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Ambient mass spectrometry employing a DART ion source for metabolomic fingerprinting/profiling: a powerful tool for beer origin recognition

Tomas Cajka · Katerina Riddellova · Monika Tomaniova · Jana Hajslova

Abstract A metabolomic fingerprinting/profiling generated by ambient mass spectrometry (MS) employing a direct analysis in real time (DART) ion source coupled to high-resolution time-of-flight mass spectrometry (TOFMS) was employed as a tool for beer origin recognition. In a first step, the DART–TOFMS instrumental conditions were optimized to obtain the broadest possible representation of ionizable compounds occurring in beer samples (direct measurement, no sample preparation). In the next step, metabolomic profiles (mass spectra) of a large set of different beer brands (Trappist and non-Trappist specialty beers) were acquired. In the final phase, the experimental data were analyzed using partial least squares discriminant analysis (PLS-DA), linear discriminant analysis (LDA), and artificial neural networks with multilayer perceptrons (ANN-MLP) with the aim of distinguishing (i) the beers labeled as Rochefort 8; (ii) a group consisting of Rochefort 6, 8, 10 beers; and (iii) Trappist beers. The best prediction ability was obtained for the model that distinguished the group of Rochefort 6, 8, 10 beers from the rest of beers. In this case, all chemometric tools employed provided ≥95% correct classification. The current study showed that DART–TOFMS metabolomic fingerprinting/profiling is a powerful analytical strategy enabling quality monitoring/authenticity assessment to be conducted in real time.

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1 Introduction

At the time of its emergence, metabolomics was mainly viewed as an advanced, specialized tool of analytical biochemistry enabling innovative research on plants and other organisms. Recently, this "omics" strategy centered around detection of the broadest possible range of small molecules (<1500 Da) in complex biological matrices using a single or small number of analyses has also emerged as a field of interest in food analysis (Wishart 2008; Cevallos-Cevallos et al. 2009). Metabolomics may be used either for "fingerprinting" of samples to perform comparative analyses aimed at detection of differences or for "profiling" in which individual, differential sample components (including secondary components originating during food processing) are identified for further analyses (Wishart 2008). In addition to food quality assessment or safety control, authentication is also a challenging application area. To date, most metabolomic studies on food have focused on commodities such as vegetable oils (Hutton et al. 1999; Ogrinc et al. 2003; Prestes et al. 2007; Vaclavik et al. 2009; Cajka et al. 2010a), fish oil (Aursand et al. 2007), fruit juices (Cuny et al. 2008, 2007; Le Gall et al. 2001), wines (Setkova et al. 2007), honey (Cajka et al. 2009), and beers (Almeida et al. 2006; Nord et al. 2004, Cajka et al. 2010b).

Beer is a very popular alcoholic beverage with high world production (approx. 1.6×10^{11} l per year) (da Silva et al. 2008). Among the hundreds of beer brands available on the market, specialty beers represent a special (and quite

expensive) product range, each with its own unique organoleptic characteristics. Up to now, only a few studies have focused on the authenticity of beer products. The methods employed in these studies were gas chromatography–isotope ratio mass spectrometry (GC–IRMS—$^{13}C/^{12}C$ ratio of CO_2), gas chromatography–flame-ionization detection (GC–FID—amino acids), high-performance liquid chromatography–fluorescence detection (HPLC–FLD—amino acids), HPLC–UV–Vis (phenolic substances), LC–MS (metabolite profiles), head-space solid-phase microextraction coupled to GC–FID or GC–MS (volatile compounds), nuclear magnetic resonance spectroscopy (D/H ratio of the methylene group of ethanol and $\delta^{18}O$ measurement of water; organic and amino acids), and Fourier transform infrared spectroscopy (da Silva et al. 2008; Calderone et al. 2007; Erbe and Bruckner 2000; Kabelova et al. 2008; Obruca et al. 2009; Rossmann 2001; Lachenmeier et al. 2005; Lachenmeier 2007; Cajka et al. 2010b; Araujo et al. 2005; Mattarucchi et al. 2010). Since highly complex data matrices are generated by these fingerprint and profiling techniques and require to be processed, powerful chemometric tools are needed to fully utilize this comprehensive information. In most cases, principal component analysis (PCA) is used for a preliminary inspection of the data structure. In the next step, various classification methods such as linear discriminant analysis (LDA), partial least squares discriminant analysis (PLS-DA), soft independent modeling of class analogy (SIMCA), or artificial neural networks (ANN) are the options most commonly used for data processing in an authenticity context (Berrueta et al. 2007).

