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Recognition of beer brand based on multivariate analysis of volatile fingerprint☆

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ABSTRACT

Automated head-space solid-phase microextraction (HS-SPME)-based sampling procedure, coupled to gas chromatography–time-of-flight mass spectrometry (GC–TOFMS), was developed and employed for obtaining of fingerprints (GC profiles) of beer volatiles. In total, 265 speciality beer samples were collected over a 1-year period with the aim to distinguish, based on analytical (profiling) data, (i) the beers labelled as Rochefort 8; (ii) a group consisting of Rochefort 6, 8, 10 beers; and (iii) Trappist beers. For the chemometric evaluation of the data, partial least squares discriminant analysis (PLS-DA), linear discriminant analysis (LDA), and artificial neural networks with multilayer perceptrons (ANN-MLP) were tested. The best prediction ability was obtained for the model that distinguished a group of Rochefort 6, 8, 10 beers from the rest of beers. In this case, all chemometric tools employed provided 100% correct classification. Slightly worse prediction abilities were achieved for the models "Trappist *vs.* non-Trappist beers" with the values of 93.9% (PLS-DA), 91.9% (LDA) and 97.0% (ANN-MLP) and "Rochefort 8 *vs.* the rest" with the values of 87.9% (PLS-DA) and 84.8% (LDA) and 93.9% (ANN-MLP). In addition to chromatographic profiling, also the potential of direct coupling of SPME (extraction/pre-concentration device) with high-resolution TOFMS employing a direct analysis in real time (DART) ion source has been demonstrated as a challenging profiling approach.

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1. Introduction

Beer represents a widely popular alcoholic beverage with a high world production rate (approx. 1.6×10^{11} l per year) [1]. Among the hundreds of beer brands available on the market, speciality beers represent a special (and quite expensive) type of beer with its own unique characteristics including flavour. The main production of speciality beers is localised in Belgium and The Netherlands. Some of these beers, so-called Trappist, are brewed by, or produced under the control of Trappist monks. Today, only seven monastery breweries producing this type of beer (six in Belgium and one in The Netherlands) are authorised to label their beers with the "Authentic Trappist Product" logo that indicates a conformity with various rules edicted by the International Trappist Association (ITA) [2].

Up to now, only a few studies have been focused on the authenticity of beer products. The earlier employed methods targeted various groups of beer components were represented by gas chromatography–isotope ratio mass spectrometry, GC–IRMS ($^{13}C/^{12}C$ ratio of CO_2), gas chromatography–flame-ionisation detection, GC–FID, or high-performance liquid chromatography–fluorescence detection, HPLC–FLD (amino acids), HPLC–UV–vis (phenolic substances), head-space solid-phase microextraction coupled to GC–FID (volatile compounds), nuclear magnetic resonance spectroscopy (D/H ratio of the methylene group of ethanol and $\delta^{18}O$ measurement of water), and Fourier transform infrared spectroscopy [1,3–9]. It should be noted that only relatively small number of samples was examined in these published studies.

In addition to these approaches, examination of the volatile profiles might be considered as a strategy enabling beer authentication/recognition since its composition (including volatiles) is known to vary depending on a raw material, technology employed for particular beer production as well as the storage conditions [10]. During recent years, solid-phase microextraction (SPME) in combination with the gas chromatography–mass spectrometry (GC–MS) has been implemented as the key method of choice for the analysis of food volatiles pattern including beer. This inexpensive, solvent-free sampling technique enables isolation not only of low molecular weight volatile analytes by their extraction from the beer head-space (HS), but also pre-concentration of higher molecular weight fraction of semi-volatiles using direct fibre immersion (DI) into aqueous beer solution [1,10–13].

Typically, large volume of data generated by the fingerprint techniques is to be processed, thus, smart chemometric tools are required to fully utilise this comprehensive information. In most

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cases, the 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 modelling of class analogy (SIMCA), or artificial neural networks (ANN) are the most commonly considered options for data processing assessment [14–18].

The current case study has been conducted within the EU-funded TRACE project (www.trace.eu.org) aiming at development of cost-effective traceability methods and systems, which provide consumers with added confidence in the authenticity of food at the European market. Alike for other food matrices examined within the TRACE project, the selected tool for recognition of speciality beers is based on recording fingerprints, in this particular case on GC profiles of volatiles collected by HS-SPME and then separated/detected by GC–TOFMS. Advanced chemometric strategies represented by PLS-DA, LDA, and ANN were employed for interpretation of acquired data set. As a challenging, straightforward profiling way of beer components pre-concentrated by DI-SPME, ambient mass spectrometry was employed for the examination of fibre surface. Only recently introduced new type of ion source, direct analysis in real time (DART) hyphenated with a high-resolution TOF mass spectrometer was used for obtaining mass spectral fingerprints (MS profiles).

2. Experimental

2.1. Beer samples

The first batch (delivered in 04/2008) contained 123 beer samples, while the second batch (delivered in 11/2008 and 01/2009) consisted from 142 samples. The samples were stored in a refrigerator (4 °C) between the delivery and their analysis. Before analysis, the samples were conditioned to a room temperature (2 h). An overview of beers examined within this study is shown in Table 1. Samples were collected over a relatively long production season (continuously over 1 year) to cover possible seasonal variability of the products.

