initial/final retention time, 0.00/7.50 min; low/high mass, 50/1000 Da; mass tolerance, 0.02 Da; peak width at 5% height, 10 s; peak-to-peak baseline noise, 10; mass window, 0.04 Da; and retention time window, 0.2 min. Relative retention time and noise elimination level were not used, while smoothing and deisotopization processes were applied. To compensate for the different scan-time used in the two batches of analysis, the intensity threshold was settled at 90 and 180 counts, respectively, in the first and second analytical sessions.

For each batch of analysis, the intensities of the extracted variables were normalized dividing them by the sum of the intensities of the common

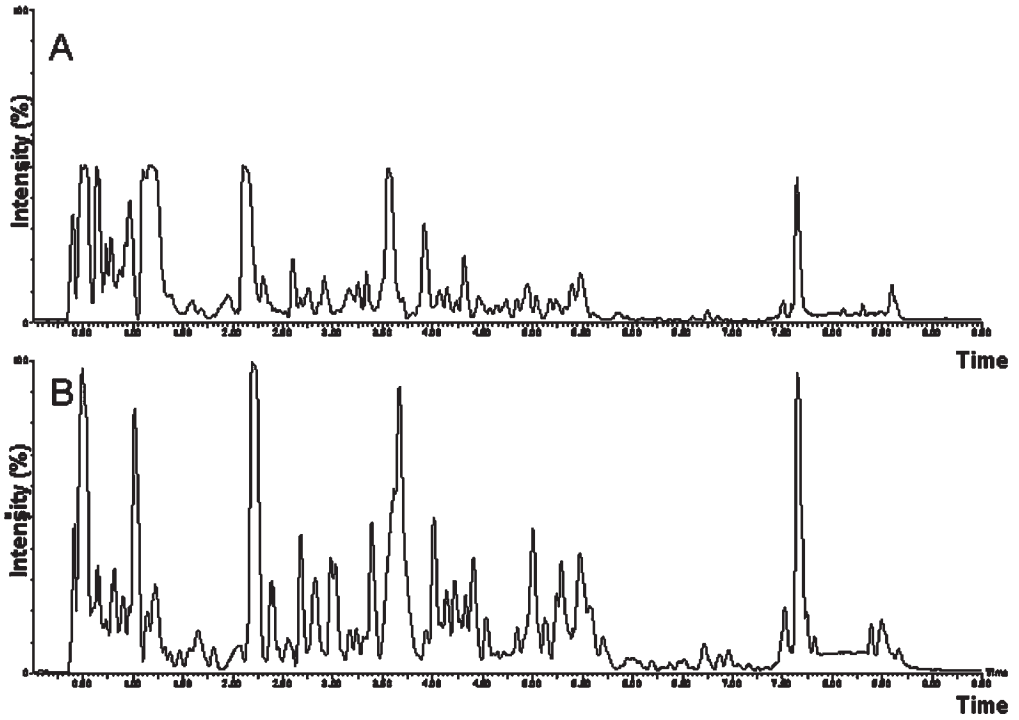

Figure 1. Typical chromatograms of the test mix sample injected at regular intervals in the first (panel **A**) and second (panel **B**) batches of analysis.

variables (e.g., variables detected in all the samples), the so-called "total useful MS signal" (*16*). Normalized variables were then averaged over the three replicates. Following this procedure, two separate data sets were generated and individually investigated through multivariate data analysis.

Multivariate Data Analysis. Extracted variables were treated through mean centering and Pareto scaling, and then analyzed by principal component analysis (PCA) and orthogonal projection to latent structures−discriminant analysis (O2PLS-DA) (*17*). PCA was mainly used to have an overview of the extracted data sets, while, for each batch of analysis, two chained O2PLS-DA models were used to generate the classifier. O2PLS-DA models were built up using representative training sets selected with Onion D-optimal design applied to the latent space spanned by the principal components of two independent PCA models for Rochefort and NR samples. O2PLS-DA models were built by variable selection performed on the analogous' PLS-DA models, considering only the variables having values of the VIP parameter (*18*) greater than the threshold that allows the maximum value of Q^2. Each regression model was validated by a permutation test to exclude overfitting. Onion D-Optimal design was performed by MODDE 8 (Umetrics AB, Umeå, Sweden) and multivariate data analysis by SIMCA P+12 (Umetrics). In order to introduce a probabilistic framework for O2PLS-DA classification, a Naïve Bayes classifier based on the representation of the sample space obtained using the predictive score vector was built by using WEKA 3.4.11 (University of Waikato, New Zealand). This strategy prevented the use of empirical decision rules for the classification of samples.