In recent years, a large number of novel ambient desorption ionization techniques, such as desorption electrospray ionization (DESI), atmospheric-pressure solids analysis probe (ASAP), direct analysis in real time (DART), and many others have become available (Takats et al. 2004; McEwen et al. 2005; Cody et al. 2005). Their main advantages compared to conventional techniques involve the possibility of direct sample examination in the open atmosphere, minimal or no sample preparation requirements and remarkably high sample throughput. DART, which was investigated in this study, represents one of the atmospheric pressure chemical ionization (APCI)-related techniques employing a glow discharge for the ionization. Metastable helium atoms, originating in the plasma, react with ambient water, oxygen, or other atmospheric components to produce the reactive ionizing species (Cody et al. 2005). A DART ion source was shown to be efficient for soft ionization of a wide range of both polar and non-polar compounds. Until now, several papers have been published describing DART applications for rapid analysis of explosives, pharmaceuticals, flavor and fragrances, fatty acid methyl esters originating from bacterial

lipids, soft drinks, olive oil quality assessment, and pesticide residues (Venter et al. 2008; Weston 2010; Hajslova et al. 2010).

The current study has been conducted within the EU-funded TRACE project (www.trace.eu.org), which aimed at the development of cost-effective traceability methods and systems to provide consumers with added confidence in the authenticity of food in the European market. The approach adopted in this work for recognition of specialty beers was based on recording metabolomic fingerprints or profiles of ionizable compounds generated under the conditions of ambient MS employing a DART ion source. That this is an innovative approach is shown by the fact that a comprehensive review on metabolomics in food science published less than two years ago (Wishart 2008) did not list ambient mass spectrometry among the instrumental techniques discussed.

2 Materials and methods

2.1 Beer samples

The first batch (delivered in April 2008) contained 123 beer samples; the second (delivered in November 2008 and January 2009) consisted of 142 samples. Samples were stored in a refrigerator (4°C) between the delivery and analysis. Before analysis, the samples were conditioned to room temperature (2 h). An overview of beers examined within this study is shown in Table S1 (Supplementary material). Samples were collected over a relatively long production season (continuously over one year) to cover possible seasonal variability of the products.

2.2 DART–TOFMS analysis

For DART–TOFMS analyses, the system consisted of an ambient DART ion source (IonSense, Danvers, MA, USA), an AccuTOF LP high-resolution TOF mass spectrometer (JEOL (Europe) SAS, Croissy sur Seine, France), and an AutoDART HTC PAL autosampler (Leap Technologies, Carrboro, NC, USA). For a mass drift compensation needed for accurate mass measurement and subsequent elemental composition calculation, polyethylene glycol with an average molecular weight of 600 Da (Sigma–Aldrich, Steinheim, Germany) at a concentration of 200 µg/ml in methanol was introduced using a Dip-it sampler (IonSense, Saugus, Massachusetts, USA) at the end of each analysis.

MassCenter (JEOL) software (v. 1.3.0) was used for instrument control, data acquisition, and data processing. Mass spectral data were obtained by averaging of the mass spectra recorded during the exposure of the sample to the

DART gas beam; background ions were subtracted and a mass drift was corrected.

2.2.1 Sample preparation

After opening each bottle, approx. 20 ml of beer sample were degassed in an ultrasonic bath for 5 min at 5°C. A volume of 0.7 ml of beer sample was put into the sampling hole of a deep well micro-plate for direct analysis.

2.2.2 DART(+) ionization

Ion mode: positive; helium flow-rate: 2.9 l/min; needle voltage: 3000 V; discharge electrode: +150 V; grid electrode: +250 V; gas beam temperature: 250°C; sampling time: 5 s.

2.2.3 DART(−) ionization

Ion mode: negative; helium flow-rate: 2.9 l/min; needle voltage: 3000 V; discharge electrode: −150 V; grid electrode: −350 V; gas beam temperature: 250°C; sampling time: 5 s.

2.2.4 TOFMS detection

Mass range: m/z 50–600; peaks voltage: 600 V; detector voltage: −2400 V (positive ion mode); +2200 V (negative ion mode); acquisition rate: 5 spectra/s.

2.3 Chemometric analysis

Chemometric analysis included principal component analysis, formation of an artificial neural networks model, and partial least squares discriminant analysis employing the software package STATISTICA "Neural Networks" (v. 6, 2003, StatSoft, Inc., Tulsa, OK, USA; www.statsoft.com). For linear discriminant analysis, statistiXL (v. 1.8, 2008, statistiXL, Broadway—Nedlands, Australia, www.statistiXL.com) was used.

