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Rapid analysis of selected beer volatiles by atmospheric pressure chemical ionisation-mass spectrometry

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ABSTRACT

For rapid determination of some key aroma compounds in fermented beer, headspace was analysed by atmospheric pressure chemical ionisation-mass spectrometry (APCI-MS) and the ion intensities used to predict beer aroma content. To achieve this aim, potential interference from ethanol during ionisation was overcome by using ethanol as the charge transfer reagent ion and the cone voltage was varied to induce fragmentation of the compounds and provide more information to build a robust correlation between headspace analysis and concentration in the beer itself. Ten solutions containing different ethanol concentrations (0.5–5 mL/100 mL), and different concentrations of the key aroma compounds, were analysed by APCI-MS at cone voltages between 12 and 21 V. Linear regression models were created for each compound to correlate the ion intensities measured by APCI-MS at the different cone voltages with the in-solution concentration of the compounds (average $R^2 = 0.95$). Of the 14 compounds studied, six could be quantified unequivocally, six compounds were quantified as pairs of isobaric compounds and two could not be reliably analysed. To test the linear regression model equations, the headspace above samples of fermenting wort or commercial beer and cider products were analysed by APCI-MS and the predicted concentrations of twelve aroma compounds in the samples were compared with the actual values measured by extraction and GC–MS analysis; correlation coefficients ranged between 0.753 and 0.979.

1. Introduction

Direct mass spectrometry techniques like atmospheric pressure chemical ionisation-mass spectrometry (APCI-MS) and proton transfer reaction mass spectrometry (PTR-MS) have been developed to monitor the concentrations of known volatile compounds in air [1,2]. Applications range from aroma release in foods to monitoring volatile pollutants in the atmosphere. However the techniques have limitations when a mixture of compounds is analysed due to common ions that can originate either from isobaric compounds, or from fragments produced from other compounds [3–6] and, in these situations, reliable quantification cannot always be achieved. Previous work has investigated the use of accurate mass analysers [7] or the use of GC using dual EI and APCI/PTR detectors to respectively identify compounds and then assign ions to those compounds [8,9], so that reliable quantification can be achieved. However, these procedures are time consuming and this paper describes an alternative APCI-MS method that could be used as a high throughput analysis to quantify individual compounds in mixtures. The methodology was developed for analysis of alcohols and esters that are found in beer during fermentation.

During fermentation, yeasts form alcohols and esters as major aroma active metabolites which contribute to the characteristic beer flavour. Of all the secondary metabolites formed by yeasts, the higher alcohols are produced in the highest concentrations in fermentation, where propanol, isobutyl alcohol, isoamyl alcohol, and 2-phenylethyl alcohol are the predominant aroma compounds [10,11]. In comparison, although esters are produced in much lower concentrations than the alcohols, they have lower odour thresholds and therefore have a greater impact on the aroma and flavour profile. The major esters formed by yeasts are ethyl acetate, isoamyl acetate, ethyl hexanoate, ethyl octanoate and 2-phenylethyl acetate [10–12]. If these compounds can be measured rapidly during beer fermentation, the effects of substrate, yeast and environmental conditions on flavour formation can be studied.

Conventional analyses of beer, using extraction and gas chromatography–mass spectrometry (GC–MS), provide high quality, qualitative and quantitative data, but are time consuming. In contrast, a direct mass spectrometry process can be used for quantitative analysis of known volatiles using APCI coupled with mass spectrometry [2,13–15]. The soft ionisation of APCI leads to the for-

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mation of predominantly molecular ions with little fragmentation and, as it involves direct introduction of samples into the source with no prior separation, detection and analysis are instantaneous allowing for rapid sample profiling. To apply such a technique to fermentation samples that contain significant amounts of ethanol, compared to the levels of volatile compounds present (e.g., up to 50 g/L of ethanol compared to 0.1–100 mg/L of higher alcohols and esters), modification of the proton transfer reaction is necessary to ensure consistent and quantitative ionisation of all compounds. This can be achieved by maintaining a constant ethanol concentration in the source via the make-up gas so that ionisation proceeds using the protonated ethanol dimer as the predominant charge transfer ion [16,17].