One of the advantages of O2PLS-DA models is that the information relevant for the discrimination is collected in a limited number of predictive components; when samples belong to two classes, simple models having only one predictive component can be obtained and interpreted by using the so-called S-plot (*19*). In these plots, the loading p representing the magnitude of each variable is plotted against the correlation loading p(corr), corresponding to its reliability. S-plots were used to interpret models in terms of relevant measured variables; only variables having p values considerably different from 0 and significant values of p(corr) were considered important for the models. The standard error of p was estimated by jack-knifing from all rounds of cross-validation, while a permutation test was applied in order to estimate the level of significance of p(corr). The class index was randomly permutated to obtain completely random classification models. Then, the distribution of the p(corr) values for these random models was characterized to determine the level of significance for different thresholds of p(corr). A value of 0.60 was estimated to have a significance level of higher than 0.999 for all of the O2PLS-DA models considered in this study.

RESULTS AND DISCUSSION

A total of 232 samples of beer were dispatched and thus analyzed during two different analytical sessions, more than one year apart. Injections at regular intervals of a test sample containing sulfadimetoxine (isotopic $[M + H]^+$ ion at 311.0814) revealed a mass accuracy of −1.66 and −2.03 ppm., and a shift in the retention time in the range of 0.06 and 0.02 min, respectively, for the first and second batches of analysis. **Figure 1** shows the typical chromatographic profiles of a test mix of 9 representative beers. Between the two analytical sessions, the scan time was increased from 0.15 to 0.3 s, while the number of scanned functions was decreased from two to one. These changes were introduced to increase the intensity of the signals, maintaining the width of the chromatographic peaks on an average of 20/25 scans at the base of the peak. As a result, the better signal-to-noise ratio of the second batch of analysis improved the reliability of the peak picking process and thus increased the number of extracted variables. A total of 1255 and 1534 variables were extracted from the first and the second batches, respectively.

The size of the two data sets confirmed that LC-MS was a valuable technique to represent the complexity of the samples under analysis. However, most of the variables brought nonpredictive or orthogonal information with respect to the class separation (structured noise) as shown by a preliminary PCA (**Figure 2**) and then by the O2PLS-DA models. The overall variability was further amplified by the heterogeneity of the NR group, which contained different types of beers characterized by specific sets of variables. For these reasons, an elevated number of principal components was necessary in the PCA models to explain approximately 50% of the variability. In detail, 4 and 6 principal components explained, respectively, 52.3% and 57.5% of the total variance of the first and second data sets.

Four of the samples under analysis belonged to a very particular, traditional Belgian beer (Geuze) produced by spontaneous fermentation (i.e., using wild strains of yeasts and bacteria).

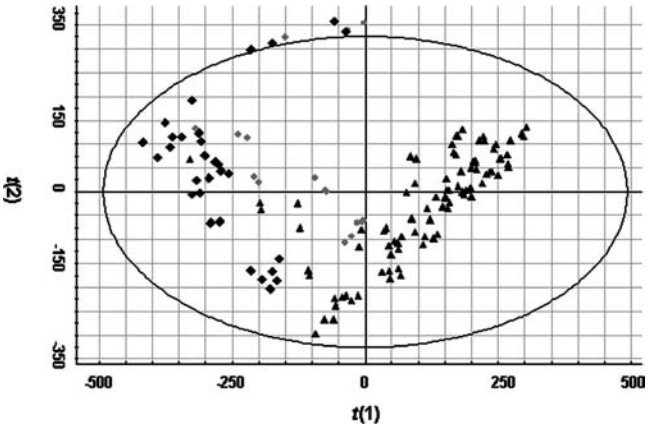

Figure 2. PCA score scatter plots considering 140 samples (2 strong outliers were removed before calculation) from the second batch of analysis: the triangles represent NR samples; the gray dots the NR8 samples; and the black diamonds the R8 samples. A similar representation (data not shown) was obtained for the first batch of analysis.

