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Headspace analysis of volatile organic compounds from ethanolic systems by direct APCI-MS

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Abstract

Measuring the dynamic release of aroma compounds from ethanolic solutions by direct gas phase mass spectrometry (MS) techniques is an important technique for flavor chemists but presents technical difficulties as the changing ethanol concentration in the source makes quantitative measurements impossible. The effect of adding ethanol into the source via the sweep gas (0–565 μL ethanol/L N_2), to act as the proton transfer reagent ion and thereby control ionization was studied. With increasing concentrations of ethanol in the source, the water ions were replaced by ethanol ions above 3.2 μL/L. The effect of source ethanol on the ionization of eleven aroma compounds was then measured. Some compounds showed reduced signal (10–40%), others increased signal (150–400%) when ionized via ethanol reagent ions compared to water reagent ions. Noise also increased in most cases so there was no overall increase in sensitivity. Providing the ethanol concentration in the source was >6.5 μL/L N_2 and maintained at a fixed value, ionization was consistent and quantitative. The technique was successfully applied to measure the partition of the test volatile compounds from aqueous and 12% ethanol solutions at equilibrium. Ethanolic solutions decreased the partition coefficient of most of the aroma compounds, as a function of hydrophobicity.

Keywords: APCI; API; Ethanol; Volatiles; Ionization

1. Introduction

Measuring the real time release of aroma compounds from foods by direct mass spectrometry (MS) techniques provides flavor chemists and flavorists with important information on the behavior of food systems as they are consumed. From the data obtained, links between aroma release and flavor perception can be established [1]. Direct MS of these samples is accomplished by sampling air from above the food or sampling air from the exhaled air of people as they consume the food. The air containing mixtures of aroma compounds is led directly into the ionization source where conditions are set to achieve consistent ionization of all molecular species. Following ionization, mass analysis of the ions gives a "real time" picture of the dynamics of aroma release either in model systems or in vivo [2]. Since it is the dynamics of release that are important, the MS system is used to monitor known aroma compounds rather than identify unknowns in the mixture.

In systems which contain ethanol, aroma release is changed, due partly to a change in air–liquid partition, but also due to other physicochemical effects such as micelle formation [3] and surface tension effects [4]. It would be interesting to study aroma release as a function of the physical chemistry of the system (and with time) to establish whether aroma release correlated with the perception of flavor from alcoholic beverages. However, samples containing more than 4% ethanol cause significant changes to the ionization behavior of aroma compounds in the direct MS techniques and, in

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our experience, consistent data cannot be achieved. The use of APCI-MS to monitor the effect of ethanol on aroma compound partitioning has been reported but no mention of this effect was made [5].

The direct mass spectrometry methods developed for real time aroma release are based on Atmospheric Pressure Chemical Ionization (APCI)-MS [6] or Proton Transfer Reaction (PTR)-MS [7]. Both methods rely on transfer of a proton from water reagent ions to the analyte molecule to form a protonated ion from the aroma compound.

$$(H_2O)_nH^+ + M \rightarrow [M + H]^+ + nH_2O \quad (1)$$

In APCI-MS, source operating conditions are set to provide a constant concentration of H_2O via the make up gas flow, to optimize $[M+H]^+$ formation and to minimize fragmentation [8]. For charge transfer to occur, the Proton Affinity (PA) of the analyte molecule has to be greater than the donating species and the greater the difference in Proton Affinities (ΔPA), the easier the charge transfer. With water as the donating species, any compound with a PA > 691 kJ/mol, will be ionized [9]. For the analysis of volatile aroma compounds in the air, the use of water as the proton transfer medium is effective as the major air constituents (nitrogen, oxygen) are not ionized but the aroma compounds of interest are. With sufficient charge available, quantitative ionization of most species in a mixture is achieved [8]. The ions produced by charge transfer are then detected using mass analyzers.

The techniques work adequately within certain defined limits but one constraint is that when a large excess of one particular volatile compound is present in the source, the ionization of other trace compounds is suppressed, leading to non-quantitative results. Wine is the alcoholic beverage of interest, with an ethanol concentration in the range 10–15% (v/v). At these levels, ethanol is present in an excess of 10^3 to 10^6 compared to the aroma compounds and the ionization processes in the APCI source are different with the result that data from alcoholic solutions cannot be compared with results from aqueous solutions.

The approach adopted in this paper was to provide a constant ionization environment by using ethanol as the charge transfer medium so that ionization would be independent of the sample ethanol content. This was to be achieved by introducing ethanol into the source at a fixed level. Ethanol has a PA of 776 kJ/mol which is similar to water (691 kJ/mol) but some important aroma compounds (e.g., acetaldehyde; PA 768 kJ/mol and methylsulfide; PA 773 kJ/mol) will not be analyzed under these conditions. In addition to the protonated monomers, reagent ions can also form clusters (dimers, trimers) and this may affect ionization processes due to different reaction energies. Initially, the effects of source ethanol concentration on the proportion of water/ethanol ions were studied, then the ionization behavior of eleven wine aroma compounds was tested with ethanol as the charge transfer agent to establish the feasibility of this approach.