A major drawback when using direct MS techniques to analyse complex mixtures, such as fermentation samples, is the formation of common ions formed from different analytes, thus making it difficult to assign ions to compounds for quantification [18]. Such overlapping ions can sometimes be differentiated by inducing limited fragmentation of the compound by altering the potential difference between the sampling cone and the skimmer in the intermediate pressure region (the cone voltage) as the ions pass from the source into the mass analyser [19–22]. The concept in this paper was to use cone voltage fragmentation to provide more spectral information on the key aroma compounds in beer headspace and then use regression procedures to relate the ion intensities measured in the headspace with the concentrations measured in the liquid phase in solution. The goal was to establish a simple and fast headspace analysis to determine the concentration of esters and alcohols in samples during and post-fermentation.

2. Materials and methods

2.1. Reagents

2.1.1. Chemical reagents

2-Phenylethyl acetate and 3-heptanone were supplied by Acros Organics (Loughborough, UK). 1-Propanol, 2-methyl-1-propanol (isobutyl alcohol), 1-butanol, 3-methyl-1-butanol (isoamyl alcohol), ethyl acetate, ethyl propionate, 2-methylpropyl acetate (isobutyl acetate), 2-methylbutyl acetate (amyl acetate), ethyl hexanoate, 2-phenylethyl alcohol and dichloromethane were obtained from Sigma Aldrich (Poole, UK). 2-Methyl-1-butanol (amyl alcohol), ethyl butyrate and ethyl octanoate were sourced from Fluka (Poole, UK). Ethanol (analytical reagent grade, 99.99%) was purchased from Fisher Scientific (Loughborough, UK). All volatile compounds were of analytical grade with 98% purity or greater.

2.1.2. Training set samples

Ten model solutions were prepared containing ethanol (0.1–5 mL/L) along with the 14 alcohols and esters in different amounts (Table 1). The compounds were present within the concentration range typically found during fermentation and in finished beer samples. These solutions were used as a training set for developing regression models that related the APCI-MS measured headspace ion intensities to the solution concentrations of the compounds. These solutions were also used for calibration of the GC–MS analysis. A standard solution (Sample C in Table 1) was also prepared and was used for the standardisation of APCI-MS data.

2.1.3. Test set samples

A commercial wort sample (supplied by SAB Miller) was fermented at 15 °C under continuous stirring and seven samples were collected at 4 h, 16 h, 24 h, 40 h, 72 h, 96 h and 137 h after the initial inoculation with a brewing yeast. Samples were also taken from seven commercially available beers and ciders, purchased from a local retail shop. Aroma content of all fourteen samples was analysed by extraction and GC–MS, as well as by headspace APCI-MS, and used as test samples to evaluate the model generated using the training set of model solutions.

2.2. Modification to the APCI-MS source

A MS Nose interface (Micromass, Manchester, UK) fitted to a Platform LCZ mass spectrometer (Micromass) was operated as described previously [15] but with ethanol introduction into the APCI-MS source [16]. In this set up, a proportion of nitrogen make-up gas (70 mL/min) was bubbled via a sinter into a 2 mL/100 mL ethanol solution (400 mL) placed inside a 500 mL flask (Schott bottle; Fisher Scientific). This flow (70 mL/min) was combined with the bulk nitrogen flow (total flow of 10 L/min) before it entered the APCI-MS source. The ethanol solution was renewed every 4 h to avoid significant depletion of ethanol from the solution. This set up ensured that ethanol was present in the source at 11.3 μL/L of the nitrogen make-up gas and the protonated ethanol dimer was the dominant reagent ion in the APCI-MS source [16].

2.3. Headspace analysis by APCI-MS

Aliquots of the training or test set samples (50 mL) were placed in 100 mL flasks fitted with a one port lid. After a 2 h equilibration period at room temperature (22 °C), the headspace was drawn into the APCI-MS source at a rate of 5 mL/min. The samples were analysed in selected ion mode, monitoring 24 ions: m/z 43, 45, 55, 57, 59, 61, 69, 71, 73, 75, 87, 89, 92, 99, 103, 105, 115, 117, 129, 131, 145, 160, 165, and 173. The intensity of these ions was measured at 4 cone voltages: 12, 15, 18 and 21 V, with a dwell time of 0.05 s.

