Electrospray ionization mass spectrometry fingerprinting of beer

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After just simple degassing, dilution, pH adjustment and direct flow injection, characteristic fingerprint spectra of beer samples have been obtained by fast (few seconds) electrospray ionization mass spectrometry (ESI-MS) analysis in both the negative and positive ion modes. A total of 29 samples belonging to the two main beer types (lagers and ales) and several beer subtypes from USA, Europe and Brazil could be clearly divided into three groups both by simple visual inspection of their ESI(+)-MS and ESI(−)-MS fingerprints as well as by chemometric treatment of the MS data. Diagnostic ions with contrasting relative abundances in both the positive and negative ion modes allow classification of beers into three major types: P = pale (light) colored (pilsener, pale ale), D = dark colored (bock, stout, porter, mild ale) and M = malt beer. For M beers, samples of a dark and artificially sweetened caramel beer produced in Brazil and known as Malzbiers were used. ESI-MS/MS on these diagnostic beer cations and anions, most of which are characterized as arising from ionization of simple sugars, oligosaccharides, and iso-alpha-acids, yield characteristic tandem mass spectra adding a second and optional MS dimension for improved selectivity for beer characterization by fingerprinting. Direct ESI-MS or ESI-MS/MS analysis can therefore provide fast and reliable fingerprinting characterization of beers, distinguishing between types with different chemical compositions. Other unusual polar components, impurities or additives, as well as fermentation defects or degradation products, could eventually be detected, making the technique promising for beer quality control.

Introduction

Beer is an amber aqueous solution of highly complex composition containing CO_2, ethyl alcohol, several inorganic salts and a blend of more than 800 organic compounds. Inorganic salts come primarily from water and the brewing grains such as barley and other cereals. The organic compounds, which come mainly from the brewing materials and as by-products of yeast metabolism, are responsible for most of the flavor characteristics that are so unique to beer.[1]

Important stages of beer processing are malting, brewing and fermentation, followed by maturation, filtering and bottling. During malting and mashing, polysaccharides from the cereals are hydrolyzed to low molecular weight sugars (such as glucose, sucrose and maltose) as well as maltooligosaccharides consisting of 3 to 10 glucose units.[2] Fermentation then converts most of the low molecular weight sugars into alcohol, but maltotetrose and higher oligosaccharides (dextrins) are not fermented by most yeast strains.[2]

Although brewed from similar materials, beers throughout the world have distinctive tastes. Their uniqueness comes mainly from differences in the mineral content of the water, the types of ingredients and variations in brewing methods that form different subtypes of beers such as pilsener, pale ales, bock, stout, mild ale, porter and malt (caramel) beers. In a strict sense, there are two classical beer styles, top (ale) or bottom (lager) fermented. Ales are fermented using *Saccharomyces cerevisae* at temperatures between 16 and 24 °C. Lagers are fermented using a different species of yeast and at lower temperatures.[2]

Flavor is an important quality factor of beer. Therefore many efforts have been undertaken to control and improve the brewing process and to enhance flavor quality and stability.[3] Compounds formed by oxidative processes in beer[4] as well as compounds formed due the elevated temperatures during packing, storing and transportation, influence the flavour and smell of the product.[5,6] For instance, the characterization of lager, dark and low alcoholic content beers based on their mineral content as measured by inductively coupled plasma optical emission spectrometry (ICP-OES) has been demonstrated.[7] Electrospray ionization mass spectrometry (ESI-MS) after liquid chromatography (LC)[8] or capillary electrophoresis (CE)[9] has been used to identify specific classes of compounds in beer. The content of maltooligosaccharides influences the characteristics of beers, and recently qualitative and quantitative analysis of saccharides in beer samples have been demonstrated using direct flow injection ESI-MS, with no chromatographic separation.[10]