In the first stage of the data processing, the raw data (265 samples × 19 selected signals in positive ion mode, 265 samples × 18 selected signals in negative ion mode) in the form of absolute peak intensities were pre-processed using *range transformation*—for each sample the lowest value of given variable was assigned to "0" and the highest one to "1" with the remaining entries being numbers between these values; this procedure transformed all the data to a uniform range of variability. While all the data were subjected to PCA, in the case of the supervised pattern recognition techniques (PLS-DA, LDA, and ANN-MLP), the data were randomly split into a calibration (training) set (2/3 of samples) with the remaining samples (1/3) being used as a test set (Table S2 (Supplementary

material)). In the case of ANN-MLP the calibration data set was randomly divided by the software into two subsets: (i) *training subset* (2/3 of calibration data), which was used to accomplish the network model training; (ii) *selection subset* (1/3 of calibration data) for checking the network within the training process to avoid network overtraining. The *test subset* (i.e., remaining data) was used to assess the quality of the generated model.

Classification results were presented in terms of recognition and prediction abilities, and sensitivity and specificity. *Recognition ability* represents the percentage of samples in the training set, which were correctly classified. *Prediction ability* is the percentage of samples in the test set correctly classified by using the model developed during the training step. *Sensitivity* of a class model is the percentage of objects belonging to a given class, which is correctly identified by the model. *Specificity* is the percentage of objects foreign to the modeled class that are classified as foreign (Berrueta et al. 2007).

3 Results and discussion

3.1 DART–TOFMS mass spectra of beers

In the first part of our experiments, the relationship between the setting of various DART operating parameters and features of mass spectra generated under particular conditions was investigated. Helium beam temperature, flow rate, and desorption time were the major parameters affecting DART ion formation and transmission into MS.

The impact of gas beam temperature was monitored for temperatures of 150, 250, and 350°C (Fig. 1). At low temperature (150°C), desorption and ionization of mainly low molecular-weight compounds in both positive and negative ion modes occurred; at high temperature (350°C), the observed ion intensity of some of these compounds decreased markedly. At the same time, a population of "new" lower m/z ions (probably degradation and/or reaction products originating from sample components) appeared in the mass spectrum. A temperature of 250°C was therefore seen as optimal, allowing the monitoring of ions across a wide m/z range. In general, the direct measurement of beer samples provided fingerprints with ions of m/z values <200 Da. To allow monitoring of higher molecular weight compounds, their pre-concentration using direct immersion solid-phase microextraction (DI-SPME) can be employed. Under these conditions, the DART heated gas stream is used for desorption and direct introduction of the ionizable sample components thermally released from the SPME fiber into a mass spectrometer. This approach permits collection of a fingerprint of higher molecular weight (less volatile) components up to approx.
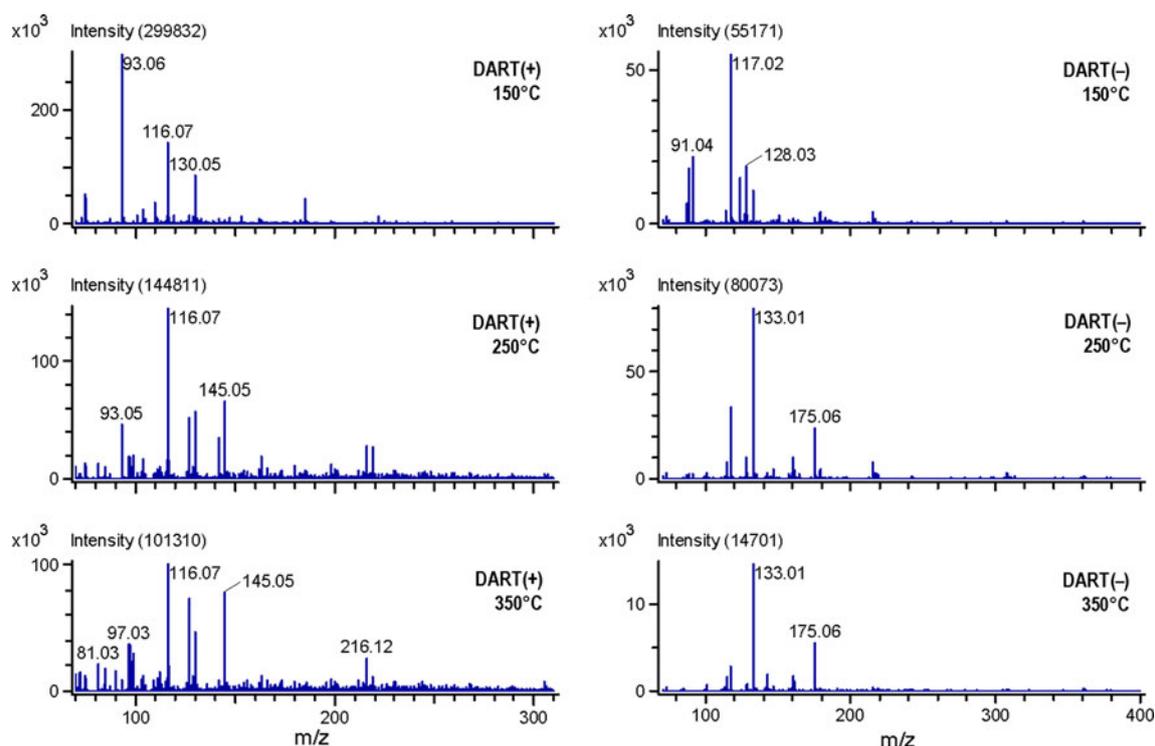
Fig. 1 The impact of DART gas beam temperature on the ion profiles of beer sample (Rochefort 8, desorption time 5 s)