2. Materials and methods

2.1. Reagents

All volatile compounds were of analytical quality. Ethyl butyrate was obtained from Firmenich (Geneva, Switzerland); diacetyl, octanal, furfuryl alcohol, c-3-hexenol, ethyl isovalerate, ethyl octanoate, linalool and 3-methyl butanol were supplied by Sigma-Aldrich (Poole, UK); limonene and ethanol (analytical reagent grade, 99.99%) were purchased from Fisher Scientific (Loughborough, UK) and 1-octen-3-one from Lancaster (Morecambe, England).

2.2. Solutions

Individual solutions of the 11 volatile compounds were prepared either in water or 12% ethanol/water (v/v) solution at concentrations shown in Table 1. To ensure the aroma compounds with low water solubility were dissolved in the aqueous solutions the solutions were left on a flask shaker overnight. Final water and ethanolic solutions were prepared by diluting the initial solution with the same quantity of water or ethanol, respectively.

2.3. Headspace analysis by GC–MS

Aliquots (200 mL) of pure ethanol, 2% and 12% (v/v) ethanol in water were placed in 250 mL flasks fitted with a one port lid that allowed headspace sampling. The samples were left at 22 °C overnight to equilibrate. Portions (200 μL) of the headspace were removed with a gas tight syringe and injected at 150 °C in splitless mode into a Gas Chromatograph (GC8000 Fisons Instruments, Manchester, UK) coupled with a mass spectrometer MD800 (Fisons). The column was a DB-5 (J&W scientific, Folsom, CA), 30 m, 0.25 mm i.d. and 1 μm film thickness. The temperature program was: 45 °C for 2.5 min, then 25 °C/min to 150 °C and 150 °C for 5 min. Carrier gas was helium at 2.5 psi. Spectra were recorded in scan

Table 1
Volatile compounds, log *P* values, main ion, range of solution concentrations used for the analysis (μL/L) and optimum cone voltage (*V*) with water reagent ions

Compound	log *P*[a]	*m/z*	μL/L	*V*
Diacetyl	−0.348	87	20–80	27
Furfuryl alcohol	0.099	81	150–300	30
c-3-hexenol	0.929	101	20–200	18
3-methyl butanol	1.351	71	30–100	21
Ethyl butyrate	1.443	117	1–10	21
Ethyl isovalerate	1.801	131	0.5–5	21
Linalool	2.13	137	30–150	21
1-octen-3-one	2.434	127	2–20	21
Octanal	2.856	129	5–25	15
Ethyl octanoate	3.211	173	5–10	21
Limonene	3.604	137	1–30	24

[a] Values calculated using MOE (Chemical Computing Group Inc, Montreal, Canada).

mode, scanning from *m*/*z* 17 to 100 (EI+). Ethanol was measured at *m*/*z* 45 and identity confirmed by chromatography of an authentic standard which showed the same retention time. The GC–MS signal from headspace above 99.99% ethanol was given a value of 100%. The ethanol headspace signals from the ethanol/water mixtures were expressed relative to the 100% value. Three replicate headspace samples were analyzed for each ethanol concentration.

2.4. Modifications to the API source

A Platform LCZ mass spectrometer fitted with an MS Nose interface (Micromass, Manchester, UK) was used. The APCI source was operated as described previously [6] but ethanol was added to the nitrogen make-up gas by passing a stream of nitrogen (0–100 mL/min) through a sinter into an ethanolic solution (200 mL) placed inside a 250 mL flask (Schott bottle; Fisher Scientific) (Fig. 1). This flow was then combined with the bulk nitrogen flow (total flow 10 L/min) before entering the source. The concentration of ethanol entering the source was calculated assuming equilibrium between the ethanol solution and the nitrogen passing through it. The ethanolic solution was renewed every 4 h to avoid significant depletion of ethanol from the solution (assuming equilibrium throughout, the ethanol concentration in the flask decreased by less than 5% over the 4 h period).

2.5. Headspace analysis by APCI

For APCI-MS analysis, aliquots of volatile solutions (40 mL) were placed in 100 mL flasks fitted with a one-port lid. After equilibration for at least 1 h at ambient temperature (22 °C), headspace was sampled through this port into the APCI-MS with sample flows from 3 to 10 mL/min. For Selected Ion Monitoring (SIM) analysis, cone voltages and ions monitored for each volatile are shown in Table 1.