Electrospray (ESI) is a soft and wide-ranging ionization technique that has revolutionized the way molecules are ionized and transferred to mass spectrometers for mass measurement and structural characterization.[11] ESI has greatly expanded the applicability of mass spectrometry to a variety of new classes of molecules with thermal instability, high polarity and mass. Direct injection ESI-MS has also been shown to be suitable for fast fingerprinting of complex mixtures such as

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plant extracts,[12] propolis,[13] wine[14] and whisky.[15] ESI, with direct sample introduction, is likely therefore to be a convenient technique for fingerprinting and fast quality control of beer with very little, simple sample manipulation and direct injection into a mass spectrometer. This is so because key components of the blend of beer organic compounds bear acidic or basic sites that are therefore likely to be detected by direct injection ESI-MS as either protonated or deprotonated molecules and form a set of diagnostic beer ions. Tandem MS/MS with collision-induced dissociation (CID) of such diagnostic ions could also be used to add a second MS dimension in very selective beer fingerprinting, allowing for the structural characterization of the precursor molecules.

Sensory properties such as taste, smell and sight are unique characteristics of food and drinks. These properties are multivariate for they involve a combination of sensations, which are useful for classification, but are also subjective and can lead to evaluation and classification errors. Fast and objective instrumental analytical measurements, such as the ESI-MS fingerprinting method exemplified herein for beer, represent an attractive alternative for quality control of foods and drinks, particularly when associated with multivariate statistics. These statistics provide a set of tools that help deal with the complexities and subtleties confronted in the characterization and sensory classification process. Treating two or more variables simultaneously requires the use of the mathematical apparatus of matrix algebra. Techniques such as principal component analysis (PCA)[16] can serve to order known samples and have been applied to MS data to classify unknown samples.[17]

In this work, ESI-MS with direct flow injection is tested as a fast method (a few seconds) for the fingerprint characterization and quality control of beer samples. Samples of internationally famous brands belonging to the two main types (ales and lagers) and of several subtypes of beers from USA, Europe and Brazil were analyzed. These beer samples produce characteristic ESI-MS data in both the positive and negative ion modes. Both simple visual inspection and chemometric treatment of data place the samples in three distinct groups owing to very diagnostic (marker) ions.

Experimental

Beer samples

Twenty-nine samples of beer (Table 1) belonging to the two main types (ales and lagers) and several beer subtypes (pilsener, pale ales, bock, stout, porter, mild ale, malt) from USA, Europe, and Brazil were were analyzed by ESI-MS in both positive and negative ion modes. For malt beer, samples of a dark and artificially sweetened malt (caramel) Brazilian beer known as Malzbiers were used.

General experimental procedures

A Q-TOF mass spectrometer (Micromass, Manchester, UK) was used for fingerprint ESI-MS analysis. Typical ESI-MS conditions were: source temperature 100 °C, desolvation temperature 120 °C, capillary voltage 3.0 kV and cone voltage 40 V. The beer samples (250.0 μL) were degassed in order to eliminate CO_2, and diluted in a flask with a 1:1 solution of water: methanol up to a final volume of 1.0 mL. Formic acid (2 μL) was added to each sample for the positive ion mode ESI(+)-MS analysis whereas 2 μL of ammonium hydroxide were added for the negative ion mode ESI(−)-MS analysis. The samples were injected at a flow rate of 15 μL min^{-1} using a syringe pump (Harvard Apparatus). Mass spectra were acquired over a 50–1000 *m*/*z* range.

Table 1 Origin and types of beers analyzed. P = pale (light) colored, M = malt "Malzbier" and D = dark colored beer

Origin	Type	Origin	Type
Brazil	P1	Europe	D1
Brazil	P2	Europe	D2
Brazil	P3	Brazil	D3
Brazil	P4	Europe	D4
Brazil	P5	Europe	D5
Brazil	P6	Europe	D6
USA	P7	Brazil	D7
USA	P8	Brazil	D8
Europe	P9	Brazil	D9
Europe	P10	Brazil	D10
USA	P11	Europe	D11
Brazil	P12	Brazil	D12
Brazil	M1	Brazil	D13
Brazil	M2		
Brazil	M3		
Brazil	M4		