Table 1
Overview of speciality 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

2.2. Chemicals and materials

2.2.1. SPME

The SPME fibres tested were as follows: (i) 100 μm polydimethylsiloxane (PDMS); (ii) 65 μm polydimethylsiloxane/divinylbenzene (PDMS/DVB); (iii) 65 μm Carbowax/divinylbenzene (CW/DVB), (iv) 50/30 μm divinylbenzene/Carboxen/polydimethylsiloxane (DVB/CAR/PDMS); and (v) 85 μm polyacrylate (PA). All of them were supplied by Supelco (Bellefonte, PA, USA). Prior to use, all fibres were conditioned following the manufacturer's recommendations.

2.2.2. GC

For GC–TOFMS experiments, a SolGelWAX, polyethylene glycol (SGE, Austin, TX, USA) column; 30 m × 0.25 mm id, 0.25 μm film thickness was employed.

A mixture of *n*-alkanes (C_8–C_{20}) dissolved in *n*-hexane, which was employed for the retention index determinations, was supplied by Supelco (Bellefonte, PA, USA). The calculations were done for components eluting under experimental conditions between *n*-octane and *n*-eicosane. For those compounds with elution after C20, extrapolation using C18–C20 alkanes was used.

2.3. HS-SPME–GC–TOFMS analysis

A TruTOF HT system consisting of an Agilent 7890A gas chromatograph equipped with a split/splitless injector (Agilent Technologies, Palo Alto, CA, USA), a PalCombi autosampler for automated SPME (Leap Technologies, Carrboro, NC, USA), and a TruTOF high-speed time-of-flight mass spectrometer (Leco Corp., St. Joseph, MI, USA) was used.

ChromaTOF (LECO Corp.) software (v. 3.35) was used for instrument control, data acquisition, and data processing. The GC–TOFMS records were baseline corrected, followed by peak finding (S/N = 5:1). Identification of compounds was based on a NIST 2005 mass spectra library search and was further confirmed by comparing linear retention indexes available in NIST 2008.

Sample preparation. After opening a bottle, approx. 20 ml of beer sample were degassed in an ultrasonic bath for 5 min at 5 °C. A volume of 2 ml of degassed beer sample was placed into a 10-ml vial for SPME containing 2 ml of distilled water and 1.7 g NaCl, the vial was sealed with a magnetic cap with PTFE/silicon septum.

HS-SPME sample extraction. Incubation time: 5 min; incubation temperature: 30 °C; agitator speed: 500 rpm; extraction time:

5 min; desorption temperature: 250 °C; desorption time: 60 s (splitless).

GC separation. Oven temperature program: 45 °C (3 min), 10 °C/min to 180 °C, 30 °C/min to 270 °C (1.50 min); carrier gas: helium (purity 99.9999%); column flow: 1.0 ml/min.

TOFMS detection. Electron ionisation mode (70 eV); ion source temperature: 220 °C; mass range: *m*/*z* 30–300; acquisition rate: 10 spectra/s; detector voltage: 2600 V.

2.4. SPME–DART–HRTOFMS analysis

For DART–HRTOFMS analyses, the system consisting of a 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) was used. For a mass drift compensation needed for accurate mass measurement and subsequent elemental composition calculation, a 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 manually using a Dip-it sampler (IonSense, Saugus, MA, USA) at the end of each analysis. Before the SPME sampling, the fibre was thermally cleaned for 5 min in the GC injector (250 °C; 50 ml/min helium flow).

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 SPME fibre to the DART gas beam; background ions were subtracted and a mass drift was corrected.

2.4.1. HS-SPME–DART–TOFMS

Sample preparation. After opening a bottle, approx. 20 ml of beer sample were degassed in an ultrasonic bath for 5 min at 5 °C. A volume of 2 ml of degassed beer sample was placed into a 10-ml vial for SPME containing 2 ml of distilled water and 1.7 g NaCl, the vial was sealed with a magnetic cap with PTFE/silicon septum

HS-SPME sample extraction. Incubation time: 5 min; incubation temperature: 30 °C; agitator speed: 500 rpm; extraction time: 15 min.

DART ionisation. 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: 20 s.

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

2.4.2. DI-SPME–DART–TOFMS

Sample preparation. After opening a bottle, approx. 20 ml of beer sample were degassed in an ultrasonic bath for 5 min at 5 °C. A volume of 8 ml of beer sample was placed into a 10-ml vial for SPME, the vial was sealed with a magnetic cap with PTFE/silicon septum.

DI-SPME sample extraction. Incubation time: 2 min; incubation temperature: 30 °C; agitator speed: 500 rpm; extraction time: 15 min.

DART ionisation. 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: 20 s.

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

2.5. Chemometric analysis

Chemometric analysis included the 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 the software statistiXL (v. 1.8, 2008, statistiXL, Broadway–Nedlands, Australia, www.statistiXL.com) was used.

Classification results are presented in terms of recognition and prediction abilities, and sensitivity and specificity. *Recognition ability* represents a percentage of the samples in the training set successfully classified. *Prediction ability* is a percentage of the samples in the test set correctly classified by using the model developed during the training step. *Sensitivity* of a class model is the rate of objects in the test set belonging to the class, which is correctly identified by the model. *Specificity* is the rate of objects foreign to the class that are classified as foreign in the test set [19].

3. Results and discussion

In the first phase of this case study, the HS-SPME procedure was optimised for obtaining as broad as possible representation of volatile compounds released from beer samples. In the follow-up part, this extraction strategy was employed for the GC–TOFMS and DART–HRTOFMS examination of a large set of beers, which were presumed to differ in profiles of volatiles/semi-volatiles, depending on the brand. The feasibility of employing this approach for the recognition purpose of some speciality beers is presented in the following sections.