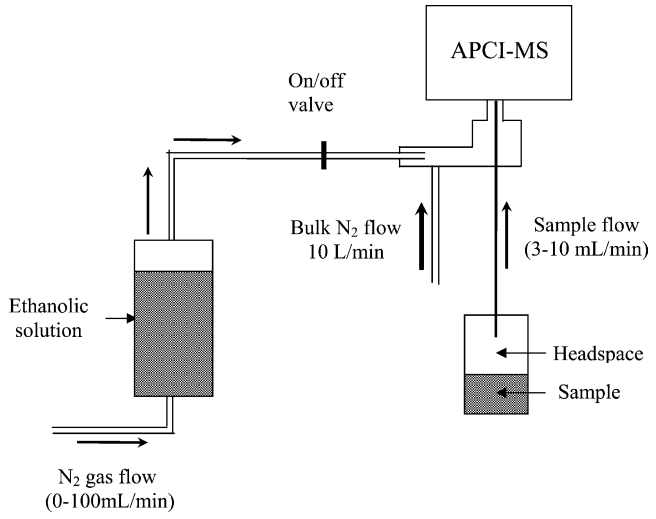

Fig. 1. Apparatus for the addition of ethanol into the APCI source via the make-up gas flow.

Cone voltages were selected as the optimum cone voltages for the volatiles in aqueous systems, and ions monitored corresponded to the protonated molecules $[MH]^+$ except for 3-methylbutanol, furfuryl alcohol and linalool where $[M - H_2O + H]^+$ was monitored due to dehydration of the molecules.

2.5.1. Effect of introducing ethanol into the source on reagent ions

To check the effect of ethanol on reagent ions in the source, different ethanol concentrations were introduced via the make-up gas. By bubbling nitrogen (0–100 mL/min) through a 2% ethanolic solution, and adding this flow to the main make up gas flow, ethanol concentrations in the range 0–16.2 μL of ethanol vapor per liter of make-up gas were achieved.

2.5.2. Effect of ethanol reagent ions on analyte response

Ethanol was introduced into the bulk make-up gas flow entering the APCI source (0–16.2 μL/L N_2) while volatiles were sampled from the headspace above wholly aqueous solutions at 10 mL/min. To check the influence of a very high ethanol source concentration on analyte response, 565 μL ethanol/L N_2 was also added to the source using a pure ethanol solution in the flask and a nitrogen flow of 70 mL/min.

2.5.3. Effect of ethanol on the pattern of ionization of volatiles

For these experiments, data were collected in scan mode (*m*/*z* 15–350) and volatiles were analyzed at eight different cone voltages from 12 to 33 V (for furfuryl alcohol the range had to be increased to 42 V). Analysis was done with: no ethanol in the system, two medium ethanol concentrations (6.5 and 11.3 μL/L N_2) and a high ethanol concentration (565 μL/L N_2) added in the make-up gas. Sample flow was 10 mL/min.

2.5.4. Effect of ethanol on volatile partitioning

Individual solutions of each volatile were prepared in water and in 12% ethanol, then the headspace above them sampled at 5 mL/min into the APCI source. Water solutions were sampled whilst adding 11.3 μL of ethanol per liter of make up gas and 12% ethanol solutions were sampled whilst adding 6.5 μL ethanol per liter of make-up gas. For the ethanolic solution, the ethanol in the source was 4.8 μL ethanol/L N_2 from the sample plus 6.5 μL ethanol/L N_2 added in the make-up to make a total of 11.3 μL/L N_2. Therefore, the final concentration of ethanol in the source was the same for both solutions.

The same experiment was repeated for seven of the volatile compounds with a sample flow of 3 mL/min. In this situation, water solutions were sampled adding 9.3 μL of ethanol per liter of make up gas and 12% ethanol solutions were sampled adding 6.4 μL ethanol per liter of make-up gas. This combined with the ethanol from the sample again resulted in a final ethanol concentration of 9.3 μL ethanol/L N_2.

For these experiments, the optimum cone voltage for the compounds in systems with ethanol reagent ions was used.

These values corresponded well with the optimum cone voltages for water systems for most of the compounds (Table 1) but 3-methylbutanol and octanal required cone voltages of 27 and 21 V, respectively, to optimize ion formation.

3. Results and discussion

3.1. Preliminary data

Volatile compounds were chosen to represent aroma compounds with different polarities and volatilities, as demonstrated in Table 1 where the hydrophobicity values (log *P*) ranged from −0.348 (diacetyl) to 3.604 (limonene). Most of the important chemical families were represented in the 11 volatiles; acids were excluded as the acid–base equilibrium of these species would influence their partition behavior as well as ethanol content. Since ethanol vapor was introduced by bubbling make up gas through ethanol–water solutions (Fig. 1), the ethanol–water partition was also studied using GC–MS analysis of headspace from solutions of 2 and 12 % ethanol/water to test for linearity. For a 2% ethanol solution, the measured headspace concentration was 1.4% (±0.5) of the headspace signal of pure ethanol (note 99.9% ethanol was used), and the headspace signal from a 12% ethanol solution was 12.0% (±0.1) of the headspace signal of pure ethanol. Therefore, the data showed that partition across the range 2–12% ethanol was constant and linear in the system. Consequently, the amount of ethanol entering the source of the mass spectrometer (during headspace sampling or via make-up gas ethanol addition) was directly proportional to the ethanol content of the solution.