2.3.1. GC–MS analysis of solutions and samples

Aliquots of the training or the test set samples (10 mL) were placed in glass vials (25 mL) and equilibrated at room temperature (22 °C) for 2 h. Using a gas tight syringe, 250 μL of the headspace was injected into the injector port (150 °C) of a Trace GC ULTRA, (Thermo Scientific, Massachusetts, USA) in splitless mode using a Combi PAL autosampler (CTC Analytics, Zwingen, Switzerland). The column was a ZB Wax, 30 m × 0.25 mm i.d. × 1 μm film thickness (Phenomenex, Macclesfield, UK). The temperature programme for the oven was: 40 °C for 5 min, 10 °C/min to 100 °C and then 100 °C for 2.5 min (column head pressure 18 psi, helium carrier gas). Single ion chromatograms were recorded using a DSQ mass spectrometer (Thermo Scientific) operating in selected ion mode, monitoring ions m/z 15, 29, 31, 41, 42, 43, 45, 46, 56, and 59.

2.3.2. GC–MS analysis by solvent extraction

Aliquots (10 mL) of the training or test set samples were added to 1 mL of dichloromethane containing 25 μg of 3-heptanone (used as internal standard) in a screw topped tube and mixed on a roller bed (Stuart Scientific, Stone, UK) for 1 h at room temperature (22 °C). After leaving the mixture to stand for 1 min, the lower dichloromethane layer was collected in 1 mL glass vials and stored at −20 °C for analysis by GC–MS. Aliquots (1 μL) of the dichloromethane extracts were injected in splitless mode using an AS 3000 autosampler (Thermo Scientific) into the injector port (240 °C) of a Trace GC ULTRA (Thermo Scientific) fitted with a ZB-5 column, 30 m × 0.25 mm i.d. × 1 μm film thickness (Phenomenex). The initial oven temperature was maintained at 40 °C for 1 min after injection and then ramped at 8 °C/min to 250 °C (column head pressure 18 psi, helium carrier gas). The compounds were detected using a DSQ mass spectrometer (Thermo Scientific) operating in full scan mode, from 30 to 250 m/z at 2 scans/s.

Table 1
Concentration of ethanol (mL/100 mL) and aroma volatiles (mg/L) in the solutions used for training set and model development. Sample C was a standard solution run in parallel with the training set mixtures and used to normalise the data.

Compounds	Solutions										
	1	2	3	4	5	6	7	8	9	10	C
Ethanol	1	5	1	2	0.5	0.1	0.5	0.1	2	5	–
Ethyl acetate	2	0.5	5	40	25	50	25	50	2	10	0.1
Ethyl propionate	0.1	0.5	0.1	0.25	0.4	0.5	0.25	0.05	0.075	0.05	0.1
Ethyl butyrate	0.2	0.15	0.25	0.1	0.2	0.15	0.05	0.05	0.25	0.1	0.1
Isobutyl acetate	0.25	0.05	0.5	0.25	0.5	0.1	0.075	0.05	0.4	0.1	0.1
Amyl acetate	0.5	2.5	1	4	5	0.2	5	0.05	2.5	0.2	0.1
Ethyl hexanoate	0.4	0.05	0.05	0.25	0.25	0.075	0.1	0.5	0.5	0.1	0.1
2-Phenylethyl acetate	2	0.5	1.5	0.2	1	0.05	0.1	0.5	1.5	0.05	0.1
Ethyl octanoate	0.075	0.05	0.25	0.5	0.05	0.1	0.5	0.4	0.1	0.25	0.1
1-Propanol	20	15	5	5	10	20	7.5	15	10	1	10
1-Butanol	10	4	5	5	2	1	2	0.5	7.5	10	10
Isobutyl alcohol	15	5	20	10	10	7.5	1	20	5	15	10
Amyl alcohol	10	1	25	50	40	5	0.5	1	10	40	10
Isoamyl alcohol	100	1	50	75	20	5	75	50	10	10	10
2-Phenylethyl alcohol	10	40	1	10	25	40	0.5	1	5	50	10

2.3.3. Calibration curves of volatile compounds in GC–MS analysis

Using the training set of solutions, which contained known amounts of the volatile compounds, the relationship between the amount of volatile in solution and the GC–MS response was established as a series of calibration curves. The peak areas from GC–EI-MS were corrected by reference to the 3-heptanone internal standard (IS).