Table 2. Features of the O2PLS-DA Models That Made up the Classifiers of the Two Data Sets

	batch I		batch II	
	first model	second model	first model	second model
principal components	1	1	1	1
orthogonal components	4	2	3	2
R^2	0.98	0.92	0.97	0.80
Q^2	0.89	0.79	0.90	0.49

Metabolic profiles of Geuze showed a series of distinctive compounds; all of the four samples of this type of beer proved to be strong outliers on the basis of the Hotelling's T2 test (significance of 0.95), and they were excluded for further investigations. As shown in **Figure 2**, PCA was not sufficient to highlight a clear separation between classes, whereas there is evidence of clustering between NR and Rochefort beers when they are considered together (i.e., R8 and NR8 samples). These observations suggested that a classifier for R8 beer could be built up by combining two chained O2PLS-DA models: the first level model to separate NR beers and the second level model allowing the discrimination of R8 samples within the restricted group of Rochefort beers.

For each batch, representative training sets of Rochefort and NR samples were selected and used to generate the corresponding O2PLS-DA models. Training sets of the first and the second batches consisted of 55 (38 NR, 9 NR8, and 8 R8) and 86 (57 NR, 10 NR8, and 19 R8) samples, respectively. The complements of the whole batches were considered as test sets. For each data set, the features of the two O2PLS-DA models that were used to build up the classifier are reported in **Table 2**. No sample falls outside the applicability domain of the classifiers. In the first and the second batches of analysis, respectively, one and two samples of the test sets, were wrongly predicted (**Table 3**), while all the samples of the training sets were correctly classified.

The most relevant measured variables capable of distinguishing Rochefort from NR beers were highlighted by S-plots (**Figure 3**). No single relevant measured variable able to distinguish R8 from the other types of Rochefort beers (e.g., Rochefort6 and Rochefort10) was highlighted. Thus, the overall group of Rochefort beers can be well defined by a few variables which have the character of markers, while the different types of Rochefort beers can be discriminated only on the basis of a particular pattern of many variables (i.e., the whole fingerprint).

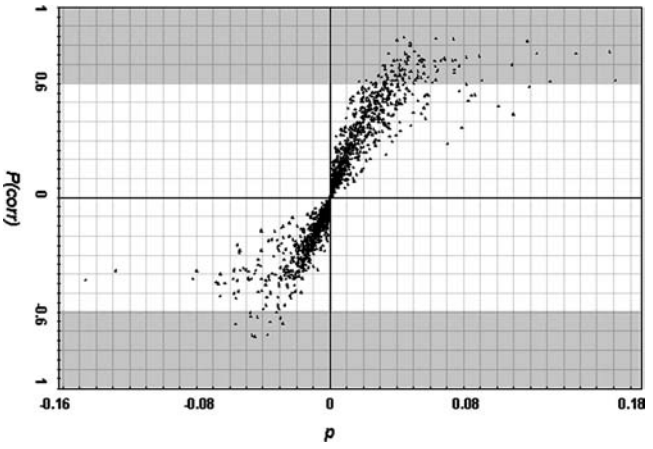

Figure 3. S-plot highlighting, in the gray boxes, the most relevant variables in the Rochefort/NR O2PLS-DA model of the second data set. A similar representation (data not shown) was obtained for the first batch of analysis.

Table 3. Confusion Matrix of the Beer Samples Belonging to the Test Sets of Each Batch of Analysis

	batch I			batch II		
class/class pred	NR	NR8	R8	NR	NR8	R8
NR	23	0	0	36	0	0
NR8	0	2	0	0	7	0
R8	0	1	7	0	2	9

This observation was already evident from the scatter plot reported in **Figure 2**, and it is likely to be the consequence of the fact that all of the three different types of Rochefort beers are brewed according to the same recipe.

A data integration approach based on O2PLS was used to distinguish the part of the information useful to discriminate Rochefort beers from that due to the batch-to-batch variability observed for these hand crafted beers. A direct integration of the two data sets was not possible because the composition of samples, the scan-time parameter and the sets of variables used for normalization, were different for the two analytical sessions. Thus, relevant variables were selected for each single data set on the basis of their influence in the O2PLS-DA models (absolute value of p(corr) greater than 0.60). A total of 100 and 85 relevant variables were extracted, respectively, from the first and second batches. The use of the same chromatographic settings and the level of accuracy and reproducibility of the LC-MS system used in this study allowed for the direct comparison of the m/z and retention time values of the two different analytical sessions. As result, a subset of the fingerprint composed of 27 common relevant variables (i.e., variables with the parallel presence of relevant signals in both data sets) was selected. For these variables, the median and the spread of distribution of the intensities between Rochefort and NR beers are represented in **Figure 4**. Quite large spreads of distribution were observed, especially in the second batch of analysis. For example, the variable with m/z 100.112 (one of the most relevant since it is present only in Rochefort beers) has a relative spread of distribution of about 40% and 110% in the first and second batches, respectively. This finding is probably due to the batch-to-batch variability of Rochefort beers.