3.2. Effect of ethanol on reagent ions in the APCI source

Different ethanol concentrations were introduced into the APCI source (0–16.2 μL ethanol/L N_2) and the relative amounts of water and ethanol ions were monitored (Figs. 2 and 3). With no ethanol being introduced into the APCI source, protonated water reagent ions were observed on the tune page of the mass spectrometer at *m/z* 37 and 55, corresponding to the protonated dimer and trimer, respectively (monomer is not usually seen, the dimer is typically the predominant species). When the headspace from above ethanolic solutions (at concentrations greater than 0.5%) was sampled into the APCI source, ethanol monomer *m/z* 47, $(C_2H_5OH)H^+$, dimer *m/z* 93, $(C_2H_5OH)_2H^+$, and the trimer *m/z* 139 $(C_2H_5OH)_3H^+$ were observed on the tune page. A water–ethanol adduct (*m/z* 65) was also observed, but only at low ethanol concentrations. The ethanol cluster ion distribution shifted towards larger clusters as the proportion of ethanol in the make-up gas increased. The distribution of H_3O^+ clusters as a function of water concentration [9] and ion source temperature [10] has been described. It was found that clusters of $n = 2$ and 3 were dominant under the source conditions used. In our source, the ethanol dimer (*m/z* 93) was the predominant species and above 4 μL ethanol/L N_2 its signal was so high, it exceeded the MS detector scale so it was not possible to follow its true evolution. Previous studies showed similar behavior in the gas-phase proton transfer reactions when methanol was used as a cosolvent with water in liquid MS [11,12].

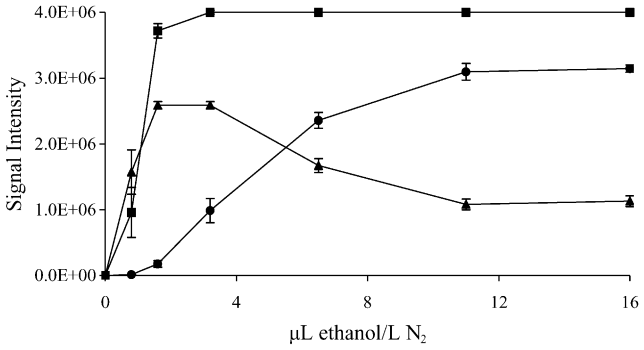

Fig. 2. Intensity of ethanol ions when ethanol make-up gas concentration increased ((▲) *m/z* 47 monomer; (■) *m/z* 93 dimer; (●) *m/z* 139 trimer). Each marker point is the mean of three measurements and standard deviation is shown with error bars.

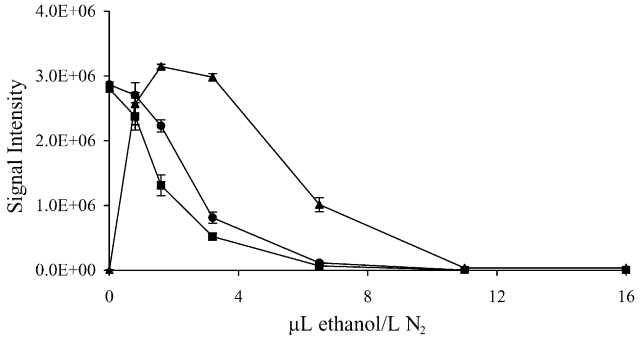

Fig. 3. Intensity of water ions when ethanol make-up gas concentration increased ((●) *m/z* 37 dimer; (■) *m/z* 55 trimer; (▲) *m/z* 65 ethanol–water adduct). Each marker point is the mean of three measurements and standard deviation is shown with error bars.

For ionization of aroma compounds, one species of reagent ion is needed to achieve consistent ionization. Water ions were absent from the tune page at ethanol concentrations over 6 μL ethanol/L N_2 whereas ethanol ions were dominant at ethanol concentrations above 10 μL ethanol/L N_2. Further experiments were carried out to determine the ionization behavior of aroma compounds under these different reagent ion conditions.

3.3. Effect of ethanol reagent ions on analyte response

The relative intensity of the signal for the volatile compounds at the different ethanol make-up gas concentrations were compared to the signal obtained with water reagent ions (Table 2). From the values in Table 2, it is clear that the response of the aroma compounds to ethanol was very

Table 2
Relative intensity of the volatile compounds main ions when ethanol was added in the make-up gas normalised to the intensity with water reagent ions (% CV for three replicate analyses of a sample at any given ethanol content was less than 4%)

μl eth/L N_2	Diacetyl	Furfuryl alcohol	c-3-Hexenol	3-Methyl butanol	3-Methyl butanol[a]	Ethyl butyrate	Ethyl isovalerate	Linalool	1-Oct3one	Octanal	Octanal[a]	Ethyl octanoate	Limonene
0	100	100	100	100	100	100	100	100	100	100	100	100	100
1.6	68	321	150	225	623	300	254	131	308	146	188	175	64
3.2	27	386	170	198	695	317	263	160	317	98	175	171	45
6.5	7	381	177	165	580	321	278	174	346	70	132	169	21
11.3	3	347	170	129	461	330	284	185	357	49	95	173	11
16.2	2	310	159	104	372	321	295	210	378	38	74	175	7
565	0	105	68	3	nd	243	239	312	331	7	nd	185	6