2.4. Computational analysis programmes

Models were generated using Design-Expert v.7.1.6 (Stat-Ease Inc., Minneapolis, USA), where ion intensity of key ions at different cone voltages from APCI-MS analysis were correlated with the analyte concentration of the training set samples by backward multiple regression. The "Prob > F" value was less than 0.01 for all the models obtained.

The co-correlation of the concentrations of volatile compounds in the training set was evaluated by principal component analysis (PCA) using Unscrambler v.9.0 (Camo Process AS., Norway). The air/water partition coefficients of phenyl ethyl alcohol and phenyl ethyl acetate were estimated from the KowWIN program v.1.67 (EPI Suite, Environmental Protection Agency, USA)

3. Results and discussion

3.1. Training set solutions

To construct models that could evaluate the solution concentration of the volatiles in a mixture, a training set was designed that consisted of ten solutions containing varying concentrations of fourteen volatile aroma compounds (Table 1). The training set had several features:

(a) It consisted of the main esters and alcohols that are typically formed during fermentation of wort by yeast.
(b) The composition range of the volatile compounds in the training set represented the range that is present during fermentation and in post-fermentation beer samples.
(c) Any effect of ethanol on the partitioning efficiency of the volatile compounds into the headspace or the ionisation in the APCI-MS source was evaluated by including ethanol as an additional factor in the training set.
(d) The composition of the ten solutions was specifically designed so that the concentrations of the compounds had minimum co-correlation with each other (PCA analysis of co-correlation gave principal components of <25%). This differs from other approaches which may use samples to compare two techniques, where the concentrations of the volatiles often co-correlate.

A standard solution (Sample C in Table 1) containing known concentrations of volatile compounds was also measured during APCI-MS analysis of the training set samples. In this solution, the alcohols were present at 100-fold excess relative to the esters, reflecting the differences in their partition coefficients. The result was that the signal intensity from all the compounds on APCI-MS was roughly equal. The intensity of each ion from the training set sample was expressed relative to the ion intensity of the standard solution, thus allowing for standardisation of the data that was carried out on different days and to account for any day to day variations. Models developed using signal intensities relative to this standard solution could potentially be used in further experiments to predict volatile concentrations in samples containing unknown amounts of the volatiles.

3.2. Volatile analyses and calibration curves

APCI-MS analysis of the headspace above training set samples successfully detected all 14 volatiles at ethanol levels of 0.1 to 5 mL/100 mL. Headspace above the training set solutions (and test set samples) was sampled using a low flow rate (5 mL/min) so that the ethanol from the sample had a minimal effect on the ethanol in the APCI source, provided by the make-up gas. At a sample flow rate of 5 mL/min, the maximum increase in in-source ethanol, from a sample containing 5 mL/100 mL ethanol, would be less than 20% relative to the ethanol content of the make-up gas. Consistent results were obtained from training set solutions with ethanol contents from 0.1 to 5 mL/100 mL as reported previously [16].

In comparison to the APCI-MS, GC analysis using headspace samples from the training set solutions could only detect seven compounds (1-propanol, isobutyl alcohol, 1-butanol, ethyl acetate, isobutyl acetate, 2-methyl 1-butanol and 3-methyl 1-butanol) due to the low concentrations of some compounds in the headspace caused by unfavourable partition coefficients. Solvent extraction followed by GC–MS led to some peaks eluting under, or too close, to the solvent peak but the remaining seven compounds could be reliably quantified. Thus to determine the amounts of the 14 volatiles in the liquid phase, a combination of the headspace and solvent extraction GC data were used.

The training set data was used to correlate the signals from GC–MS (headspace and solvent extraction methods) with the actual liquid phase concentrations to form a series of calibration curves.

Table 2
The ion distribution (relative % abundance) at cone voltages of 12 V and 21 V, with ethanol in ionisation source (11.3 μL/L of nitrogen make-up gas). The limits of detection (LOD) of the main ion used in model development are shown at a s/n ratio of 3:1 from calibration curves of individual compounds. The LOD is in mg/L in solution.