m/z 500 Da containing mainly polar compounds (e.g., cohumulone, xanthohumol, humulone/adhumulone, lupulone/adlupulone) originating from hops (Cajka et al. 2010b).

Helium flow rates were also observed to have an influence on the DART–TOFMS fingerprint patterns. The number of metabolites detected increased with increased flow rate but gas flow rates >3 l/min led to dispersion of the sample (liquid) spread on the sample stick and some solvent droplets were split off towards the inlet orifice of the mass spectrometer, causing contamination.

Another important factor optimized was (thermo) desorption time; values tested include 1, 5, and 20 s; it was observed that 5 s provided sufficient intensity of ions in mass profiles in beer samples for subsequent analysis. Longer desorption time led only to slightly increased detection of higher *m/z* ions present in beer samples.

In principle, beer DART–TOFMS examination can be realized either as non-targeted fingerprinting or targeted profiling analysis. Tentative identification of compounds whose presence is anticipated or the estimation of the most probable elemental composition of ions of unknowns (the best hit in terms of mass accuracy suggested by the software) was enabled by exact mass measurement.

In positive ion mode, amino acids and various derivatives of saccharides were detected; analysis in negative ion mode mainly provided information on organic acids including typical bitter hop components. It should be noted that free amino acids transferred to wort from barley malt are metabolized by yeast during fermentation and are involved in biosynthetic pathways that lead to formation of important flavor components such as higher alcohols, esters and sulfur compounds. The profile of amino acids and organic acids in beer is a reflection of wort composition and yeast metabolism (Nord et al. 2004). Monitoring of batch-to-batch fluctuation of characteristic component profiles may help to conveniently assess the constancy of the beer production process (Wishart 2008). Examples of various beer components detected in beer samples by DART–TOFMS are shown in Table 1. Both the original components (metabolites) transferred from raw materials and products originating during malting and brewing (in addition to the above mentioned products of fermentation, such as sugar degradation products like maltol, derivatives of furan) are represented here. In this way, a broader portfolio of potentially differential components is obtained compared to a beer authentication approach based on ^{1}H NMR profiling of only free amino acids (Ala, His, Phe, Tyr, Val) and organic acids (acetic, citric, lactic, malic, pyruvic, succinic) reported previously (Nord et al. 2004).

3.2 Chemometric analysis for beer origin recognition

3.2.1 Selection of the markers

For chemometric analysis, several potential markers (ions) of the beer samples acquired in positive and negative ion

Table 1 Analytical data of selected beer ions (markers) determined by DART–TOFMS

m/z	Estimated formula	Tentative identification	RSD (%)[d]
(A) DART positive ion mode			
81.03	C_5H_5O	Furan-2-ylmethanol[a]	13.1
85.03	$C_4H_5O_2$	2H-furan-5-one[b]	22.7
93.05	$C_3H_9O_3$	Propane-1,2,3-triol[b]	20.7
97.03	$C_5H_5O_2$	Furan-2-carbaldehyde[b]	18.4
99.04	$C_5H_7O_2$, $C_4H_7N_2O$		21.7
116.07	$C_5H_{10}NO_2$	Proline[b]	–
127.04	$C_6H_7O_3$	Maltol[b]	11.8
130.05	$C_5H_8NO_3$	5-Oxoproline[b]	8.9
145.05	$C_6H_9O_4$		9.8
163.06	$C_6H_{11}O_5$		10.8
180.09	$C_{10}H_{14}NO_2$	Maltoxazine[b]	7.4
198.10	$C_{10}H_{16}NO_3$	1-Ethenylpyrrolidin-2-one ethenyl acetate[b]	7.3
216.12	$C_{10}H_{18}NO_4$		10.5
219.09	$C_{10}H_{11}N_4O_2$, $C_9H_{14}O_6$		4.6
268.10	$C_{10}H_{14}N_5O_4$	Adenosine[b]	10.2
278.12	$C_{11}H_{20}NO_7$		10.2
289.10	$C_{13}H_{13}N_4O_4$, $C_{12}H_{16}O_8$		5.3
298.13	$C_{15}H_{16}N_5O_2$, $C_{14}H_{19}NO_6$		14.7
305.13	$C_{13}H_{17}N_6O_3$, $C_{12}H_{21}N_2O_7$, $C_{17}H_{21}O_5$		10.7