Conditions of analysis taken from Table 1. nd: values not determined.
[a] Compounds measured at the optimum cone voltages for systems with ethanol reagent ions.

different. The diacetyl and limonene signal decreased when the ethanol concentration increased in the make-up gas, the largest changes occurring at the lowest ethanol concentration, from 0 to 3.2 μL ethanol/L N_2. When a high ethanol concentration was added (565 μL ethanol/L N_2), the signal of these molecules almost disappeared. The signal for octanal and 3-methyl butanol increased at very low ethanol concentrations (1.6 μL/L N_2) and decreased at higher concentrations. For the rest of the molecules, adding ethanol to the make-up gas improved the signal compared to ionization with water as the reagent ion. There was an increase of approximately 300–400% in the signal for furfuryl alcohol, ethyl butyrate, ethyl isovalerate and 1-octen-3-one, and slightly less (around 150–200%) for c-3-hexenol, linalool and ethyl octanoate. For all of these compounds the signal decreased at very high ethanol source concentration, except for ethyl octanoate (where the signal remained stable) and linalool (where the signal kept increasing as ethanol increased).

To obtain a better understanding of the effect of source ethanol concentration on ionization behavior, the signal intensity from some representative volatiles is plotted in Fig. 4. The data are expressed relative to the signal intensity obtained for the volatile compounds in aqueous solution. Large changes in signal intensity occur mainly in the first stages as water reagent ions are replaced by ethanol reagent ions. Thereafter, the response is largely linear, although not constant. These results suggest that volatile compounds could be analyzed quantitatively if ethanol concentration in the source could be controlled.

Besides studying the change in signal from the aroma compounds, the noise emanating from ethanol was also monitored Table 3 shows the relative increase in the signal of the volatiles and in the noise when ethanol was added to the make-up gas (6.5 μL of ethanol/L N_2) compared to the signal and noise in a water reagent ion system. Generally, an increase in noise was observed which was greater than the increase in signal; only for furfuryl alcohol and 1-octen-3-one was the increase

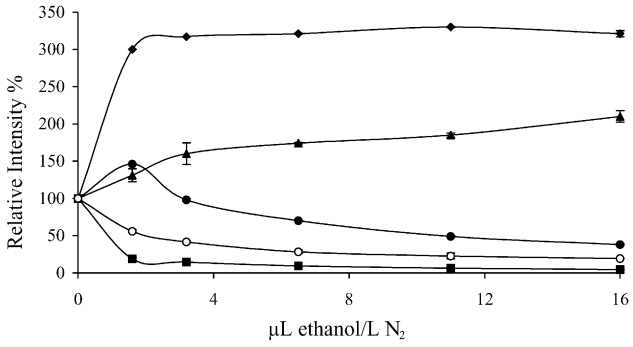

Fig. 4. Intensity of the major ions of selected compounds as ethanol make-up gas concentration increased. The results are expressed relative to the signal observed for APCI with water reagent ions (100%) ((●) octanal; (■) diacetyl; (▲) linalool; (♦) ethyl butyrate; (○) limonene). Each marker point is the mean of three replicate measurements and standard deviation is shown with error bars; S.D. was small and often cannot be seen over the size of the marker points.

Table 3
Percentage change in signal intensity and noise for the volatile compounds' main ions when ethanol was added to the make-up gas (6.5 μL ethanol/L N_2) relative to the intensity and noise in a system with only water reagent ions

Volatiles	Intensity (%)	Noise (%)
Diacetyl	−90	470
Furfuryl alcohol	280	130
c-3-Hexenol	80	860
3-Methyl butanol	60	180
Ethyl butyrate	220	350
Ethyl isovalerate	180	300
Linalool	70	170
1-Octen-3-one	250	70
Octanal	−30	170
Ethyl octanoate	70	100
Limonene	−80	170

Conditions of analysis taken from Table 1.

in noise smaller than that of the volatile signal. Therefore, only two compounds would be detected with a better sensitivity in the presence of ethanol. For the majority, a decrease in sensitivity of around 150% occurred when ethanol was added to the source.