Compound	MW	Ion (*m*/*z*)	Rel. % abundance		LOD (mg/L)	
			12 V	21 V	12 V	21 V
Ethyl acetate	88	61	9.3	100.0	–	–
		89	100.0	44.4	0.008	0.006
Ethyl propionate	102	75	0.7	100.0	–	–
		103	100.0	50.7	0.004	0.004
Ethyl butyrate	116	89	8.2	100.0	–	–
		117	100.0	53.7	0.002	0.002
Isobutyl acetate	116	57	77.7	100.0	0.002	0.005
		61	10.7	40.7	–	–
		117	100.0	6.1	–	–
Amyl acetate	130	61	24.8	54.7	–	–
		71	100.0	100.0	0.003	0.005
		131	69.6	2.4	–	–
Ethyl hexanoate	144	73	26.3	26.3	–	–
		117	10.4	100.0	–	–
		145	100.0	73.0	0.002	0.002
2-Phenylethyl acetate	164	103	9.6	0.9	–	–
		105	100	100.0	0.177	0.047
Ethyl octanoate	172	117	5.1	3.6	–	–
		145	4.2	100.0	–	–
		173	100.0	0.0	0.002	–
1-Propanol	60	43	21.5	100.0	2.080	1.830
		61	100	12.4	–	–
		65	26.5	4.1	–	–
1-Butanol	74	57	100.0	100.0	0.130	0.280
		73	5.8	9.0	–	–
		121	64.2	2.8	–	–
Isobutyl alcohol	74	57	100.0	100.0	0.130	0.320
		73	67.4	44.7	–	–
		121	67.0	0.5	–	–
Amyl alcohol	88	71	100.0	100.0	0.120	0.190
		135	42.1	2.4	–	–
Isoamyl alcohol	88	71	100.0	100.0	0.140	0.180
		135	51.5	3.1	–	–
2-Phenylethyl alcohol	122	65	100.0	13.3	–	–
		105	31.9	100.0	8.330	1.560

This approach compensated for the differences in partition coefficients and extraction efficiencies for each of the techniques, respectively. The data obtained showed that all the volatile compounds in the training set were detected by the GC–MS techniques consistently showing a linear relationship ($R^2 > 0.95$) with their liquid phase concentrations (data not shown). Because of the composition of the training set samples, ten point calibration curves were obtained for each compound.

3.3. Effect of cone voltage on APCI spectra

Individual ion profiles of eight esters and six alcohols in APCI-MS were obtained at four different cone voltages (12–21 V at 3 V increments). Typically, at low cone voltages, the protonated molecular ion was the major ion (Table 2). As the cone voltage increased, the compounds tended to fragment, forming smaller *m*/*z* ions. The alcohols and esters showed some characteristic trends in their fragmentation pattern. The alcohols readily formed ions corresponding to their dehydrated protonated molecular ion $[M\text{-}H_2O+H]^+$. Propanol (MW 60), butanols (1-butanol and isobutyl alcohol; MW 74), methyl butanols (amyl alcohol and isoamyl alcohol; MW 88) and 2-phenylethyl alcohol (MW 122) generated ions of 43, 57, 71 and 105 *m*/*z*, respectively (Table 2). The esters fragmented at their ester linkage, producing ions corresponding to their "alcohol" and "acid" fragments. It was further observed that the acetate esters fragmented more easily than esters of other alkyl groups. This can be seen in the ion profiles of ethyl butyrate and isobutyl acetate (Table 2). At 12 V, ethyl butyrate showed little fragmentation, predominantly producing ion *m*/*z* 117 corresponding to its protonated molecular ion, while isobutyl acetate generated two ions of *m*/*z* 89 and *m*/*z* 117 of similar proportions. Such differences may help in building models using the APCI-MS ion profile to differentiate between isomer pairs, which have the same molecular mass.

The ionisation in APCI-MS is a low energy process, which may lead to different degrees of fragmentation due to small changes in the potential difference between the sampling cone and the skimmer region in the MS source. Thus it is necessary to standardise the cone voltage settings that will produce defined fragmentation conditions. To attain such standardisation, the ion intensity ratios in Table 2, which show the impact of cone voltage on fragmentation, may be used to set the voltage settings in other APCI-MS instruments and apply the models generated in this work to other instruments.

3.4. APCI-MS limits of detection (LOD)

LOD values of the volatile compounds analysed by APCI-MS were determined at a signal to noise ratio of 3:1 from the calibration curves of individual compounds and were expressed as their solution concentrations (Table 2). The LOD of esters and alcohols using APCI-MS were found to be below 0.01 mg/L and 0.15 mg/L respectively, which is a reflection of the higher air/water partition coefficients of esters compared to alcohols. These LOD values compare well with the concentrations of most esters and alcohols in beer which typically reach concentrations between 0.2–0.5 mg/L and 5–20 mg/L respectively [23,24], although some esters and alcohols are produced at a higher concentration (ethyl acetate 20–30 mg/L, amyl acetates 2–3 mg/L, amyl alcohols 5–20 mg/L) [23,24].