These 27 variables were used to create a simplified classification model based on PCA. For each data set, the class of the Rochefort beers was represented through the training set previously used for the corresponding O2PLS-DA model. Thus, all of the samples were classified by analogy with these training sets

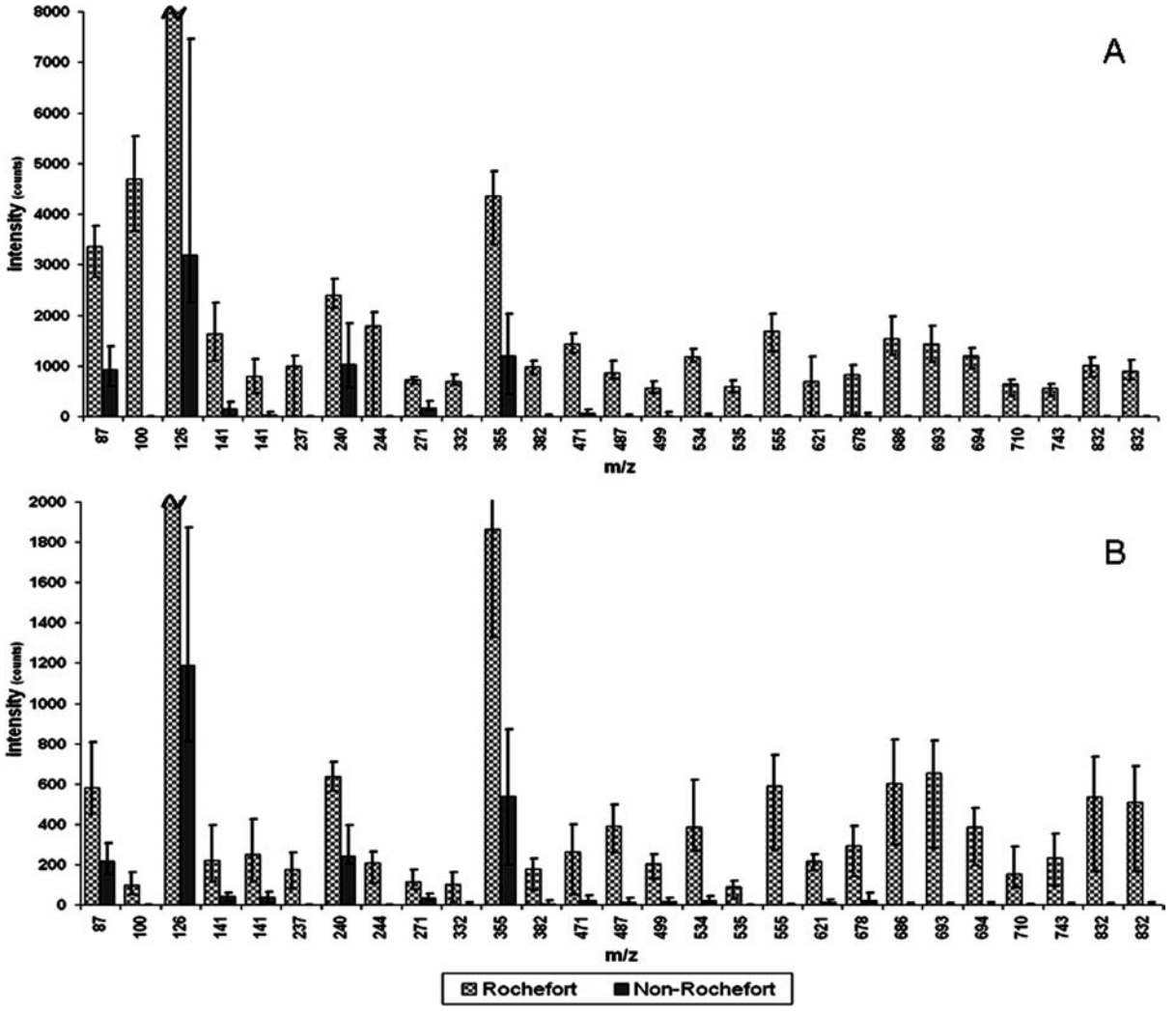

Figure 4. Bars represent the median of the intensities between the group of Rochefort and NR for each of the 27 relevant variables in common to the first (**A**) and the second (**B**) data sets. The spread of the distribution was represented by the 25th to the 75th percentile. The bar of the variable with $m/z = 125.99$ was cut off because it was out of scale.

on the basis of the augmented distance to model (DModX+) (*18*) with a significance of 0.95. Graphical overviews of the results were reported in **Figure 5**; all of the samples of the first data set were classified correctly, even if two samples of the same brand of Trappist beer were just above the threshold. In the second data set, the prediction capability of the model appeared reduced. Most of the NR samples were classified correctly, but for some of them, the distance to the model was just above the threshold. Furthermore, 4 samples of 2 different brands of NR Trappist beers were predicted as Rochefort beer. The second data set is likely to be affected by an increased variability within its training set. This hypothesis is supported by the fact that the second batch of Rochefort bottles have been collected in different periods of the year, thus are more representative of the variability typical of these hand crafted products. The classifier based on chained O2PLS-DA models proved to be more robust with respect to this effect. However, the fact that even the simplified PCA model allowed an adequate classification of the samples, confirmed the relevance of the 27 variables with respect to the problem under analysis and the overall value of the approach here presented.