Statistical data treatment

To classify the beer samples after ESI-MS fingerprint analysis, principal component analysis (PCA) was performed on the respective ESI-MS results. Data matrixes were constructed using the information of the mass spectra obtained in both ESI(+)-MS and ESI(−)-MS modes. Ions producing the 20 major peaks in the MS of each of the 29 samples analyzed were selected as the variables. Exploratory data analysis was applied to the data matrix constituted of the 29 samples (as rows) and this group of variables (as columns). Einsight and Pirouette, both from Infometrix (Seattle,WA), were used to perform the Principal Component Analysis (PCA) using Mean Centering as data pre-treatment.

Results and discussion

As exemplified by the mass spectra shown in Figs. 1 and 2, simple visual inspection of all 29 mass spectra obtained in both the positive and negative ESI ion modes reveals 3 very characteristic groups that directly correspond to the three major types of beers: P = pale (light) colored (pilsener, lager, pale ales), D = dark colored (bock, stout, mild ale) and M= malt "Malzbier" beers. Principal component chemometric analysis of both the ESI(+)-MS and ESI(−)-MS data (Fig. 3A and B) also clearly divide the samples into the same P, D, and M groups, confirming the clear but subjective visual interpretation of the ESI-MS fingerprints.

ESI(+)-MS fingerprints

Fig. 1A shows an ESI(+) mass spectrum of very typical of P beers, with intense "beer cations" in the *m*/*z* range of 70 to 705

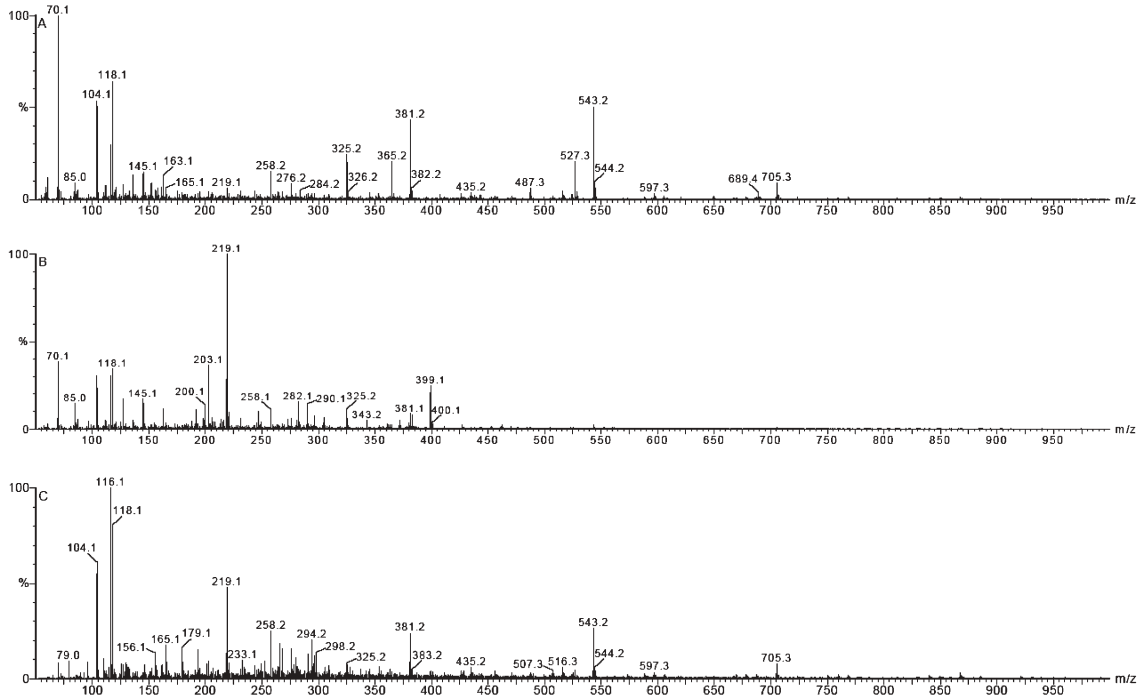

Fig. 1 ESI(+) fingerprint spectra representative of (A) pale (light) colored (P), (B) malt ''Malzbier'' (M), and (C) dark colored beers (D).