Table 3
Model equations and regression coefficients that correlate APCI-MS headspace signals and the solution concentration of individual compounds in the training set samples.

Compound	Model equation regression coefficient × *ion* (*cone voltage*)	R^2 [a]	Range (mg/L)[b]
Ethyl acetate	−0.26 + 1.04 × *89m/z* (*12*)	1.000	0.05–50.00
Ethyl propionate	0.02 + 0.12 × *103m/z* (*12*) −0.01 × *173m/z* (*18*) −0.01 × *165m/z* (*12*)	0.993	0.05–0.50
Ethyl butyrate + isobutyl acetate	−0.16 + 0.25 × *117m/z* (*12*)	0.881	0.10–0.75
Amyl acetate	−0.06 + 0.13 × *131m/z* (*12*)	0.987	0.05–2.50
Ethyl hexanoate	0.03 + 0.10 × *145m/z* (*12*) −0.01 × *173m/z* (*12*) −0.01 × *117m/z* (*12*)	0.995	0.05–0.50
2-Phenylethyl acetate	NM		
Ethyl octanoate	−0.04 + 0.37 × *145m/z* (*21*) −0.12 × *145m/z* (*12*)	0.935	0.05–0.50
1-Propanol	0.49 + 12.17 × *43m/z* (*12*) −0.35 × *61m/z* (*21*)	0.925	1–20
1-Butanol + isobutyl alcohol	2.26 + 21.68 × *57m/z* (*21*) −4.15 × *117m/z* (*12*)	0.817	3–25
Amyl alcohol + isoamyl alcohols	2.85 + 24.09 × *71m/z* (*21*) −3.92 × *131m/z* (*12*)	0.995	2–125
2-Phenylethyl alcohol	43.74 + 89.85 × *105m/z* (*21*) −6.12 × *117m/z* (*12*) −94.95 × *105m/z* (*18*) +5.52 × *173m/z* (*18*)	0.942	0.05–2.00

NM: no model could be generated.
[a] Correlation coefficient for the values predicted from the model and the actual concentrations.
[b] Range of concentration of the compounds in the training set.

2-Phenylethyl acetate and 2-phenylethyl alcohol showed a comparatively higher LOD of 0.047 and 1.56 mg/L respectively (cone voltage 21 V); this was thought to be due to their lower air/water partition coefficient compared to other compounds analysed. However, they could still be detected using the APCI-MS technique in the fermentation and post-fermentation samples where 2-phenylethyl acetate and 2-phenylethyl alcohol reached a concentration of 0.5 mg/L and 30 mg/L, respectively. Hence at a sampling rate of 5 mL/min, used in this study, all the esters and alcohols in the fermentation and post-fermentation samples have the potential to be analysed directly by the APCI-MS technique without the need for sample work-up.

3.5. Regression models

A backward multiple linear regression was used to generate equations to relate the concentration of each volatile compound present in the ten training solutions to the relative ion intensities observed during analysis by APCI-MS. This allowed the development of models whose interpretation and application are simple, and also allowed the selection of important variables from a large data set (intensity of 24 ions at four cone voltages) available for model development. A total of 10 model equations were produced using multiple linear regression, which predicted the concentration of the volatile compounds in the aqueous solution (Table 3). The typical models for the esters determined the concentration by using the relative ion intensity of the molecular ion $[M+H^+]$ at low cone voltage and subtracting the contribution of other compounds which may fragment to form ions with a similar m/z value. The models for the alcohols used the predominant dehydrated protonated molecular ion $[M\text{-}H_2O + H^+]$ to predict their in-solution concentration.

The regression coefficients for most models were good with an average R^2 for all ten models of 0.947. Seven compounds were modelled on their own to predict their solution concentrations (Table 3). Six compounds had to be modelled in pair-wise combinations as they had similar molecular weights and produced common ions (Table 3). One compound, 2-phenylethyl acetate, could not be modelled adequately. Most models were based on the signal intensity of two fragment ions, typically at minimum or maximum cone voltages representing minimum or maximum fragmentation with respect to the experimental range. All the models exhibited linear "$y = x$" relationships between the amount predicted and the actual amount in the solution with slopes of 1.00 and R^2 values of 0.995.