3.1. Optimisation of HS-SPME–GC–TOFMS method

All the key parameters that may affect the SPME performance, such as a type of fibre coating, extraction temperature and time, ratio of beer and water, and addition of NaCl, were subject of careful optimisation carried out within the first part of this study. For the method development, Rochefort 8 beer was used.

Among the tested fibres (see Section 2.2.1), the complexity (*i.e.* number of extracted and detected compounds) of obtained beer profiles decreased in order: 50/30 μm DVB/CAR/PDMS ≈65 μm PDMS/DVB > 65 μm CW/DVB > 85 μm PA > 100 μm PDMS. On this account, the first fibre with combined sorbents was used in subsequent experiments.

Extraction temperatures of 30, 40, 50, 60, 70, and 80 °C, with a 5 min extraction period (+5 min incubation time at selected temperature) were employed to test 50/30 μm DVB/CAR/PDMS fibre sorption efficiency. For most of the volatiles, an increase in extraction temperature up to 80 °C led to a growth in the MS signal intensity, presumably due to their quicker transfer (higher diffusion coefficient) into the head-space. However, it is rather difficult (or even impossible) to distinguish whether the increased signal results from enhanced transfer of analytes into the head-space due to increased volatility, or whether the formation of particular compounds from precursors present in beer samples is enhanced. To elucidate these phenomena, beer samples (Rochefort 8) were conditioned at temperatures 30, 40, 50, 60, 70, and 80 °C for 10 min and, after cooling, volatiles were extracted at 30 °C. Largely differing GC–TOFMS volatiles patterns were observed for those samples that were conditioned at temperatures above 60 °C. The main differences were noted in relative abundances of esters and organic acids. Therefore, in the case when less sensitive mass spectrometers (*e.g.* quadrupole mass analyser in full scan mode) are considered for the analysis of compounds thermally desorbed from SPME fibre, we recommend to use extraction temperature up to 60 °C. However, in our study, a sampling temperature of 30 °C was selected in the final method, mainly not to exceed the linearity range of the TOF detector, and, consequently, to avoid distortion of volatiles pattern. It should be noted, however, that thermal induction of

artefacts formation, which may occur at higher incubation temperatures, does not necessarily hamper achieving the objective of profiling experiment. Different volatile signatures due to the differences in matrix composition, *i.e.* volatiles precursors, may provide the valuable information needed for classification of the samples.

In the next step, extraction time was tuned. The growth of the detector signal was observed for sampling times 1, 5, 10, 20, and 30 min at 30 °C (with a 5 min incubation period). However, since, as explained below, the detection sensitivity did not appear to be a limiting factor in our study, only a 5 min extraction could be used in subsequent experiments to enable reasonable sample throughput.

Also, the optimal ratio of beer:water (4:0; 3:1; 2:2; 1:3; v/v) was investigated. In this case, the best (the most intensive) volatiles profile was obtained when only beer (4 ml) was analysed (no water addition). However, when testing this parameter together with the addition of NaCl (0; 0.5; 1; 1.5; 1.7; 2 g), the intensity of volatiles was higher when using the ratio of 2 ml beer and 2 ml water containing 1.7 g of NaCl compared to the analysis of only beer (4 ml) with 1.7 g NaCl.

The further aspect considered was the effect of ethanol (obviously dominating organic component in the beer head-space) on the extraction efficiency of other beer volatiles. The ethanol content in the speciality beers examined in this study ranged between 5% and 11.3% (v/v). Therefore, a standard addition of 5% (minimal value), 8.2% (average value of all beers), and 11.3% (maximal value) ethanol (v/v) was tested using a non-alcoholic beer as a "blank" matrix. For the SPME experiment, the samples were prepared using 2 ml of beer + the corresponding alcohol volume, 2 ml of water, and 1.7 g NaCl (see Section 2.2.1). Thus, diluting the original beer by a factor of 2, the ethanol content decreased to 2.5%; 4.1%; 5.7% (v/v). In general, increasing the level of ethanol decreased the signal intensity of minor volatiles; nevertheless, the decrease was not dramatic. For instance, taking the absolute signal intensity in beer with 2.5% (v/v) ethanol as 100%, the abundance of 3,7-dimethylocta-1,6-dien-3-ol (linalool) in beers with 4.1% and 5.7% (v/v) ethanol decreased to 78% and 66%, respectively. These results accord with the work of Luan et al., who found only minor influence of ethanol on the extractability of *trans*-resveratrol from wine within the range from 0% to 20% (v/v) of ethanol [20]. Since the proposed SPME method was considered as a profiling tool, no adjustment by ethanol to a reference level prior the SPME analysis was carried out. Actually, the ethanol content itself is one of the important characteristics of the particular beer groups tested, thus this parameter cannot be excluded from the complex evaluation of the samples.

Relatively good repeatability of peak height measurements (average RSD of 7.5%) of the optimised HS-SPME–GC–TOFMS procedure was obtained within a series of ten consecutive analyses of Rochofort 8 beer for all 45 selected volatile compounds (Table 2).

