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SPECIALITE CHIMIE ANALYTIQUE

ÉPREUVE N° 3

Traduction d'un document technique rédigé en langue anglaise ;réponses **en langue anglaise** à des questions portant sur les documents..

(durée 2 h - coefficient 2)

Cette épreuve comporte 5 pages.

1 - Traduire les deux premiers paragraphes de l'introduction figurant dans l'encadré.

2 - Questions

Give a concise summary of the main idea of the text – no more than a hundred words.

Give one of the advantages of electrospray ionization mass spectrometry.

What are the characteristics of an ESI(+) mass spectrum representative of malt “Malzbier” beer?

Electrospray ionization mass spectrometry fingerprinting of beer

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Introduction

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. 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 plant extracts, propolis, wine and whisky. 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) can serve to order known samples and have been applied to MS data to classify unknown samples.

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 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 CO₂, 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.

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 very typical of P beers, with intense “beer cations” in the m/z range of 70 to 705 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 maltotetraose (m/z 689 and 705), as indicated by their tandem MS spectra and comparison with reported data. Therefore, as these oligosaccharides are not totally consumed during fermentation, 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 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. 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 characteristic “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, maltotetraose and maltopentose, respectively, as also indicated by ESI-MS/MS and comparison with reported data. 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 maltotetraose, as previously suggested. 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 dextrans and less sugar, whereas higher temperatures produce less dextrans and more sugars.

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. 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 anhydrohexose. 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.

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		

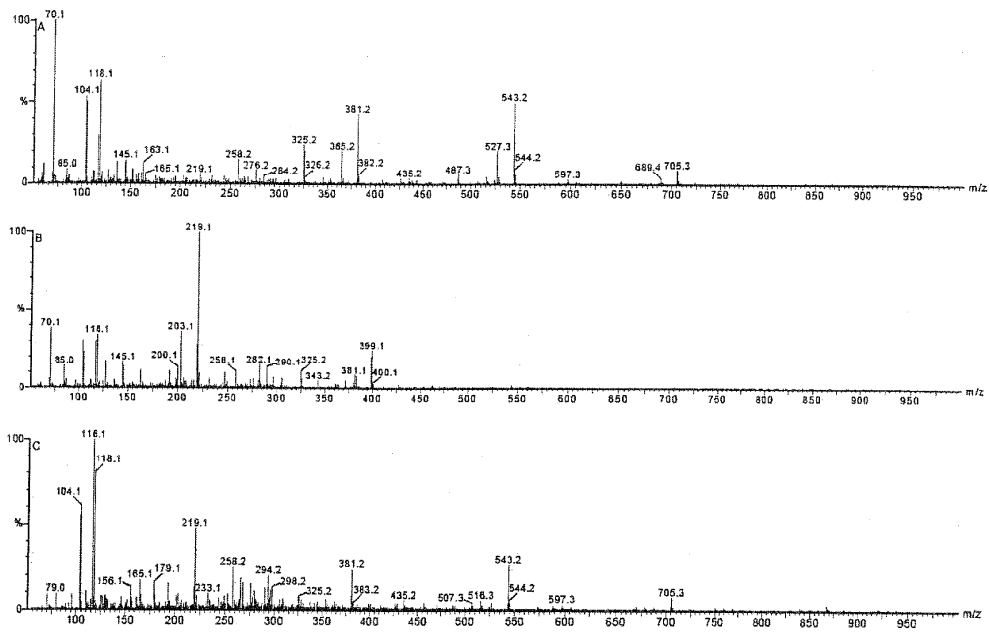


Fig. 1 ESI(+) fingerprint spectra representative of (A) pale (light) colored (P), (B) malt "Malzbier" (M), and (C) dark colored beers (D).