The different fragmentation patterns of the isomeric compounds (Table 2) were insufficient to generate individual models for each of the compounds. Combined models were thus produced by summation of their solution concentration, which was then correlated with the ion intensities to develop their regression models. Assumptions for combined models were that the compounds had similar partition coefficients in aqueous ethanolic solution and had similar ionisation and fragmentation efficiencies in the APCI-MS source. For instance, amyl alcohol and isoamyl alcohol were modelled in combination because they are positional isomers with similar air/water partition coefficients and similar fragmentation patterns. The model that predicted the combined in-solution concentrations of these two compounds gave a very good correlation ($R^2 = 0.995$). Combined models were also produced for the isomer pairs, isobutyl acetate and ethyl butyrate as well as isobutyl alcohol and 1-butanol. However, due to interferences from other ion fragments or due to differences in structure, fragmentation patterns or partition coefficients, lower correlation coefficients for the models were obtained (0.881 and 0.817 for isobutyl acetate/ethyl butyrate and isobutyl alcohol/1-butanol models, respectively). The lack of a good model for the phenyl ethyl ester was probably due to interference with its predominant ion at m/z 105, which was also a major feature of the 2-phenylethyl alcohol model. In this case, a simple combined model to predict the concentration of both 2-phenylethyl alcohol and 2-phenylethyl acetate could not be formed because of their different partition coefficients (K_{aw} 1.18×10^{-5} and 7.68×10^{-4}

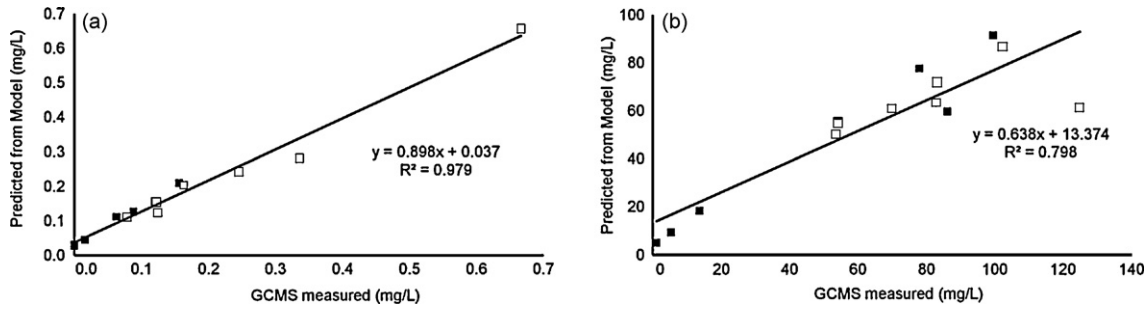

Fig. 1. Correlation between concentration predicted by the model equations (Table 3) and the sample concentration found by GC–MS in fermented wort and commercial beer and cider samples. Ethyl hexanoate (a); combined model: amyl alcohol + isoamyl alcohol (b). Fermentation samples ■; beer samples □.

for 2-phenylethyl alcohol and 2-phenylethyl acetate respectively), thus the sum of their aqueous concentration would not be directly proportional to the sum of their headspace concentration.

The approach described above, overcame some of the problems of common ions but with the ethyl esters, another issue was identified. Fragment ions were formed due to loss of C_2H_4 and these ions then overlapped with the molecular ion of compounds in the homologous series. For example ethyl octanoate (MW 172) lost C_2H_4 and produced ion m/z 145 that overlapped with the molecular ion of ethyl hexanoate (MW 144) (Table 2). In this case, the regression equation (Table 3) estimated the concentration of ethyl hexanoate using the intensity of m/z 145 at low cone voltage (12 V), where very little fragmentation of ethyl octanoate occurred. In comparison, the equation for the concentration of ethyl octanoate was generated using the intensity of m/z 145 at high cone voltage (21 V), a cone voltage at which all the ethyl octanoate fragmented to form m/z 145. A similar scenario concerning ethyl esters was also seen between ethyl butyrate and ethyl acetate, where the ion intensity of m/z 89 at cone voltage 12 V could be used alone to derive the latter's concentration. A good correlation ($R^2 = 1$) was obtained, showing insignificant interference from ethyl butyrate in this case. The use of different cone voltage was also important in determining the concentration of the alcohols. Most of the alcohols form a dehydrated protonated molecular ion, which is favoured at a high cone voltage. All the models for alcohols used this type of ion except for propanol, as m/z 43 is a typical interference by other compounds at high cone voltage (21 V). The models for alcohols also take account of the overlapping ion formed by their corresponding acetate esters, where the latter is accounted for by the ester's molecular ion formed at low cone voltage (12 V). The data in Table 3 are valid for the APCI instrument tested and the general principles of ion fragmentation with cone voltage do transfer between APCI sources. However, the degree of fragmentation may differ in different APCI sources and it may be that each machine will need calibration to define the numerical values in Table 3.