3.3.1. Potential explanations for ionization behavior with ethanol

If we take the simple, theoretical assumption that ionization through proton transfer depends solely on the ΔPA value, we might expect water and ethanol to produce similar patterns of ionization across the 11 test compounds. The fact that ethanol causes increases in the ion intensity of some compounds relative to water and decreases in others may be due to the differences in the cluster ion distribution. Charles et al. [11] used methanol as the reagent ion and reported that ionization of methyl salicylate occurred only through protonation from the monomer. The methanol dimer was not able to transfer charge because the binding energy of the proton to the dimer was 135 kJ/mol and, under these conditions, the reaction became endothermic rather than exothermic. For example, in the case of methyl salicylate (PA 855 kJ/mol) reacting with the methanol monomer (PA 755 kJ/mol), the reaction is exothermic by 100 kJ/mol. With the dimer, the additional binding energy of 135 kJ/mol makes the reaction endothermic by +35 kJ/mol. According to the literature, ethanol cluster ions show a similar binding energy [13] (http://webbook.nist.gov/), so a similar effect may be occurring with the aroma compounds in our study. Unfortunately PA values for all 11 aroma compounds are not easily available so it is not easy to determine whether the change in behavior is due to exo- or endo-thermic reactions in all cases. For the compounds where data is available, the calculations support the idea of ionization being dependent on the ethanol cluster distribution. For example, diacetyl has a reported PA of 810 kJ/mol so proton transfer from ethanol is exothermic 775 − 801 = −26 kJ/mol but is endothermic from the ethanol dimer 775 − 801 + 135 = 109 kJ/mol. The same is true for limonene (PA 875; [14]) which also shows decreased signal intensity as the proportion of ethanol dimer increases in the source (compare Figs. 2 and 4). No PA value for ethyl butyrate (which shows a significant increase in signal with ethanol as the reagent ion) could be found in the literature but data are published for ethyl formate and ethyl acetate (799 and 835 kJ/mol, respectively). Using a modification of the formula which predicts PA values for a homologous series of fatty acid methyl esters [15], we conclude that ethyl butyrate has a PA around 890 kJ/mol. This value would permit charge transfer from the ethanol monomer and is close to the ethanol dimer PA value.

Although the discussion above explains some of the observed differences, it does not account for the fact that some compounds were ionized more efficiently with ethanol rather than water as the proton transfer reagent ion. Again, Charles et al. [11] remarked that the ionization of methyl salicylate was not in step with the decrease in proton charge reagents and suggested that some other unknown mechanisms were involved. These factors may include the interaction of the analyte with a physically larger reagent ion or differences due to functional groups, but, with the limited range of compounds studied in this paper, it was not possible to establish positive correlations. The other explanation is that ionization through ethanol changes the fragmentation and/or adduction pattern and, for some compounds, a greater proportion of $[M+H]^+$ is formed from ethanol compared to water. This concept was tested as described below.

3.4. Effect of ethanol on the fragmentation pattern of aroma compounds

Full scan analysis of the volatile compounds at different cone voltages and with different ethanol proportions in the make-up gas was carried out to check how the ethanol reagent ions affected the pattern of ionization (fragmentation) of volatile compounds and how this related to the increase or decrease in their signal.

Four compounds (diacetyl, 3-methyl butanol, octanal and limonene) exhibited a decrease in signal when ethanol was added to the source. For two of them (3-methyl butanol and octanal) the dominant ions (*m*/*z* 71 (dehydrated molecular ion) and 129 (molecular ion)) were formed at different cone voltages with ethanol reagent ions, compared to the water reagent ion system. The optimum voltages increased from 21 to 27 V for 3-methyl butanol and from 15 to 21 V for octanal. Cone voltage affects fragmentation of ions and a value is usually chosen to maximize a particular ion, thus enhancing signal and sensitivity. For diacetyl, limonene and the rest of the volatile compounds, no shift in the optimum cone voltage was observed with ethanol reagent ions compared to water reagent ions.

To further investigate factors that may relate the change in the signal intensity to the ethanol concentration in the source, fragmentation and adduct formation of the volatile compounds were checked. Table 4 shows the relative inten-

Table 4
Relative intensity (related to the base peak of the spectrum) of the volatile compound's main ions, fragments and adducts under different source conditions: 0, 6.5, 11.3 and 565 μL/L N_2 ethanol added to the make-up gas

Compound (cone voltage)	*m/z*	Ion	0 μL/L	6.5 μL/L	11.3 μL/L	565 μL/L
Diacetyl	87	MH^+	100	100	100	nd
	115	$(MEtOH - H_2O)H^+$	<10	16	17	nd
c-3-Hexenol	101	MH^+	23	28	28	28
	83	$(M - H_2O)H^+$	100	100	100	100
	147	$(MEtOH)H^+$	<10	16	17	20
3-Methylbutanol	71	$(M - H_2O)H^+$	100	100	100	86
	159	$(2M - H_2O)H^+$	10	24	25	100
	135	$(MEtOH)H^+$	<10	44	50	34
3-Methylbutanol[a] (27 V)	71	$(M - H_2O)H^+$	100	100	100	100
	159	$(2M - H_2O)H^+$	<10	<10	<10	29
	135	$(MEtOH)H^+$	<10	<10	<10	<10
Ethyl butyrate	117	MH^+	100	100	100	100
	89	$(M - ethene)H^+$	62	24	25	14
Ethyl isovalerate	131	MH^+	100	100	100	100
	103	$(M - ethene)H^+$	57	21	21	12
Linalool	137	$(M - H_2O)H^+$	100	100	100	100
	81	$(M - R)H^+$	96	78	73	68
Octanal	129	MH^+	100	92	97	73
	175	$(MEtOH)H^+$	<10	100	100	100
	111	$(M - H_2O)H^+$	92	18	20	17
Octanal[a] (21 V)	129	MH^+	46	100	100	100
	175	$(MEtOH)H^+$	<10	33	32	52
	111	$(M - H_2O)H^+$	100	73	75	87
Ethyl octanoate	173	MH^+	100	100	100	100
	145	$(M - ethene)H^+$	29	13	12	<10
Limonene	137	MH^+	100	100	100	100
	166	MNO^+	58	93	95	29
	121	$(M - R_1)H^+$	27	13	10	<10
	107	$(M - R_2)H^+$	35	30	36	37
	81	$(M - R_3)H^+$	70	62	60	48
	155	$(MH_2O)H^+$	19	11	11	<10