m/z	Estimated formula	Tentative identification	RSD (%)[d]
(B) DART negative ion mode			
73.03	$C_3H_5O_2$	Propionic acid[c]	16.3
89.03	$C_3H_5O_3$	Lactic acid[c]	32.1
101.02	$C_4H_5O_3$	Ketobutyric acid[c]	18.0
115.00	$C_4H_3O_4$	Maleic acid[c]	4.8
117.02	$C_4H_5O_4$	Succinic acid[c]	12.0
128.03	$C_5H_6NO_3$	5-Oxoproline[c]	16.9
129.02	$C_5H_5O_4$	Methylmaleic acid[c]	11.3
133.01	$C_4H_5O_5$	Malic acid[c]	–
143.03	$C_6H_7O_4$		21.4
161.04	$C_6H_9O_5$	Anhydrohexose[c]	11.6
175.06	$C_7H_{11}O_5$	1,2-Diacetylglycerol[c]	3.6
179.06	$C_6H_{11}O_6$	Glucose[c]	13.7
197.03	$C_{12}H_5O_3$		12.7
215.04	$C_7H_7N_2O_6$		8.0
217.04	$C_7H_9N_2O_6$		9.5
269.09	$C_{10}H_{13}N_4O_5$		11.6
308.10	$C_{12}H_{14}N_5O_5$		7.6
377.13	$C_{21}H_{29}O_6$	(Iso)humulinone[c]	15.2

[a] $[M–H_2O+H]^+$

[b] $[M+H]^+$

[c] $[M–H]^-$

[d] Relative standard deviation (RSD) of peak area, $n = 10$. The intensities of detected ions normalized to the intensity of the most abundant ion (in positive ion mode m/z 116.07, in negative ion mode m/z 133.01)

modes were selected after careful inspection of DART–TOFMS profiles. Depending on the ion detection threshold, even hundreds of ions were automatically assigned by the software in each sample, typically more in positive ion mode. Under these conditions, selection of ions for chemometric analysis can become a bottleneck; therefore, a specially-developed Excel macro was used to export the target ions (in order of tens) from the raw data. The list of markers (ions, m/z) used for area measurements (data acquired in continuum mode), their tentative identification, and intra-day measurement repeatability (expressed as relative standard deviation, RSD, %; $n = 10$) is shown in Table 1.

3.2.2 Merging of variables obtained by DART(+) and DART(−)

In DART positive ion mode, 19 variables (markers) were used for a preliminary classification model employing LDA. Using leave-one-out cross-validation (LOOCV), a prediction ability of 89.8% was achieved while, using the negative ion mode with 18 variables (markers), the same procedure gave an improved prediction ability of 92.8%. The merged data containing in total 37 variables (markers) produced prediction ability of up to 95.8% for the model "Rochefort 6, 8, 10 vs. the rest". On this account, in the subsequent chemometric analysis, only results obtained using the merged data are discussed (Table 2).

3.2.3 Principal component analysis (PCA)

PCA applied for data assessment represents one of the most frequently used chemometric tools. The ability to easily project particular data from a higher to a lower dimensional space and then reconstruct them without any preliminary assumptions about their distribution represents one of its attractive features (Berrueta et al. 2007). In the preliminary analysis of the beer data, PCA was performed to investigate any potentially existing clustering based on the type of beer samples (Fig. 2). The first principal component (PC 1) accounted for 23.6% of the total variance while the second PC explained 16.9%. The nine most important PCs (with eigenvalues >1) explained 80% of total variance. In the case of beers originating in the Czech Republic (Primator 21, Primator 24) PCA showed a discrete cluster separate from the other beers produced in Belgium and The Netherlands. Figure 3 shows a comparison of DART–TOFMS profiles of Rochefort 8 (Trappist) and Primator 24 (non-Trappist) beer samples.
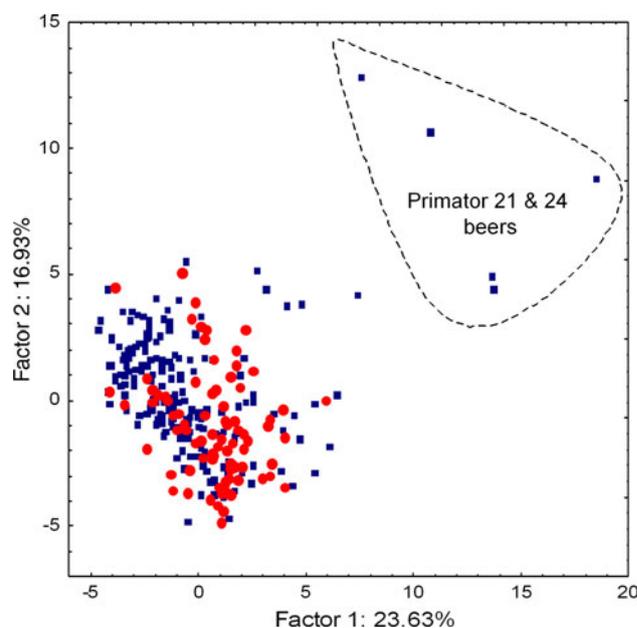