This reduced subset of measured variables was also used to further analyze the influence of batch-to-batch variability on the representation of the two data sets. Only those types of beers analyzed in both the analytical sections were considered (i.e., bottles with the same commercial label in the two data sets). The same number of Rochefort samples (16 R8 and 11 NR8 samples) were selected by D-Optimal design and PCA in each data set. Thus, two reduced data sets composed of 27 common variables and 84 corresponding samples were obtained. The two data sets were compared by O2PLS: the R8 samples and the NR8 samples were permutated within their classes, and the consequent models were analyzed to extract the common information. Most of these models showed a single parallel component and a single orthogonal component for both batches of analysis. The score scatter plot represented in **Figure 6** shows that the common information is represented by a single parallel latent variable responsible for the discrimination of Rochefort beers. This variable accounts for approximately 60% of the total variance into the data sets, while 20% of the variance is unique within each single data set. Orthogonal components (the vertical axis in the plot in **Figure 6**) are mainly due to the variability internal to each single batch of analysis and are responsible for the observed different representations of the two data sets. The study of the loadings corresponding to the orthogonal and the parallel components (data not showed) confirmed that the most influential variables useful for discriminating the Rochefort beers were the same for the two batches. Our analysis demonstrated that the batch-to-batch variation acts orthogonally to the effects produced by the different types of beer.