For the separation of beer volatiles, a polar, narrow-bore GC column (polyethylene glycol) was employed. Although the profile of volatiles of beer samples was rather complex (Fig. 1), only 21 min were needed for the separation of compounds isolated by HS-SPME. This relatively short GC run, which is approx. 2–4-times shorter than in the previously published studies employing GC [10,12], was allowed by the use of the deconvolution function of TOFMS instrument. In this way, the lower chromatographic resolution of partially coeluted compounds was resolved spectrometrically, thus, pure mass spectra of volatile compounds were obtained, allowing reliable identification based on a library search. Fig. 2 shows an example of separation of nonan-2-ol and (1*S*)-1,7,7-trimethylnorbornan-2-one (camphor). In particular case, the peak apex separation of these compounds was 1.7 s, which required relatively high acquisition rate (10 spectra/s) for automated peak finding. An additional benefit of the use of TOFMS was simultaneous acquisition of full mass spectra even at very low concentration

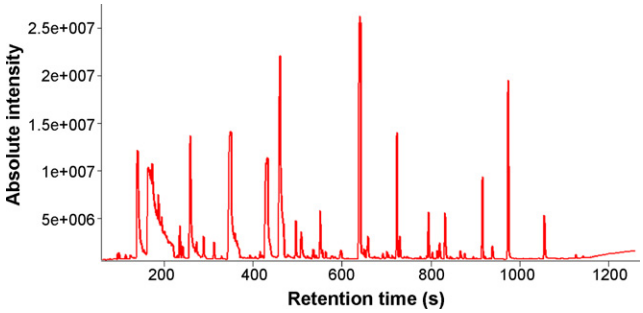

Fig. 1. The example of HS-SPME–GC–TOFMS chromatographic fingerprint (total ion current, TIC, *m*/*z* 30–300) of Rochefort 8 beer sample.

of particular compounds (due to the high mass analyser efficiency) as compared to quadrupole MS operated in full scan mode used in previous studies, thus, similar intensity of volatiles could be achieved but with shorter SPME extraction time (5 min *vs.* 30–60 min) [10,12].

3.2. Characterisation of beer volatiles and selection of markers

For the chemometric analysis, several potential markers (volatiles) were selected after careful inspection of the overlaid GC profiles of the analysed beer samples. The key selection criterion was a distinct difference in intensity of a particular peak among examined samples and known relation with beer aroma. These selected markers can be grouped as follows: alcohols, aldehydes, ketones, esters, carboxylic acids, ethers, and other compounds. Interestingly, most of these selected volatiles are typically responsible for various flavour notes. Esters are characterised by their fruit flavour and play an important role in overall flavour balance. Alcohols contribute to beer strong and pungent smell and taste. Higher alcohols are important as the immediate precursors of the more flavour-active esters. Aldehydes and ketones increase during storage, in parallel with the development of stale flavour. Carboxylic acids can contribute with fruits, cheesy, and fatty odours; they also contribute to bitterness, astringency, and rancidity. Ethers are responsible for almond, burnt sugar, sweet odour [10]. The list of the above compounds selected as markers, together with their retention times, specific masses (*m*/*z*) used for peak height measurements, RSDs, calculated retention indices, and retention indices obtained from the NIST2008 mass spectral library is shown in Table 2.

3.3. Chemometric analysis

In the first stage of the data processing, the raw data $\{265 \times 45\}$ presented in the form of absolute peak intensities were pre-processed using *range transformation*, *i.e.* the lowest value of given variable was assigned to "0" and the highest one to "1", whereas the remaining entries were numbers between these values (within the interval (0,1)) for each sample. This procedure transformed all the data to a uniform range of variability. While for PCA, all the data were used, in the case of supervised pattern recognition techniques (PLS-DA, LDA, and ANN-MLP), the data were randomly split to a calibration (training) set (2/3 of samples, $n = 166$) and the remaining samples (1/3, $n = 99$) were used as a test set (Table 3).

3.3.1. Principal component analysis

Principal component analysis (PCA) applied for data assessment represents one of the most frequently used chemometric tools. The possibility to project in a relatively easy way particular data from a higher to a lower dimensional space and then reconstruct them without any preliminary assumptions about their

Table 2
Analytical data of selected beer volatiles (markers) determined by HS-SPME–GC–TOFMS.