Fig. 2 First and second PCA scores for a group of Rochefort 6, 8, 10 beers (*dark circle*) vs. the rest (*dark square*). Graphs constructed using all samples ($n = 265$)

3.2.4 Partial least squares discriminant analysis (PLS-DA)

In principle, PLS-DA finds the variables and directions, in the multivariate space which discriminate between the classes of interest in the calibration set (Berrueta et al. 2007). For the models "Rochefort 8 vs. the rest" and "Trappist vs. non-Trappist beers", quite high recognition abilities were obtained (94.6 and 92.2% respectively) but the prediction abilities of these models were lower (84.8 and 82.8% respectively), caused by overlap of the given classes. The best recognition and prediction abilities (96.4 and 94.9% respectively) were obtained for the model

Table 2 Overall summary of PLS-DA, LDA, and ANN-MLP models

| Model/technique	Variables used	Recognition ability (%)	Prediction ability (%)	Sensitivity (%)	Specificity (%)
(A) Rochefort 8 vs. the rest					
PLS-DA	2 loadings (originated from 37 variables)	94.6	84.8	61.9	89.7
LDA	37 variables	97.0	82.8	88.9	79.4
ANN-MLP (37:37–22–1:1)	37 variables	96.4	87.9	94.4	84.1
(B) Rochefort 6, 8, 10 vs. the rest					
PLS-DA	2 loadings (originated from 37 variables)	96.4	94.9	89.2	98.4
LDA	37 variables	99.4	98.0	98.4	97.3
ANN-MLP (37:37–22–1:1)	37 variables	100	98.0	96.8	100
(C) Trappist beers vs. non-Trappist beers					
PLS-DA	2 loadings (originated from 37 variables)	92.2	82.8	76.2	94.4
LDA	37 variables	92.2	82.8	73.8	89.5
ANN-MLP (37:37–16–1:1)	37 variables	95.2	85.9	78.6	91.2
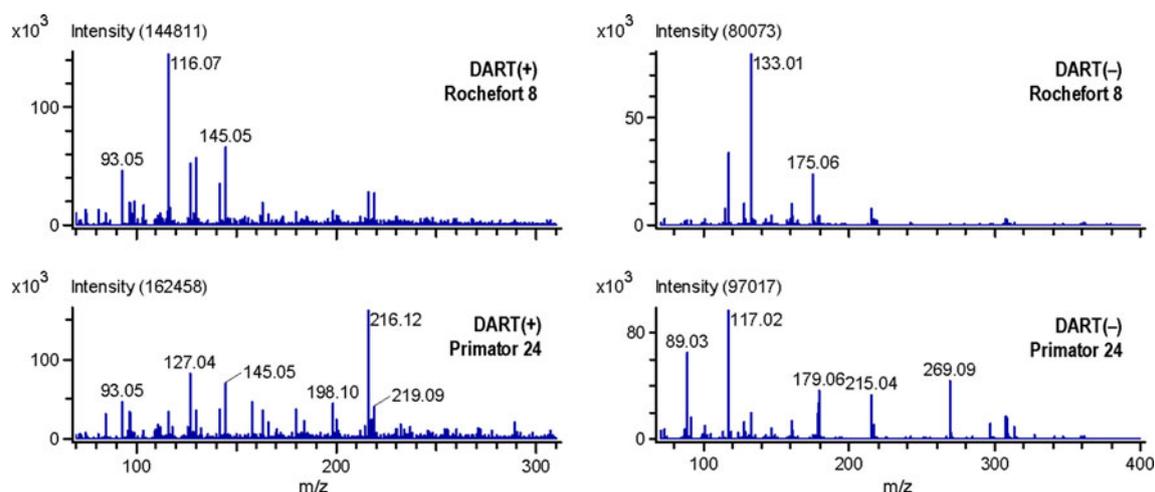
Fig. 3 Comparison of DART profiles of Rochefort 8 (Trappist) and Primator 24 (non-Trappist) beer samples

"Rochefort 6, 8, 10 vs. the rest" (Table 2). Under these conditions, the classes were well separated (Fig. 4) and good classification was obtained.