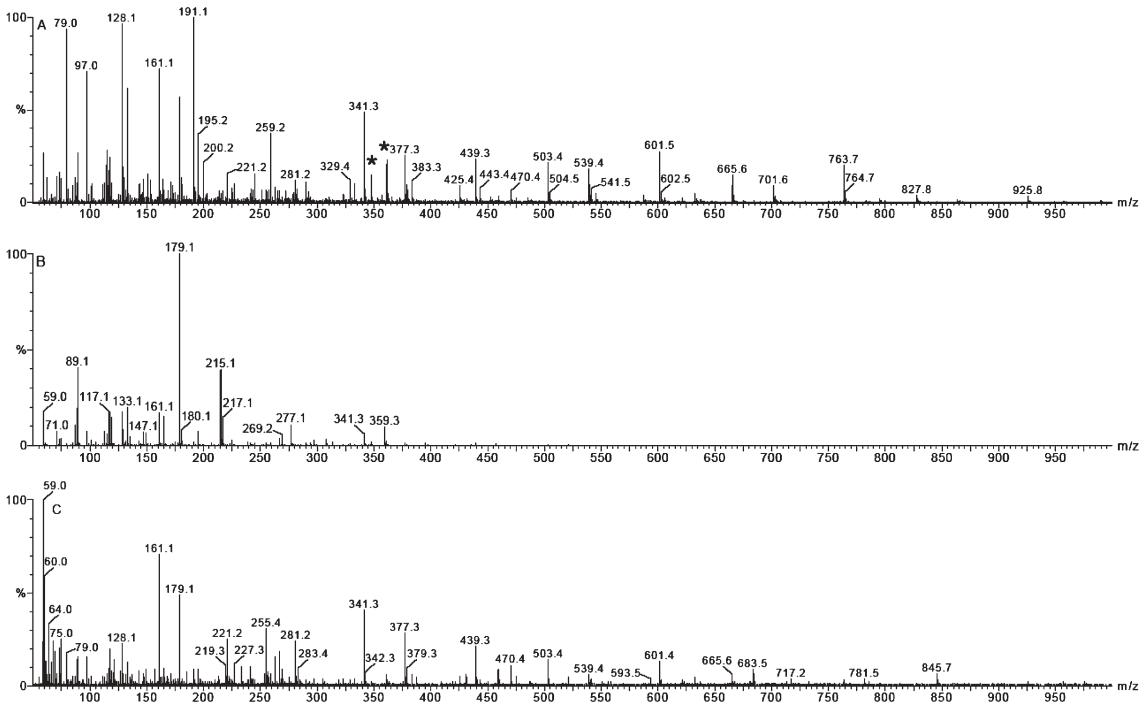

Fig. 2 ESI(−) fingerprint spectra representative of (A) pale colored (P), (B) malt ''Malzbier'' (M), and (C) dark colored beers (D). The ions marked with asterisks are those of *m*/*z* 347 and 361 which correspond to the deprotonated molecules of major iso-alpha-acids found in beer.

corresponding to sodium $[M + Na]^+$ and potassium $[M + K]^+$ adducts. Since these adducts are known to be quite resistant towards dissociation, each ion is likely to represent a single component of the mixture and not fragments of other heavier ions. A series of major cations correspond to $[M + Na]^+$ and $[M + K]^+$ adducts of maltose (*m*/*z* 365 and 381), maltotriose (*m*/*z* 527 and 543) and maltotetrose (*m*/*z* 689 and 705), as indicated by their tandem MS spectra and comparison with reported data.[2] Therefore, as these oligosaccharides are not totally consumed during fermentation,[1] they form diagnostic cationized molecules for ESI-MS typification of P beers. The $[M + K]^+$ adduct of glucose of *m*/*z* 219 constitutes, however, a