Compound	t_R (s)	*m/z*	RSD (%)[a]	*RI*	RI_{NIST}
Hexyl acetate	496	84	2.2	1273	1264
Octanal	511	84	29.2	1289	1288
Ethyl heptanoate	552	88	9.6	1334	1338
Hexan-1-ol	564	57	2.3	1347	1352
Unknown (*m/z* 43, 95, 111, 140)	577	95	3.1	1362	–
Heptyl acetate	587	61	4.6	1373	1380
Nonanal	604	98	20.1	1393	1390
Heptan-1-ol	650	56	5.4	1448	1460
Acetic acid	657	60	9.4	1457	1451
Furan-2-carbaldehyde	659	96	5.6	1460	1450
Octyl acetate	671	56	7.1	1475	1483
2-Ethylhexan-1-ol	678	83	7.5	1483	1492
Decan-2-one	685	58	6.1	1492	1491
1-(2-Furyl)ethanone	692	95	4.0	1501	1500
Nonan-2-ol	702	45	5.7	1513	1522
(1*S*)-1,7,7-Trimethylnorbornan-2-one	703	95	3.9	1515	1507
Benzaldehyde	708	106	4.7	1521	1530
3,7-Dimethylocta-1,6-dien-3-ol (linalool)	724	71	2.6	1542	1545
Octan-1-ol	730	84	5.2	1551	1557
Oxolan-2-one (butyrolactone)	785	86	12.1	1625	1631
Butanoic acid	791	60	5.9	1633	1636
Ethyl decanoate	795	101	8.2	1638	1630
2-Phenylacetaldehyde	797	92	4.0	1641	1636
2-Furylmethanol	804	98	8.8	1651	1650
Nonan-1-ol	805	56	6.1	1653	1661
3-Methylbutyl octanoate	810	127	6.7	1659	1652
Ethyl benzoate	815	105	4.1	1666	1661
Diethyl butanedioate	819	101	3.2	1673	1680
Ethyl dec-9-enoate	832	88	7.3	1691	1691
3-Methylsulfanylpropan-1-ol	844	106	19.4	1708	1702
Undecan-2-ol	848	55	8.8	1714	1712
Decan-1-ol	877	84	6.5	1756	1750
(3*S*)-3,7-Dimethyloct-6-en-1-ol (citronellol)	879	123	7.0	1759	1764
Dec-9-en-1-ol	912	55	7.1	1810	1813
Phenethyl acetate	916	91	2.5	1816	1810
(*E*)-1-(2,6,6-Trimethyl-1-cyclohexa-1,3-dienyl)but-2-en-1-one (damascenone)	921	69	6.7	1824	1816
(2*E*)-3,7-Dimethylocta-2,6-dien-1-ol (geraniol)	932	69	4.4	1840	1837
Ethyl dodecanoate	935	101	8.9	1845	1851
Hexanoic acid	939	60	6.2	1851	1850
2-Phenylethanol	974	52	7.9	1906	1905
Octanoic Acid	1055	60	3.8	2050	2050
Unknown (*m/z* 43, 55, 67, 82, 93, 107, 125, 149, 189)	1092	82	7.2	2124	–
Nonanoic acid	1095	60	21.6	2132	2110
Decanoic acid	1126	60	10.4	2199	2229
Unknown (*m/z* 41, 55, 67, 79, 91, 109, 119, 137, 157, 177)	1129	67	6.3	2205	–

[a] Relative standard deviation (RSD) of peak height, $n = 10$.

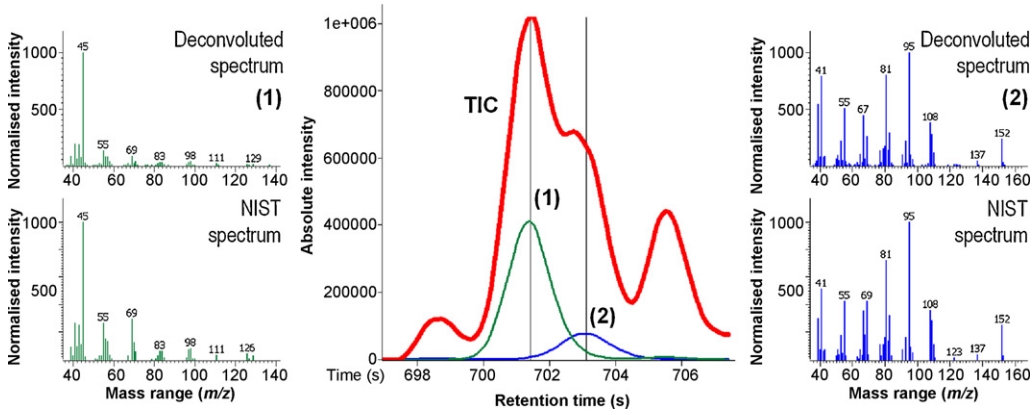

Fig. 2. Spectral deconvolution of two closely eluted beer markers of the chromatographic fingerprint shown in Fig. 1. (1) Nonan-2-ol, *m/z* 45 displayed; (2) (1*S*)-1,7,7-trimethylnorbornan-2-one (camphor), *m/z* 95 displayed.

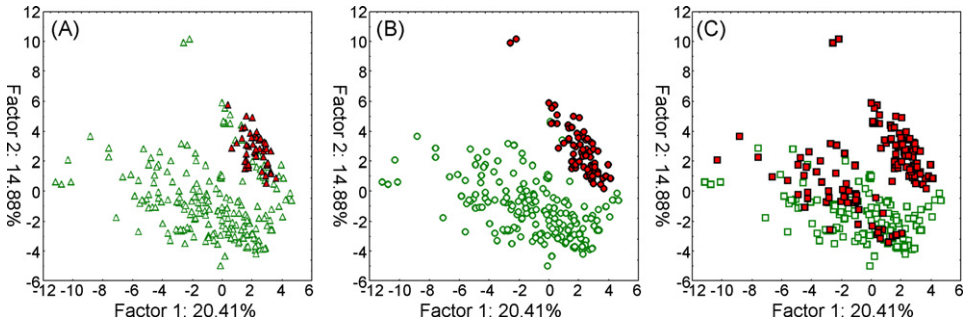

Fig. 3. First and second PCA scores for: (A) Rochefort 8 (▲) *vs.* the rest (△); (B) Rochefort 6, 8, 10 (●) *vs.* the rest (○); (C) Trappist beers (■) *vs.* non-Trappist beers (□). Graphs constructed using all samples ($n = 265$).

Table 3
Number of samples of calibration and test sets used for chemometric analysis.