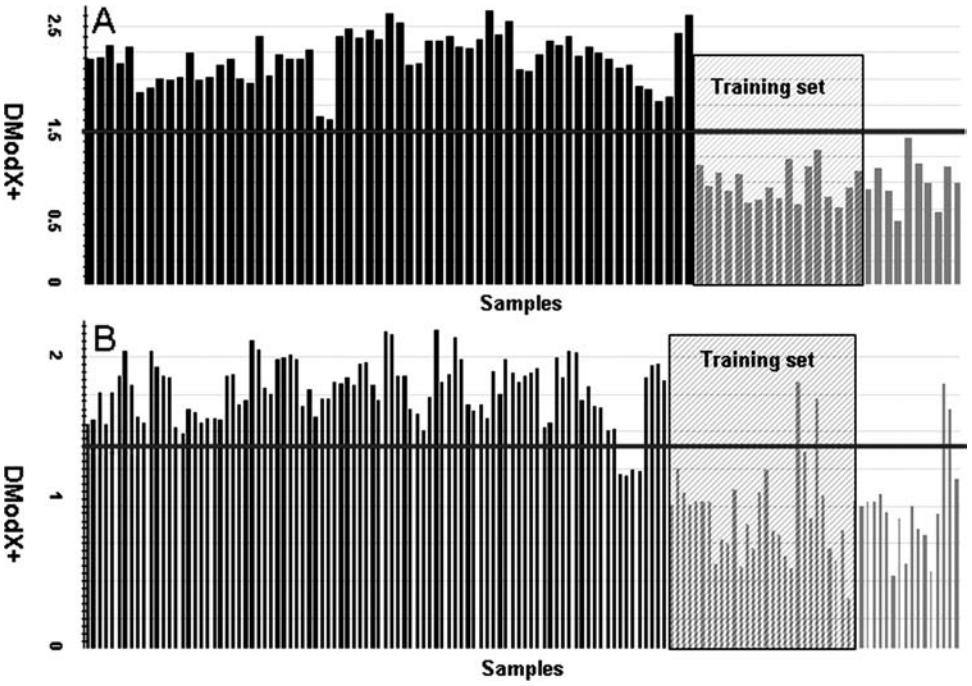

Figure 5. Classification of beer samples on the basis of the DModX+ parameter at 0.95 of significance. For the first batch (**A**), the training set of Rochefort beers (17 samples), was characterized by PCA (2 PCs, $R^2 = 0.59$). For the second batch (**B**), the training set of Rochefort beers (29 samples) was described by PCA (2 PCs, $R^2 = 0.71$). Thus, training sets were used to classify the whole sample sets. Black bars, NR samples; gray bars, Rochefort samples.

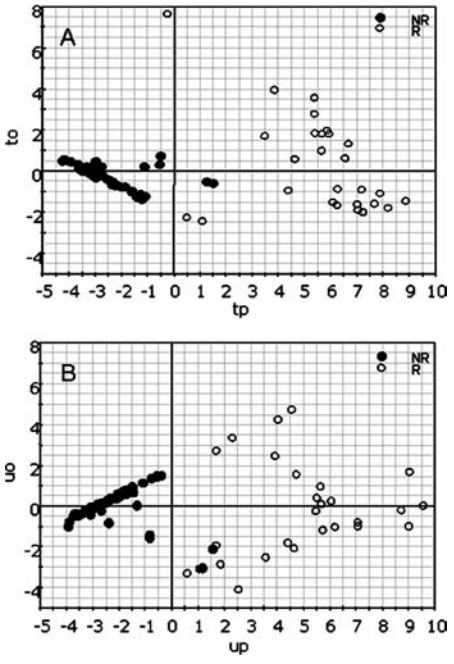

Figure 6. Score scatter plots representing the decomposition of the first reduced data set (**A**) and the second reduced data set (**B**) obtained by O2PLS. The horizontal axis (parallel component of the model) splits the samples into two groups: NR on the left-hand side and R on the right-hand side (common information). The vertical axis (orthogonal component of the model) produces different representations for the two blocks (unique information).

Our results confirmed that LC-MS fingerprints and multivariate data analysis are valuable techniques for the characterization and classification of beer and, in general, of complex biological matrices which have an elevated batch-to-batch variability. The elevated number of variables and the presence of structured noise required the use of supervised techniques such as O2PLS-DA, even if a simplified classification model based on 27 relevant variables proved to be capable of an adequate discrimination of Rochefort beers. Complete fingerprinting represents the only suitable approach to distinguish the different types of Rochefort beers, most probably because these beers are brewed according to similar processes using the same ingredients.

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LITERATURE CITED

(1) Brescia, M. A.; Di Martino, G.; Guillou, C.; Reniero, F.; Sacco, A.; Serra, F. Differentiation of the geographical origin of durum wheat semolina samples on the basis of isotopic composition. *Rapid Commun. Mass. Spectrom.* **2002**, *16*, 2286−2290.

(2) Serra, F.; Guillou, C.; Reniero, F.; Ballarin, L.; Cantagallo, I. M.; Wieser, M.; Iyer, S. S.; Héberger, K.; Vanhaecke, F. Determination of the geographical origin of green coffee by principal component analysis of carbon, nitrogen and boron stable isotope ratios. *Rapid Commun. Mass. Spectrom.* **2005**, *19*, 2111−2115.

(3) Rummel, S.; Hoelzl, S.; Horn, P.; Rossmann, A.; Schilich, C. The Combination of stable isotope abundance ratios of H, C, N and S with $^{87}Sr/^{86}St$ for geographical origin assignment of orange juices. *Food Chem.* **2010**, *118*, 890−900.