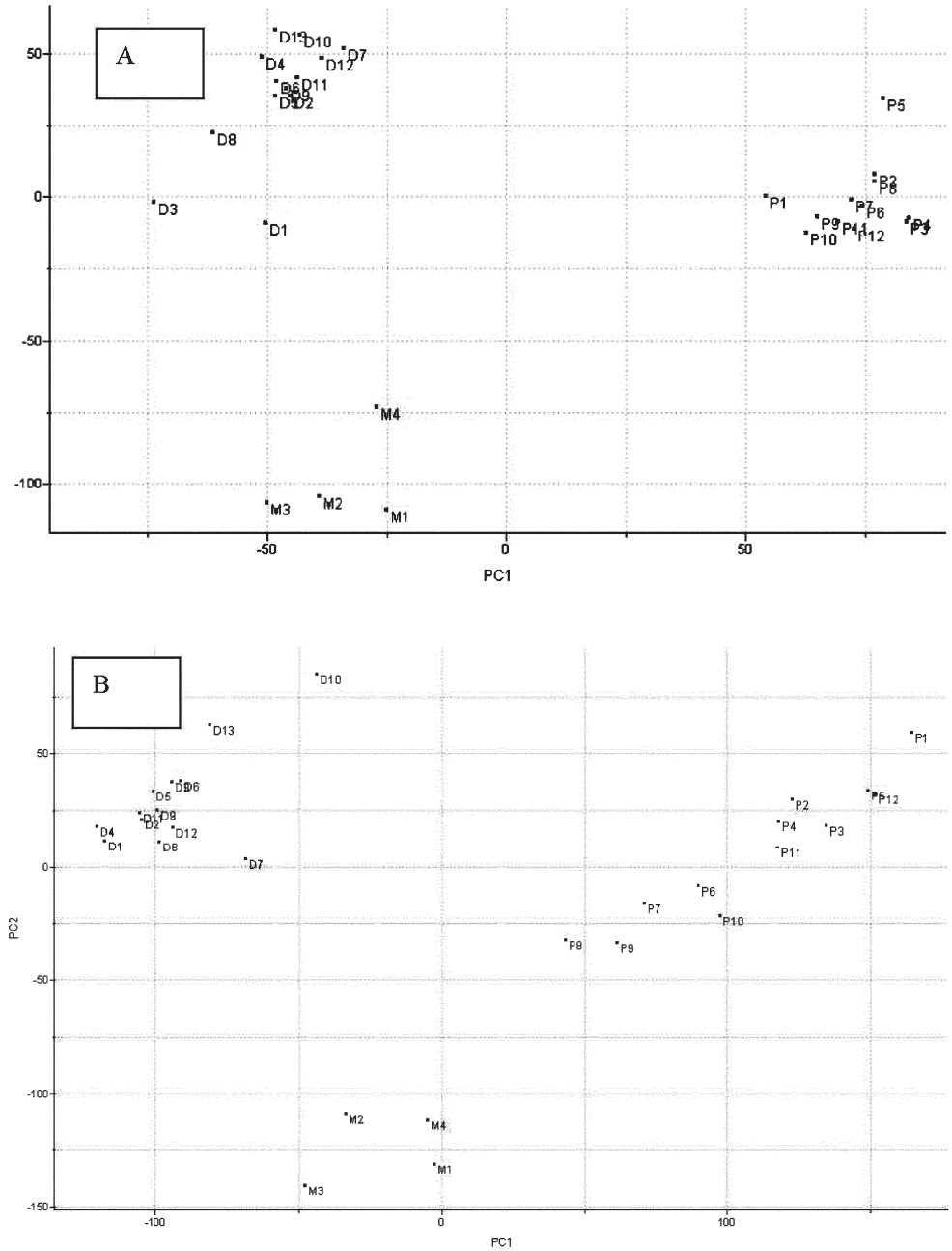

Fig. 3 (A) Scores of PC1 (42% variance) *versus* PC2 (27%) for the ESI(+)-MS data and (B) scores of PC1 (57%) *versus* PC2 (17%) for the ESI(−)-MS data of the 29 samples of beer investigated (see Table 1).