Model	Calibration set (number of samples)	Test set (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

distribution represents one of its attractive features [21]. In the preliminary analysis of the beer data, PCA was performed to investigate any potentially existing clustering based on the type of beer samples. As Fig. 3 shows, using this approach, the beer samples were divided into groups described as (A) Rochefort 8 *vs.* the rest; (B) Rochefort 6, 8, 10 *vs.* the rest; and (C) Trappist beers *vs.* non-Trappist beers. The first principal compound (PC) accounted for 20.4% variance, while the second PC contributed for 14.9%. The most important eleven PCs (with eigenvalues >1), contributed to 76% of total variance. Especially for Fig. 3A and B, some clustering behaviour that relates to recognition of Rochefort 8 and a group consisting of Rochefort 6, 8, 10 beers was observed using this exploratory technique.

3.3.2. Partial least squares discriminant analysis

In principle, partial least squares discriminant analysis (PLS-DA) finds the variables and directions (in the multivariate space) which discriminate the classes in the calibration set [19]. For the models "Rochefort 8 *vs.* the rest" and "Trappist *vs.* non-Trappist beers" quite high recognition and prediction abilities were obtained (91.6% and 97.0%, and 87.9% and 93.9%, respectively). The best recognition and prediction abilities (100% and 100%, respectively) were obtained for the model "Rochefort 6, 8, 10 *vs.* the rest" (Table 4). Under these conditions, the classes were completely separated, thus, good classification was obtained (see Fig. 4).

3.3.3. Linear discriminant analysis

Linear discriminant analysis (LDA) is another frequently used supervised pattern recognition method. In principle, LDA determines linear discriminant functions, which maximise the ratio of between-class variance and minimise the ratio of within-class variance. Contrary to PCA that selects a direction retaining a maximal structure among the data in a lower dimension, LDA selects a direction that achieves maximum separation among the given classes [19]. As Table 4 shows, the recognition and prediction abilities of model "Rochefort 8 *vs.* the rest" were 91.6% and 84.8%, respectively. The analysis of the misclassified samples showed that the LDA model presented a relatively high sensitivity (100%) but a rather low specificity (80.8%), thus, while being able to identify the Rochefort 8 samples, its ability to classify the rest of beer samples is fairly limited. The majority of these misclassified samples were Rochefort 10 beers, probably because of their relative similarity to Rochefort 8 beers. This fact documents Fig. 5 with the detail of PCA clustering that shows overlaying of Rochefort 6, 8, and 10 beer samples. Indeed, both recognition and prediction abilities improved significantly, when the model "Rochefort 6, 8, 10 *vs.* the rest" was tested (100% and 100%, respectively). Lower recognition and prediction abilities compared to the previous model were achieved for the model "Trappist *vs.* non-Trappist beers", 97.0% and 91.9%, respectively.

3.3.4. Artificial neural networks

As an alternative, ANN approach was also tested. This chemometric tool is suitable mainly in those cases when the relationship

Table 4
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 45 variables)	91.6%	87.9%	90.5%	87.2%
LDA	45 variables	91.6%	84.8%	100.0%	80.8%
ANN-MLP (45:45–21–1:1)	45 variables	96.4%	93.9%	95.2%	93.6%
(B) Rochefort 6, 8, 10 *vs.* the rest					
PLS-DA	2 loadings (originated from 45 variables)	100.0%	100.0%	100.0%	100.0%
LDA	45 variables	100.0%	100.0%	100.0%	100.0%
ANN-MLP (45:45–6–1:1)	45 variables	100.0%	100.0%	100.0%	100.0%
(C) Trappist beers *vs.* non-Trappist beers					
PLS-DA	3 loadings (originated from 45 variables)	97.0%	93.9%	90.5%	100.0%
LDA	45 variables	97.0%	91.9%	88.9%	97.2%
ANN-MLP (45:45–16–1:1)	45 variables	97.6%	97.0%	96.8%	97.2%

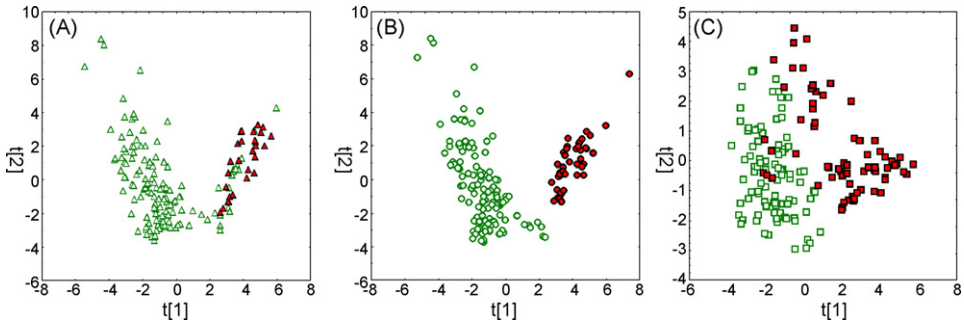

Fig. 4. First and second PLS scores for: (A) Rochefort 8 (▲) *vs.* the rest (△); (B) Rochefort 6, 8, 10 (●) *vs.* the rest (○); (C) Trappist beers (■) *vs.* non-Trappist beers (□). Graphs constructed using calibration data set ($n = 166$).

between predictor variables (independents, inputs) and predicted variables (dependents, outputs) is very complex [18,22].

The most common neural network (ANN) approach to regression-type problems is multilayer perceptrons (MLP) [23,24]. An ANN-MLP using the back propagation was employed to predict the origin of beer samples based on the pattern of their volatiles. In the first step, the calibration data set was randomly divided by the software into two subsets: (i) *training subset* (2/3 of data, $n = 110$), which is used to accomplish the network model training; (ii) *selection subset* (1/3 of data, $n = 56$) for checking the network performance within the training process and to avoid network overtraining. The *test subset* (*i.e.* remaining data, $n = 99$) represents the tool to assess the quality of the generated model.