3.2.5 Linear discriminant analysis (LDA)

LDA is another frequently used supervised pattern recognition method. In principle, LDA determines linear discriminant functions, which maximize the ratio of between-class variance and minimize the ratio of within-class variance.
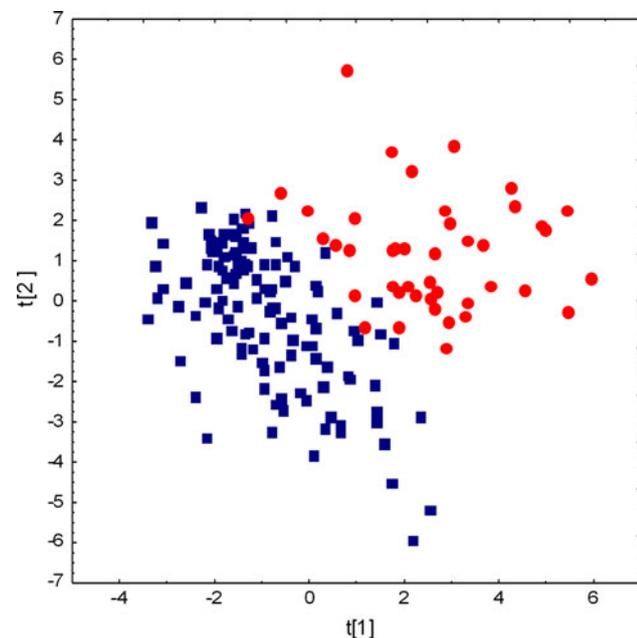
Fig. 4 First and second PLS scores for Rochefort 6, 8, 10 (*dark circle*) vs. the rest (*dark square*). Graphs constructed using calibration data set ($n = 166$)

Contrary to PCA that selects a direction retaining a maximal structure of the data in a lower number of dimensions, LDA selects a direction that achieves maximum separation between the given classes (Berrueta et al. 2007). As Table 2 shows, the recognition and prediction abilities of model "Rochefort 8 vs. the rest" were 97.0 and 82.8% respectively. Some misclassified samples were Rochefort 10 beers, probably because of their relative similarity to Rochefort 8 beers. Indeed, both recognition and prediction abilities improved significantly when the model "Rochefort 6, 8, 10 vs. the rest" was tested (99.4 and 98.0% respectively). Lower recognition and prediction abilities compared to the previous model were achieved for the model "Trappist vs. non-Trappist beers", 92.2 and 82.8% respectively.

3.2.6 Artificial neural networks with multilayer perceptrons (ANN-MLP)

As an alternative, ANN data processing approach was tested. This chemometric tool is suitable mainly in cases for which the relationship between predictor variables (independents, inputs) and predicted variables (dependents, outputs) is very complex (Cajka et al. 2009, 2010a). An ANN-MLP using back propagation was employed to predict the origin of beer samples based on the pattern of their markers. *Intelligent Problem Solver* was employed for the analysis of data set. The search for an appropriate ANN model was restricted only to MLP networks; in total, 50 alternatives were tested, the best ten of which were retained. The network architecture created for the beer data matrix included: (i) an input layer (marker compounds), (ii) one hidden layer, and (iii) an output layer providing the origin classification. The ANN was trained using selected parameters from the data sets followed by the validation

using an independent data set to estimate the beer origin. The training started with different initial random weights and was optimized during the process. Typically, the learning process continues by epoch-by-epoch (through single complete training processes) until the synaptic weights and bias level of the network stabilize. The network was trained by a back propagation algorithm (100 epochs) followed by a conjugate gradient algorithm (20 epochs). Finally, a network with the smallest error (misclassification of type of beer in particular case) was selected.

For the particular models developed, MLP networks consisted of 37 neurons in the input layer (all models), 22, 22, and 16 neurons in the hidden layer for model (a) "Rochefort 8 vs. the rest", (b) "Rochefort 6, 8, 10, vs. the rest", and (c) "Trappist vs. non-Trappist beers" respectively, and one neuron in the output layer (classification). Similarly to PLS-DA and LDA results, the best recognition and prediction abilities (100% and 98.0% respectively) were obtained for the model "Rochefort 6, 8, 10 vs. the rest". Lower recognition and prediction abilities (96.4% and 87.9% respectively) were obtained for the model "Rochefort 8 vs. the rest", and, again, the most of misclassified samples belonged to the other Rochefort beers within the group as was the case for LDA. Lower recognition and prediction abilities were also achieved for the model "Trappist vs. non-Trappist beers", 95.2 and 85.9% respectively (Table 2).