very minor ion which corresponds to the expectation that most glucose is consumed during fermentation of P beers with their characteristic bitter taste. Another diagnostic and major cation for P beers is that of *m*/*z* 325, and ESI-MS/MS shows this is likely the $[M + K]^+$ adduct of a dimer of anhydrohexose.

Fig. 1B shows an ESI(+) mass spectrum typical of M beers. As the result of artificial sweetening, by far the most diagnostic cations for the M beers are clearly the ones corresponding to the $[M + Na]^+$ and $[M + K]^+$ adducts of glucose of *m*/*z* 203 and 219, and the $[M + K]^+$ adduct of sucrose of *m*/*z* 399. These intense and diagnostic cationized glucose molecules indicate a beer additive, that is, caramel malt responsible for the pronounced sweet taste and some of the dark color characteristic of Brazilian caramel ''Malzbier'' types of beer.

Fig. 1C shows a ESI(+) mass spectrum typical of D beers. Their fingerprint mass spectra is, in general, rather similar to that of P beers, except mainly for the considerably more intense ion of *m*/*z* 219 that is clearly seen in such a spectrum. As for the M beers, this cation is likely the $[M + K]^+$ adduct of glucose indicating some degree of caramel coloring and sweetening, as normally observed for dark beers.[2] Another interesting and characteristic feature of the D beers is that only the $[M + K]^+$ of the oligosaccharides (and not the $[M + Na]^+$ adducts of *m*/*z* 365, 527 and 689 as seen in Fig. 1A) are clearly

seen as the cations of *m/z* 381, 543 and 705 (Fig. 1C). Direct infusion ESI(+)-MS seems therefore to be able to reveal, by comparison, distinct $[K^+]/[Na^+]$ concentration ratios in P and D beers. Also diagnostic but not so abundant for the D beers are the cations of *m/z* 165 and 179.

ESI(−)-MS fingerprints

Fig. 2A shows an ESI(−) mass spectrum typical of P beers. Relatively intense and characterisitc ''beer anions'' are seen in the *m/z* range from 79 to 925 corresponding to deprotonated $[M - H]^-$ molecules. Since such ions were accelerated from the ESI source to the MS using relatively low kinetic energies to avoid dissociation, the ion current is expected to represent intact molecules in their deprotonated forms rather than fragment ions. Anions of *m/z* 161, 179, 341, 503, 665 and 827 are likely the $[M - H]^-$ forms of anhydrohexose, glucose, maltose, maltotriose, maltotetrose and maltopentose, respectively, as also indicated by ESI-MS/MS and comparison with reported data.[13] Pairs of anions of *m/z* 377 and 379, 539 and 541, 701 and 703 are likely the chloride adducts (typical isotopic pattern for the ^{35}Cl and ^{37}Cl isotologues) of maltose, maltotriose, and maltotetrose, as previously suggested.[8] Other characteristic and intense anions of P beers are those of *m/z* 79, 97, 128, 191, 259, 439, 601 and 763. The ions of *m/z* 439, 601, 763, and 925 most likely indicate the presence of unfermentable higher oligosaccharides with a $\Delta m/z$ of 162 $(C_6H_{10}O_5)_n$. The concentrations of these oligosaccharides vary from beer to beer. Lower kiln temperatures during malting produce more dextrins and less sugar, whereas higher temperatures produce less dextrins and more sugars.[1]

Also noteworthy in the ESI(−) mass spectrum of Fig. 2A is the detection of the minor but relevant anions of *m/z* 347 and 361. These anions correspond to the deprotonated forms of two major iso-alpha-acids responsible for the bitter taste of beer, also playing an essential role in foam stability.[18] Direct infusion ESI(−)-MS analysis, particularly when using selective ion monitoring (SIM), may therefore also serve as a very fast method to control, compare or roughly quantitate levels of these important components in beer samples.