Intelligent Problem Solver was employed for the analysis of data. The search for an appropriate ANN model was restricted only to MLP networks. In total, 50 networks 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 optimised 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 are stabilised [24]. In this study, 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, the MLP networks consisted of 45 neurons in the input layer (all models), 21, 6, 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 100%, respectively) were obtained for the model "Rochefort 6, 8, 10 *vs.* the rest". Lower recognition and prediction abilities (96.4% and 93.9%, respectively) were obtained for the model "Rochefort 8 *vs.* the rest", and, again, the most of misclassified sample belonged to the other Rochefort beers within the group as in the case of LDA. High recognition and prediction abilities were also achieved for the model "Trappist *vs.* non-Trappist beers", 97.6% and 97.0%, respectively (Table 4).

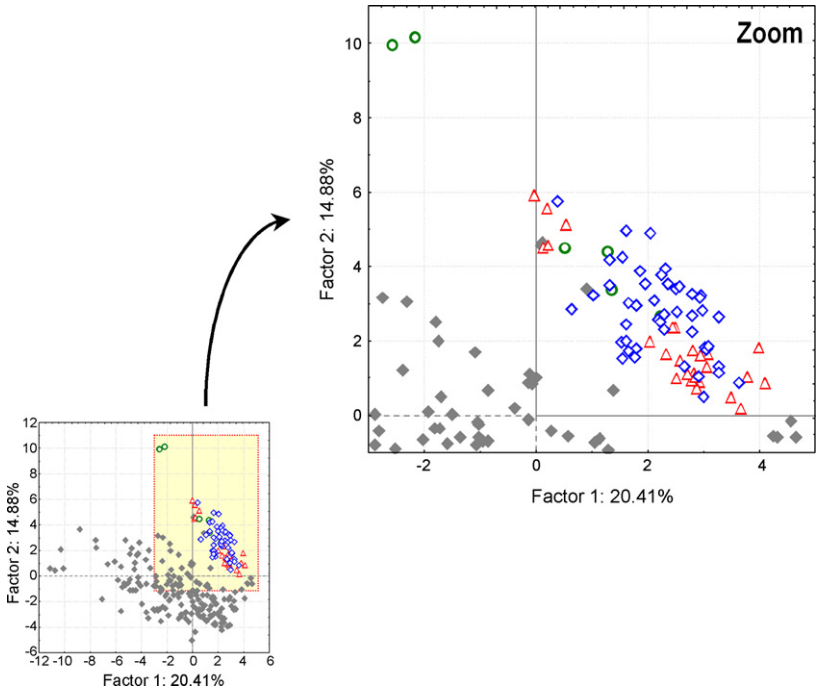

Fig. 5. First and second PCA scores for: Rochefort 6 (○), Rochefort 8 (◇), Rochefort 10 (△), the rest of Trappist and non-Trappist beers (◆). Graphs constructed using all samples ($n = 265$).

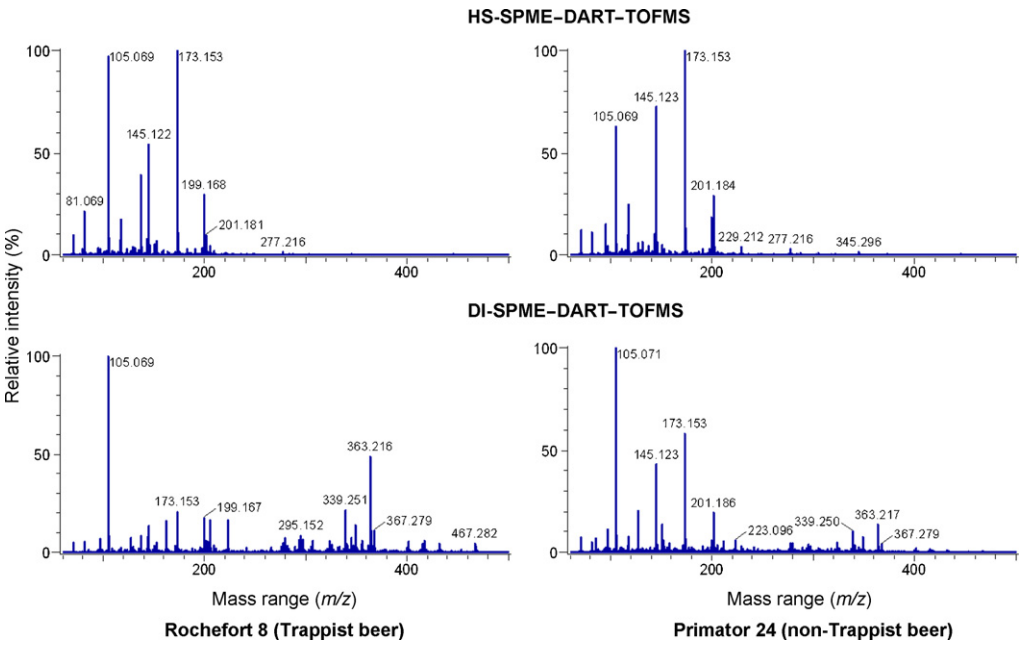

Fig. 6. Comparison of MS spectra (fingerprints) of Rochefort 8 (Trappist) and Primator 24 (non-Trappist) beers under SPME–DART–TOFMS conditions.