3.3 Comparison of metabolomic fingerprinting/profiling approaches: SPME–GC–TOFMS vs. DART–TOFMS

In our recent study (Cajka et al. 2010b), an automated head-space SPME-based sampling procedure coupled to gas chromatography–time-of-flight mass spectrometry (GC–TOFMS) was developed and employed for obtaining volatile fingerprints (GC profiles) of the identical beer sample set (265 specialty beers). Using the same chemometric tools (i.e., PLS-DA, LDA, and ANN-MLP), the best prediction ability (100%) was obtained for the model that distinguished a group of Rochefort 6, 8, 10 beers from the rest of beers. Thus, comparable results were obtained for this novel approach realized by DART–TOFMS (prediction ability 98%). However, considering the sample throughput rate, it should be emphasized that SPME–GC–TOFMS required some, although quite simple, sample preparation steps with subsequent sample incubation/extraction (10 min) and separation/detection of beer volatiles (20 min). On this basis, the time requirement for sample analysis was in fact one order of magnitude higher as compared to the direct measurement of beer samples using DART–TOFMS (<1 min is required for a single analysis).

4 Concluding remarks

The novel DART–TOFMS technique enabled differentiation of specialty beers by recording metabolomic fingerprints or profiles of ionizable compounds generated under the conditions of ambient MS. Thanks to the need for minimal sample preparation (only degassing) and automated introduction of sample spread on a Dip-it sampler in front of a DART ion source, high throughput of samples was achievable.

In general, ANN-MLP provided slightly better prediction abilities for all models tested as compared to LDA and PLS-DA. The best prediction ability (≥95% correct classification) was obtained for the model that distinguished a group of Rochefort 6, 8, 10 beers from the rest of the beers.

Supposing a comprehensive database of a wide range of (normalized) fingerprints/profiles is established and continuously up-dated over time, critical assessment of (batch-to-batch) beer quality as well as traceability of raw materials used for production and their processing conditions would be feasible using this approach.

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Electronic supplementary material

Table S1 (Supplementary material)
Overview of specialty beers used within this study.

Classification	Trade mark	Country of origin	*n*
Trappist	Achel Blond	Belgium	4
	Achel Brune	Belgium	4
	Chimay Triple Blanche	Belgium	4
	Chimay Bleu	Belgium	4
	Chimay Rouge	Belgium	4
	La Trappe Blanche	The Netherlands	4
	La Trappe Blonde	The Netherlands	3
	La Trappe Double	The Netherlands	4
	La Trappe Triple	The Netherlands	4
	La Trappe Quadruple	The Netherlands	4
	Orval	Belgium	4
	Rochefort 6	Belgium	6
	Rochefort 8	Belgium	48
	Rochefort 10	Belgium	26
	Westmalle Double	Belgium	4
	Westmalle Triple	Belgium	4
	Westvleteren 8	Belgium	3
	Westvleteren 12	Belgium	3
Non-Trappist	Affligem Triple 9.5%	Belgium	4
	Binchoise Brune	Belgium	3
	Bon Secours Ambrée	Belgium	4
	Brigand	Belgium	4
	Brugges Tripel	Belgium	4
	Charles Quint	Belgium	4
	Delirium Tremens	Belgium	4
	De Verboden Vrucht	Belgium	4
	Duvel	Belgium	4
	Grimbergen Dorée	Belgium	4
	Grimbergen Triple	Belgium	4
	Geuze Girardin 1882	Belgium	4
	Gouden Carolus Tripel	Belgium	4
	Hapkin	Belgium	4
	Hercule	Belgium	4
	Hoegarden Grand Cru	Belgium	3
	Hotteuse Grand Cru	Belgium	3
	Judas	Belgium	4
	Jupiler	Belgium	4
	Het Kapittel Watou Prior	Belgium	4
	Kwak	Belgium	3
	Leffe Blonde	Belgium	4
	Leffe 9°	Belgium	3
	Leffe Brune	Belgium	4
	Maredsous 8°	Belgium	4
	Moinette Blonde	Belgium	4
	Moinette Brune	Belgium	3
	Primator 21	Czech Republic	3
	Primator 24	Czech Republic	2
	Quintine Ambrée	Belgium	4
	St Bernardus Prior 8	Belgium	4
	St Feuillien Triple	Belgium	4
	Triple Karméliet	Belgium	4
	Val-Dieu Triple	Belgium	4
	Vondel	Belgium	1

Table S2 (Supplementary material)
Number of samples of calibration and test sets used for chemometric analysis.

Model	Calibration set	Test set
	(number of samples)	(number of samples)
(A) Rochefort 8 *vs.* the rest		
Trappist Rochefort 8	27	21
Rest (non-Trappist and Trappist without Rochefort 8)	139	78
(B) Rochefort 6, 8, 10 *vs.* the rest		
Trappist Rochefort 6, 8, 10	43	37
Rest (non-Trappist and Trappist without Rochefort 6, 8, 10)	123	62
(C) Trappist beers *vs*. non-Trappist beers		
Trappist	74	63
Non-Trappist	92	36