Fig. 2B shows an ESI(−)-MS spectrum representative of M beers. Similarly to what is revealed by ESI(+)-MS (sodium adduct of *m/z* 219 in Fig. 1B), deprotonated glucose of *m/z* 179 is by far the major anion detected (base peak) followed by the pair of isotopologue chloride adducts of glucose of *m/z* 215 and 217. As in the positive ion mode, caramel malt addition is the most likely source of glucose molecules. Other diagnostic anions for M beers are those of *m/z* 89 and 359.

Fig. 2C shows an ESI(−)-MS spectrum representative of the D beers. Many of the major anions seen for the P beers are also present in the fingerprint spectra of the D beers, but the two anions of *m/z* 161 and 179 are clearly distinguishing. As for the P beers, the anion of *m/z* 161 is likely the deprotonated molecule of anhydroxexose. As for the M beers, the ion of *m/z* 179 is the deprotonated molecule of glucose again indicating some degree of artificial sweetening by caramel malt addition. Another rather characteristic anion for D beers is that of *m/z* 255.

Chemometric analysis

PCA data treatment was also performed for the ESI-MS fingerprint mass spectra, to test its performance for statistical beer classification and quality control.

Fig. 3A shows a scatter plot of PC1 *versus* PC2 from the data matrix obtained from ESI(+)-MS data. The three types of beers are very clearly grouped. P beers are placed on the upper right side whereas the D (upper) and M beers (bottom) are grouped on the left side. Similarly for the ESI(−)-MS data (Fig. 3B), PCA analysis also clearly places the three types of beers in very well-defined groups. In the PC1 *versus* PC2 plot for the ESI(−)-MS data, the P beers are again on the upper right side whereas the D beers (upper) and M beers (bottom) are on the left side.

Conclusion

In conclusion, ESI-MS fingerprinting in both the negative and positive ion modes detects a series of diagnostic beer cations and anions providing a fast (few seconds), simple (little sample manipulation), robust and selective method for high throughput classification and quality control of beers. Samples are in that way clearly divided into three fingerprinting groups both by simple visual inspection or most particularly when PCA chemometric data treatment is used. ESI-MS/MS on these diagnostic ions, most of which are characterized as arising from ionization of simple sugars, unfermentable oligosaccharides and iso-alpha-acids, produce characteristic tandem mass spectra adding a second and optional MS dimension for improved selectivity for beer fingerprint characterization. Artificial sweetening is clearly detected by characteristic cations or anions arising from ESI of glucose. Distinct $[K^+]/[Na^+]$ concentration ratios are also revealed by ESI(+)-MS. For beer typification, the ESI-MS performance is comparable to the (more time-demanding) chemometric analysis of H-1 NMR spectra, performed in the region where major beer components resonate (3.0–6.0 ppm), which has been shown to separate beer samples into four groups: two groups characterized by the predominance of dextrins, one group of alcohol-free beers characterized by the predominance of maltose, and one group where glucose was found to predominate.[19] Direct ESI-MS or ESI-MS/MS analysis is an attractive approach since it is likely to reveal other unusual components, impurities or additives as well as fermentation defects or degradation products of beer, as long as these components are polar enough to compete favorably during ESI, avoiding ion supression, which makes the technique also promising for beer fermentation and degradation control. These abilities are being confirmed in your laboratory as the use of direct infusion ESI-MS(/MS) analysis of beer is further explored.