3.4. Future challenge: SPME–DART–TOFMS

In the final phase of our experiments, we also tested the potential of SPME coupled to a one of the novel ion sources, direct analysis in real time (DART). Typically, the SPME sampling procedure is followed by the transfer of pre-concentrated analytes into chromatographic system using either a GC injector (thermal desorption) or an SPME–HPLC interface (desorption by the solvent) [25]. In both cases, sample components are subsequently separated using most commonly, chromatographic procedure. Depending on the sample complexity, the separation step may take several minutes when only few compounds were extracted, or up to 1–2 h for very complex mixtures of volatiles such might be those occurring in some food matrices. In the latter case, chromatographic run may become a limiting factor in measurements. The possibility of overcoming this bottleneck has emerged with the introduction of ambient mass spectrometry employing novel ionisation sources such as DART. This technique allows a rapid, non-contact MS analysis of materials at ambient pressure and ground potential. Compared to GC–MS and LC–MS, as far as a high-resolution detector is available, ambient MS allows spectral fingerprinting without previous chromatographic separation [26,27].

In the preliminary experiments, the DART heated gas stream of helium was used for the desorbing and direct introduction of the ionisable sample components thermally released (evaporated) from the SPME fibre into a HRTOFMS instrument. The use of SPME–DART–TOFMS was tested employing both HS- and DI-SPME modes. As Fig. 6 shows, substantially different mass profiles (spectra) were obtained. While the HS-SPME mode provided profiles with ions in a lower mass region with most m/z values <200 Da, the DI-SPME mode allowed to obtain a fingerprint for higher molecular weight, less volatile, components up to approx. m/z 500 Da.

Regarding mass spectrum of volatiles pre-concentrated by HS-SPME mode, many of those compounds that were identified under the GC–EI-MS conditions could be found here when comparing the elemental composition of detected ions (*i.e.* volatiles). However, it has to be emphasised that in DART mass spectrum each recorded ion present can be a sum of different compounds with identical or very close elemental composition. For instance, the ion m/z 173.15 corresponding to an elemental composition of $C_{10}H_{21}O_2$ ($[M+H]^+$), based on complementary information provided by GC–MS analysis, can be a sum of following compounds: decanoic acid, ethyl octanoate, and octyl acetate, the most abundant volatiles with this elemental composition in HS-SPME–GC–TOFMS profile. Similarly, the ion m/z 145.12 corresponding to $C_8H_{17}O_2$ ($[M+H]^+$) can be a sum of octanoic acid, ethyl hexanoate, and ethyl 4-methylpentanoate. In the case of alcohols, such as 2–phenylethanol, elimination of water from the molecule led to formation of $[M-H_2O+H]^+$ ion, m/z 105.07.

One of the major advantages of DART–TOFMS technique employing the DI-SPME sampling approach was the possibility of sorption/desorption/ionisation of relatively polar compounds, typically not volatile enough for HS-SPME sampling prior to GC–MS. These polar compounds were detected as $[M+H]^+$ ions and tentatively identified as phenolic compounds: 8-prenylnaringenin (m/z 341.14), cohumulone (m/z 349.20), xanthohumol (m/z 355.15), humulone and adhumulone (m/z 363.22), lupulone and adlupulone (m/z 415.28) originating from hop [28].

As was indicated above, the coupling of SPME to DART–TOFMS represents a challenging tool for obtaining profiles that can be used for rapid authentication of food commodities (in particular case beer samples) and will be tested in our follow-up study, in which the database of various beer brands will be established. In any case, the critical parameter that can influence the mass separation (isobaric interferences) is the mass resolving power of particular mass spectrometer. While the mass resolving power of the TOFMS used in this study ranged between 3000 and 5000 FWHM (full width at half maximum) for the mass range of m/z 70–470 Da, the recently introduced orbitrap MS technology, which will be used in our next experiments, allows working with the mass resolving power as high as 100,000 FWHM [29], thus, further significant improvement of confirming the analyte identity and avoiding bias in marker ion intensity can be expected.

4. Conclusions

Fingerprinting employing SPME sampling of the head-space volatiles followed by GC separation and TOFMS detection represents a challenging analytical authentication option, since the whole process can be fully automated, and, moreover, the identity

of fingerprint components (markers) can be obtained based on the mass spectral data.

The best prediction ability was obtained for the model that distinguished a group of Rochefort 6, 8, 10 beers and the rest of beers. In this case, all chemometric tools employed, *i.e*. PLS-DA, LDA and ANN-MLP, provided 100% correct classification. Slightly worse prediction abilities were achieved for the models "Trappist *vs*. non-Trappist beers" with the values of 93.9% (PLS-DA), 91.9% (LDA) and 97.0% (ANN-MLP) and "Rochefort 8 *vs*. the rest" with the values of 87.9% (PLS-DA), 84.8% (LDA) and 93.9% (ANN-MLP).

Application of SPME–DART–TOFMS provided an interesting option to SPME–GC–MS. The mass spectral profiles obtained under the conditions of ambient mass spectrometry employing DART–TOFMS opens a door for new type of applications in food authentication, since the determination of relatively polar compounds (*e.g*. phenolics), not GC-amenable, is possible by employing DI-SPME sampling technique.

The profiling strategies demonstrated within this case study are believed to be applicable for many other authentication purposes.

Acknowledgements

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