Data measured at cone voltages from Table 1 (%CV for three replicates of each volatile at any given ethanol content was less than 2%). nd: compound not detected in the chromatogram at those conditions.

[a] Compound measured at the optimum cone voltages for systems with ethanol in the source.

sity (related to the base peak of the spectrum) of the main ions obtained from the 11 aroma compounds for water systems and systems with different proportions of ethanol. The shift in the optimum cone voltage of 3-methyl butanol and octanal could be explained with the results from Table 4. These two volatiles formed an adduct with ethanol ($[(MEtOH)H]^+$), when ethanol was added to the source. The ethanol-molecule adduct for 3-methyl butanol (*m/z* 135) reached 50% of the base peak, and for octanal (*m/z* 175) became the biggest peak in the spectrum at medium ethanol levels. Increasing the cone voltage presumably broke down the adduct so more signal was seen at the $[M + H]^+$ *m/z* value.

The other two compounds whose intensity decreased in the presence of ethanol reagent ions, diacetyl and limonene, did not show any shift in the optimum cone voltage, nor did they show any major changes in their mass spectrum profile. Even though diacetyl formed an adduct with ethanol (*m/z* 115) the proportion of this ion did not reach 20% of the base peak and would not account for the decrease observed in *m/z* 87. Limonene, whose mass spectrum showed five different ions, showed changes in the adduct MNO^+ (*m/z* 166) that increased from 58% of the base peak of the spectrum to 93–95%. In both situations the adduct formation could not explain the drastic decrease of the signal shown in Table 2. Therefore, it appears that for these compounds there was general reduction in ionization when ethanol reagent ions dominated the spectrum, and the net effect was to decrease sensitivity.

The rest of the volatiles increased their signal when the proportion of ethanol in the system increased at medium levels (3.2–16.2 μL/L N_2). One reason could be a decrease in fragmentation due to a "softer" ionization when the proportion of ethanol reagent ions increased in the source. Having a very exothermic proton transfer reactions, with water reagent ions, could have induced protonated analytes to fragment

[16,17]. For esters, the major ionic product was MH^+ [18] plus the fragment ion formed by the loss of ethene (C_2H_4) from the MH^+ ion: ethyl butyrate (*m*/*z* 89), ethyl isovalerate (*m*/*z* 103) and ethyl octanoate (*m*/*z* 145). The proportion of the fragments decreased (in a range from 16 to 38%) when the ethanol was added to the source. This behavior would partially explain the increase of the main ion MH^+ with the addition of ethanol (Table 2), but not completely, since the increase of MH^+ is much bigger than the decrease in fragment intensity. Linalool showed similar behavior, the fragment *m*/*z* 81 decreased around 25% with the addition of ethanol, yet showed an increase in MH^+ of around 200%. For c-3-hexenol, the mass spectrum did not change with the addition of ethanol, apart from the formation of an ethanol adduct (*m*/*z* 147) that simply increased to 17% of the base peak. Furfuryl alcohol and 1-octen-3-one did not show any fragment or adduct with percentages above 10% of the base peak.

Therefore, the decrease and increase of the signal intensity for the aroma compounds when ethanol was added to the make-up gas could be partially explained by looking at the changes that the addition of ethanol had in the pattern of ionization of the volatiles. However, other, unknown factors must also be involved.