Despite variations in ethanol concentration in the training set, good models were generated using the ion intensity of the compounds. This confirms that control of ethanol in the APCI source delivered consistent ionisation and that the small changes in ethanol concentration did not significantly affect the air/liquid partitioning behaviour of the aroma compounds tested.

3.6. Validation of predictive models

The ten regression models developed to measure the in-solution concentration of the volatile compounds were evaluated using 14 samples obtained from two sources. The first source comprised seven samples, obtained at different timepoints during fermentation of wort by yeast. The other source consisted of seven different commercial beers and ciders bought locally. The headspace of these samples was analysed by APCI-MS and their ion intensities used in the model equations to predict the concentration of each volatile present in the liquid phase of the sample. The actual volatile composition of the samples was measured using GC–MS (headspace injection and solvent extraction) and concentration values obtained from these techniques were compared with the predicted value obtained from the model (Fig. 1).

Seven out of ten models showed good correlation ($R^2 > 0.88$) between the predicted concentrations and the concentrations determined by GC–MS (Table 4). Models for ethyl propionate and methyl butanols (amyl alcohol + isoamyl alcohol) showed a lower correlation (R^2 0.75 and 0.80, respectively). However this lower correlation was due to a single outlier (one of the beer samples) in both cases. If the value for the outlier was excluded, the R^2 values between the model prediction and values obtained by GC–MS analysis were 0.936 and 0.946 for ethyl propionate and methyl butanols, respectively. Application of the model equation for 2-phenylethyl alcohol to the test samples, gave a poor correlation ($R^2 = 0.324$) with the amounts determined by GC–MS and this was attributed to interference from m/z 105, which was formed from both phenylethyl alcohol and phenylethyl acetate.

Given that two different MS approaches were used for comparison, a good correlation was obtained that validated the use of headspace APCI-MS techniques for rapid analysis of twelve of the fourteen compounds tested. As with any model of this type, the model is only valid for systems which contain esters and alcohols in the composition range used in the training set. Any prediction outside the tested range is invalid, but the work highlights that such an approach could be used to construct models for other systems containing volatile compounds, thus providing rapid analytical tools for the food and beverage industries.

Table 4
Correlation of predicted concentrations of the flavour compounds (model equations using APCI headspace ion signal) and the concentration obtained from GC–MS analysis of thirteen samples (wort fermented for different times as well as commercial beer and cider samples).

Compound	R^2
Ethyl acetate	0.909
Ethyl propionate	0.753
Ethyl butyrate + isobutyl acetate	0.885
Amyl acetate	0.965
Ethyl hexanoate	0.979
2-Phenylethyl acetate[a]	–
Ethyl octanoate	0.923
1-Propanol	0.934
1-Butanol + isobutyl alcohol	0.904
Amyl alcohol + isoamyl alcohol	0.798
Phenyl ethyl alcohol	0.324

[a] No model was generated.

4. Conclusion

Headspace analysis coupled to APCI-MS was used to differentiate and quantify compounds that play an important role in determining the flavour profile of fermented beers. The method could be used to follow the time course of flavour compound formation during fermentation and could also be used to differentiate beers. The high throughput capability makes this technique ideal for large scale sampling and it could potentially be developed for use in quality control and production monitoring. While the predictive models were robust for the APCI-MS system used, the transferability of the model equations to other APCI machines has not been tested. The general principles of compound fragmentation as a function of cone voltage in APCI-MS are well known but it may be that each machine has it's own "signature" and thus the model would need to be re-validated for a different APCI source.

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