(4) Cevallos-Cevallos, J. M.; Reyes-De-Corcuera, J. I. Metabolomic analysis in food science: a review. *Trends Food Sci. Technol.* **2009**, *20*, 557−566.

(5) Alonso-Salces, R. M.; Heberger, K.; Holland, M. V.; Moreno-Rojas, J. M.; Mariani, C.; Bellan, G.; Reniero, F.; Guillou, C. Multivariate analysis of NMR fingerprint of the unsaponifiable fraction of virgin olive oils for authentication purposes. *Food Chem.* **2010**, *118*, 956−965.

(6) Guillarme, D.; Schappler, J.; Rudaz, S.; Veuthey, J. L. Coupling ultra-high pressure liquid chromatography with mass spectrometry. *Trends Anal. Chem.* **2010**, *29*, 15−27.

(7) Trygg, J.; Holmes, E.; Lundstedt, T. Chemometrics in Metabonomics. *J. Proteome Res.* **2007**, *6*, 469−479.
(8) Arvanitoyannis, I. S.; Vaitsi, O. B. A review on tomato authenticity: Quality control methods in conjunction with multivariate analysis (chemometrics). *Crit. Rev. Food Sci. Nutr.* **2007**, *47*, 675−699.
(9) Beverland, M. B.; Lindgreen, A.; Vink, M. W. Projecting authenticity through advertising. *J. Advertising* **2008**, *37*, 5−15.
(10) Almeida, C.; Duarte, F. I.; Barros, A.; Rodrigues, J.; Spraul, M.; Gil, M. A. Composition of beer by ^{1}H NMR spectroscopy: Effects of brewing site and date of production. *J. Agric. Food Chem.* **2006**, *54*, 700−706.
(11) Duarte, I.; Barros, A.; Belton, S. P.; Righelato, R.; Spraul, M.; Humpfer, E.; Gil, M. A. High-resolution nuclear magnetic resonance spectroscopy and multivariate analysis for the characterization of beer. *J. Agric. Food Chem.* **2002**, *50*, 2475−2481.
(12) Lachenmeier, D. W.; Frank, W.; Humpfer, E.; Schäfer, H.; Keller, S.; Mörtter, M.; Spraul, M. Quality control of beer using high-resolution nuclear magnetic resonance spectroscopy and multivariate analysis. *Food Chem.* **2007**, *101*, 825−832.
(13) Araujo, S. A.; da Rocha, L. L.; Tomazela, M. D.; Sawaya, FHCA.; Almeida, R. R.; Catharino, R. R.; Eberlin, N. M. Electrospray ionization mass spectrometry fingerprinting of beer. *Analyst* **2005**, *130*, 884−889.
(14) Obruča, S.; Márová, I.; Pařilová, K.; Müller, L.; Zdráhal, Z.; Mikulíková, R. A Contribution to analysis of "Czech beer" authenticity. *Czech J. Food Sci.* **2009**, *27*, S323−S326.
(15) Cajka, T.; Riddellova, K.; Tomaniova, M.; Hajslova, J. Recognition of beer brand based on multivariate analysis of volatile fingerprint. *J. Chromatogr., A* **2010**, *18*, 4195−4203.
(16) Warrack, B. M.; Hnatyshyn, S.; Ott, K.; Reily, M.; Sanders, M.; Zhang, H.; Drexler, D. M. Normalization strategies for metabonomic analysis of urine samples. *J. Chromatogr., B* **2009**, *877*, 547−552.
(17) Trygg, J.; Wold, S. O2-PLS, a two-block (X-Y) latent variable regression (LVR) method with an integral OSC filter. *J. Chemom.* **2003**, *17*, 53−64.
(18) Eriksson, L.; Johansson, E.; Kettaneh-Wold, N.; Trygg, J.; Wikström, C.; Wold, S. Appendix II. In *Multi and Megavariate Data Analysis, Basic Principles and Applications*, 2nd ed.; Umetrics AB: Umeå, Sweden, 2006; Vol. 1, pp 381−398.
(19) Wiklund, S.; Johansson, E.; Sjöström, L.; Mellerowicz, E. J.; Edlund, U.; Shockcor, P. J.; Gottfries, J.; Moritz, T.; Trygg, J. Visualization of GC/TOF-MS-based metabolomics data for identification of biochemically interesting compounds using OPLS class models. *Anal. Chem.* **2008**, *80*, 115.

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