3.5. Effect of ethanol on volatile partitioning

With the performance of the APCI source now standardized for the test volatile compounds, the technique was applied to measuring the effect of ethanol on the partition of these compounds to determine whether the technique was sensitive enough and sufficiently robust for practical use. In order to avoid any significant changes in analytical sensitivity due to ethanol sampled from above ethanolic solutions, it was necessary to balance the ethanol entering the source from the sample with that of the make-up gas. Since the signal for the volatiles was reasonably stable in the range from 6.5 to 11.3 μL ethanol/L N_2 source concentration (Table 5), a minimum content of 6.5 μL ethanol/L make-up was used. The design of the experiment was such that the final content of ethanol entering the source was the same when sampling headspace above water and water–ethanol solutions. Differences in ion intensity between these samples were therefore only due to differences in partitioning. In order to check that the total ethanol added to the source could be calculated as the sum of the ethanol added to the make-up and the ethanol coming from the sampling of ethanolic solutions the experiment was carried out under two different conditions. Using two different sample flow rates and corresponding make-up gas ethanol contents, the same amount of ethanol was introduced into the source and the signals for the aroma compounds measured under both conditions. The results were essentially the same (R.S.D. = 4%), therefore demonstrating that it was the concentration of ethanol in the source that was the key factor controlling signal intensity, rather than the amount of ethanol in the make up gas or sample flow.

Table 5
Percentage change in signal intensity when ethanol concentration was increased from 6.5 to 11.3 μL/L of make-up gas (standard deviation from nine different replicates performed on 3 different days)

Volatiles	Signal change (%)
Diacetyl	−58 (±4)
Furfuryl alcohol	−7 (±6)
c-3-Hexenol	−3 (±4)
3-Methyl butanol	−17 (±5)
Ethyl butyrate	2 (±3)
Ethyl isovalerate	4 (±4)
Linalool	11 (±7)
1-Octen-3-one	6 (±5)
Octanal	−26 (±5)
Ethyl octanoate	5 (±5)
Limonene	−43 (±4)

Table 6
Percentage change in the partition coefficient of a 12% ethanolic solution relative to an aqueous solution (average and standard deviation of nine replicates performed on 3 different days)

Volatiles	Partition effect (%)
Diacetyl	−4 (±9)
Furfuryl alcohol	−8 (±6)
c-3-Hexenol	−13 (±3)
3-Methyl butanol	−20 (±4)
Ethyl butyrate	−19 (±4)
Ethyl isovalerate	−23 (±3)
Linalool	−33 (±3)
1-Octen-3-one	−33 (±3)
Octanal	−42 (±3)
Ethyl octanoate	−29 (±4)
Limonene	9 (±8)

Table 6 shows the differences in partitioning between water and 12% ethanol solutions. Volatiles behaved differently when they were dissolved in water or ethanol–water solutions, for most of them, the concentration in the headspace decreased when ethanol was present in the solution, by 4–42%. This agreed with the fact that the addition of ethanol generally increases the solubility of aroma compounds and therefore reduces their concentration in the headspace. The results are similar to the results from Fischer et al. [19], who found a similar decrease in partition coefficients as the concentration of ethanol was increased from 0.5 to 12% for a range of compounds. However, other authors reported an effect of ethanol on the partitioning of compounds only when the ethanol concentration was higher than 17% [20,21].

The relative decrease in the headspace concentration when ethanol was used as co solvent is plotted against the hydrophobicity ($\log P$) of the aroma compounds in Fig. 5. A linear correlation between the decrease of the signal and the $\log P$ values was observed for $\log P$ values smaller than 3 ($R^2 = 0.95$). Above a $\log P$ value of 3 (very non polar molecules), the addition of ethanol did not decrease the concentration of volatiles in the headspace to the same ex-

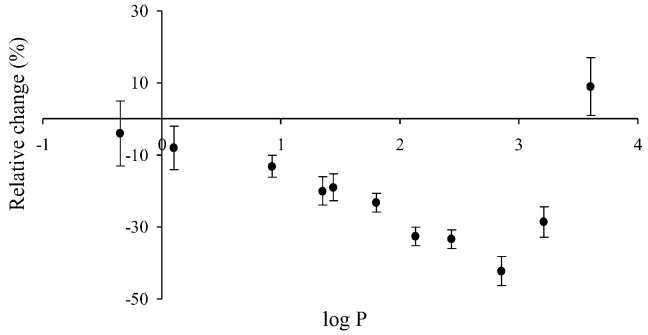

Fig. 5. Relative change (%) of volatile headspace concentration from a 12% ethanol solution relative to water solutions versus their log *P* values (average and standard deviation of nine replicates performed on 3 different days).

tent. The correlation between the decrease in the headspace concentration and the log *P* values was not linear and seemed to have a parabola-like shape. This kind of correlation with log *P* had been previously found in biological studies on molecular mobility [22]. From Fig. 5, the amount of volatile compounds in the headspace above ethanolic solutions is governed not only by the concentration of the compound but by its log *P* value. Further experiments are underway with non-equilibrium systems (for example where the headspace is continually diluted with gas) to establish if these effects are present in dynamic systems which mimic better the situation that occurs during consumption of ethanolic beverages.

4. Conclusion

The analysis of aroma compounds from ethanolic solutions by APCI-MS can be achieved although the ion chemistry is complex. For this reason, frequent calibration of the equipment using authentic standards is required as changes in the relative proportions of the ethanol clusters can affect the ionization efficiency and thus quantification. The technique allows rapid screening of partition from ethanolic solutions and shows some interesting preliminary data on the effect of hydrophobicity on partition.

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