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References

1 W. A. Hardwick, in *Handbook of Brewing*, Marcel Dekker, New York, 1995.
2 H. D. Berlitz, W. Grosch and P. Schieberle, *Food Chemistry*, 3rd. edn., Springer-Verlag, Germany, 2004.
3 S. Araki, T. Kimura, C. Shimizu, S. Furusho, M. Takashio and K. Shinotsuka, *J. Am. Soc. Brew. Chem.*, 1999, **57**, 34–37.
4 G. Lermusieau, S. Noël, C. Liégeois and S. Collin, *J. Am. Soc. Brew. Chem.*, 1999, **57**, 29–33.
5 D. Madigan, A. Perez and M. Clements, *J. Am. Soc. Brew. Chem.*, 1998, **56**, 146–151.
6 C. Shimizu, Y. Nakamura, K. Miyai, S. Araki, M. Takashio and K. Shinotsuka, *J. Am. Soc. Brew. Chem.*, 2001, **59**, 51–58.
7 A. Alcázar, F. Pablos, M. J. Martín and A. G. González, *Talanta*, 2002, **57**, 45–52.
8 (*a*) A. J. P. Hofte, R. A. M. Van der Hoeven, S. Y. Fung, R. Verpoorte, U. R. Tjaden and J. Van der Greef, *J. Am. Soc. Brew. Chem.*, 1998, **56**, 118–122; (*b*) P. Degelmann, M. Becker, M. Herderich and H. U. Humpf, *Chromatographia*, 1999, **49**, 543–546; (*c*) N. Whittle, H. Eldridge, J. Bartley and G. Organ, *J. Inst. Brew.*, 1999, **105**, 89–99.
9 C. W. Klampfl, M. Himmelsbach, W. Buchberger and H. Klein, *Anal. Chim. Acta*, 2002, **454**, 185–191.
10 P. Mauri, M. Minoggio, P. Simonetti, C. Gardana and P. Pietta, *Rapid Commun. Mass Spectrom.*, 2002, **16**, 743–748.
11 R. B. Cole, *Electrospray Ionization Mass Spectroscopy*, John Wiley & Sons Inc., New York, 1997.
12 P. Mauri and P. Pietta, *J. Pharm. Biomed. Anal.*, 2000, **23**, 61–68.
13 (*a*) A. C. H. F. Sawaya, D. M. Tomazela, I. B. S. Cunha, V. S. Bankova, M. C. Marcucci, A. R. Custodio and M. N. Eberlin, *Analyst*, 2004, **129**, 739–744; (*b*) M. N. Eberlin, I. B. S. Cunha, A. C. H. F. Sawaya, M. L. T. Rodrigues, E. C. Meurer, V. S. Bankova and M. C. Marcucci, *submitted.*
14 (*a*) H. J. Cooper and A. G. Marshall, *J. Agric. Food Chem.*, 2001, **49**, 5710–5718; (*b*) A. C. H. F. Sawaya, R. R. Catharino and M. N. Eberlin, *Analyst*, 2005, in press.
15 J. K. S. Møller, R. R. Catharino and M. N. Eberlin, *Analyst*, 2005, **130**, DOI: 10.1039/b415422c.
16 C. Zervos and R. H. Albert, Chemometrics: The use of multivariate methods for the determination and characterization of off-flavors, in *Off-Flavors in Foods and Beverages,* ed. G. Charalambous, Elsevier, 1992.
17 (*a*) C. G. Zampronio, S. P. Gurden, L. A. B. Moraes, M. N. Eberlin, A. K. Smilde and R. J. Poppi, *Analyst*, 2002, **127**, 1054–1060; (*b*) C. G. Zampronio, L. A. B. Moraes, M. N. Eberlin and R. J. Poppi, *Talanta*, 2003, **60**, 37–44; (*c*) C. G. Zampronio, L. A. B. Moraes, M. N. Eberlin and R. J. Poppi, *Anal. Chim. Acta*, 2001, **446**, 495–502.
18 G. Vanhoenacker, D. De Keukeleire and P. Sandra, *J. Chromatogr., A*, 2004, **53**, 1035.
19 I. F. Duarte, A. Barros, C. Almeida, M. Spraul and A. M. Gil, *J. Agric. Food Chem.*, 2004, **52**, 1031.