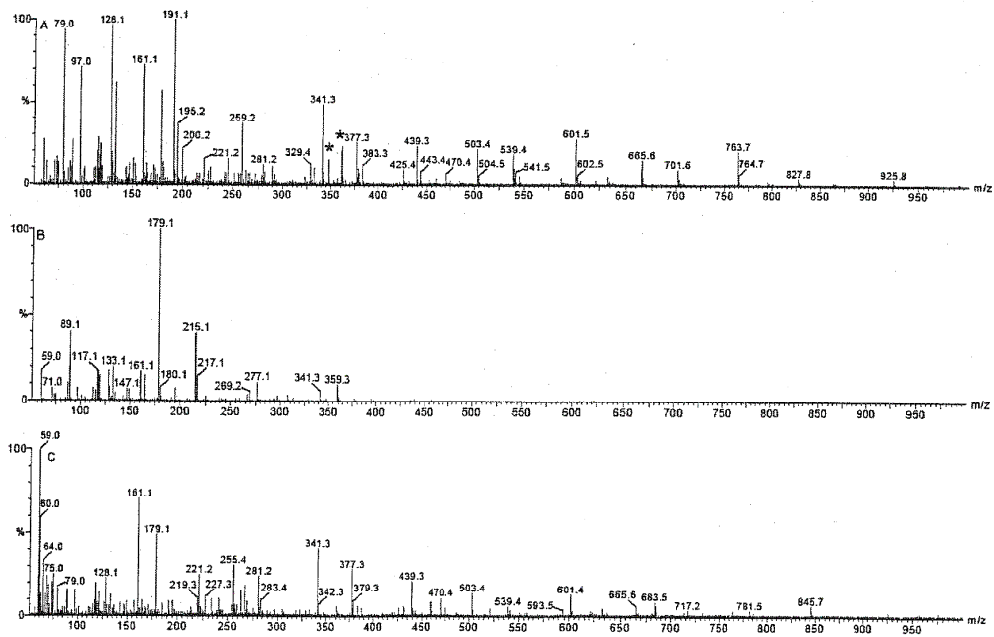


Fig. 2 ESI(-) fingerprint spectra representative of (A) pale colored (P), (B) malt "Malzbier" (M), and (C) dark colored beers (D). The ions marked with asterisks are those of m/z 347 and 361 which correspond to the deprotonated molecules of major iso-alpha-acids found in beer.

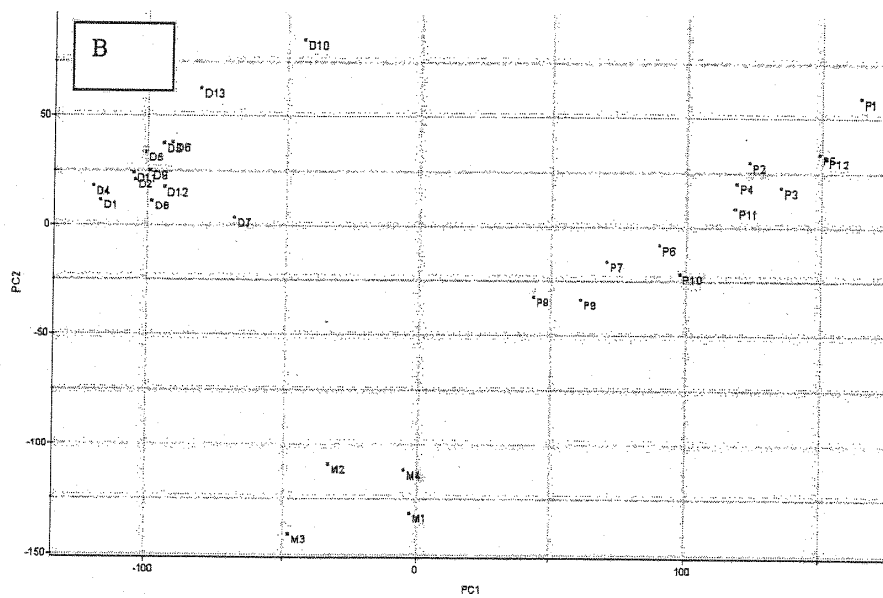
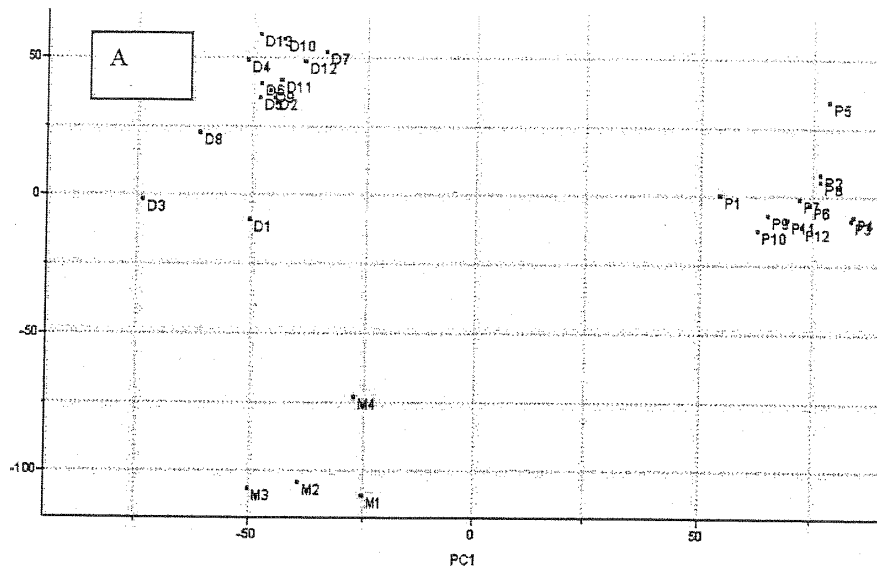


Fig. 3 (A) Scores of PC1 (42% variance) *versus* PC2 (27%) for the ESI(+)-MS data and (B) scores of PC1 (57%) *versus* PC2 (17%) for the ESI(-)-MS data of the 29 samples of beer investigated (see Table 1).