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Effect of Preparation Conditions on Release of Selected Volatiles in Tea Headspace

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The release of volatile compounds from infused tea was monitored using on-line atmospheric pressure chemical ionization (APCI) mass spectrometry. Assignment of the APCI ions to particular compounds was achieved using gas chromatography of tea headspace with dual electron ionization and APCI-MS detectors. Six ions in the APCI spectrum could be assigned to individual compounds, five ions were associated with isobaric compounds (e.g., 2- and 3-methylbutanal and pentanal) or stereoisomers (e.g., heptenals or heptadienals), and a further four ions monitored were identified compounds but with some unknown impurities. Reproducibility of infusion preparation and the analytical system was good with percentage variation values generally below 5%. The analysis was used to study the effect of infusion and holding temperatures on the volatile profile of tea headspace samples, and this was found to be compound-dependent. Both the extraction of volatiles from leaf tea and the release of volatiles into the headspace play a role in creating the aroma profile that the consumer experiences.

KEYWORDS: Infusion; partition; time; temperature; orthonasal signal

INTRODUCTION

Previous research has established the aroma composition of black tea (*Camellia sinensis*) (*1*−*7*) and the effect of production techniques on the volatile composition of black tea leaf (*8*−*10*). The number of volatiles identified in infused tea is around 600 (*7*), but a smaller number have been proposed as key contributors to Darjeeling tea aroma (*11*). The release of these volatiles into the surrounding air produces the initial, orthonasal aroma of tea before consumption and is one of the attributes by which consumers judge the quality of the beverage.

The release process is governed by the mass transfer properties of the tea volatiles, first from the tea leaf into the liquid phase and then from the liquid into the gas phase (the headspace) above the infused tea. The rate at which this overall process takes place for nonvolatile compounds is affected by a variety of factors (*12*). Although air−water partition coefficients can theoretically predict the release of volatile compounds from the liquid to the gas phase, the theory only holds good for ideal solutions and the solutes in the tea infusion may cause "nonideal" conditions (*13*). Also, the temperature of tea infusions changes from 100 °C at the point of infusion down to around 60 °C at consumption. The release profile above the tea infusion will therefore change with temperature and time. There is also the possibility that chemical changes may occur in the flavor compounds of tea after infusion (i.e., in the cup in the absence of leaf tea), which may also affect the aroma profile over time.

Conditions in the gas phase above a cup of infused tea are affected by the airflow, driven partly by the thermal effects from the hot cup as well as the natural convection currents present in atmospheric air. The concentrations of aromas in the air phase will therefore differ from region to region above the cup. To obtain comparative data on the effects of the other factors (e.g., infusion preparation method and tea type), it is necessary to control conditions in the gas phase to reduce variation and produce consistent data.

Analysis of tea volatiles in the headspace can be undertaken using either trapping followed by gas chromatography−mass spectrometry (GC-MS) or on-line methods (*14*, *15*). Each method has its advantages and disadvantages. Here, the change of headspace volatile profile over relatively short periods of time was important, so a direct mass spectrometric technique was chosen, although this limited the number of volatile compounds that could be detected due to sensitivity issues and the problem of resolving isobaric compounds. GC and combined electron impact (EI)- and atmospheric pressure chemical ionization−mass spectrometry (APCI-MS) were used to associate the ions monitored on the APCI-MS with the compounds found in tea headspace as described previously (*16*−*18*).

The aim of the current work was to develop a method to investigate the real-time release of key volatiles above a system that represented as closely as possible hot, freshly infused tea. The method was designed to mimic the aroma signal that a real consumer would perceive through the orthonasal "sniffing" route, while maintaining a level of control so that reproducible data could be obtained. The effect of infusion water temperature

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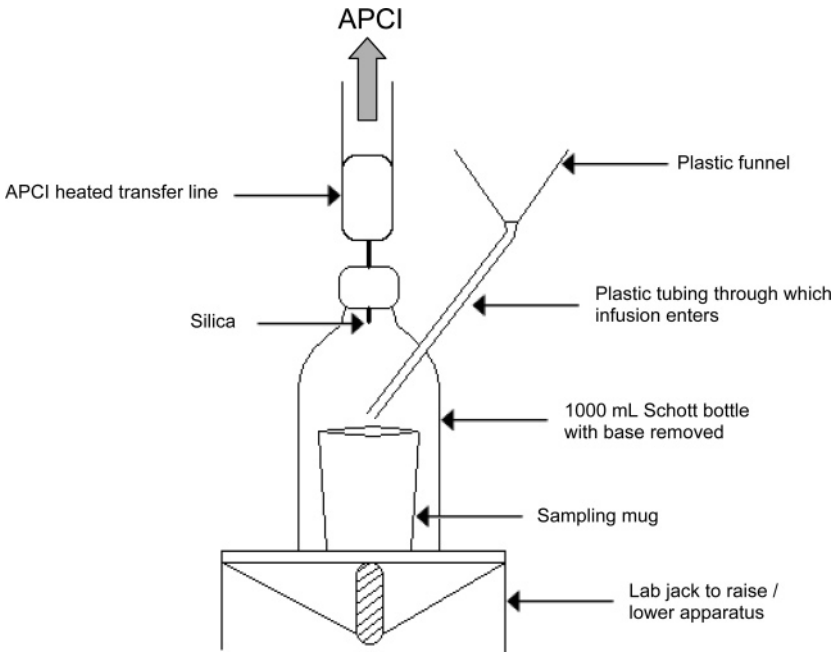

Figure 1. Schematic diagram of tea infusion headspace sampling apparatus.

(the temperature of water as it is mixed with dry tea leaves) on volatile release from the complete infusion was studied to test the new technique. The results were compared with previous work that studied the effect of selected temperatures and times on the extraction of volatile compounds from tea leaf to aqueous infusion (*11*, *19*). Some simple hypotheses on the overall mechanisms governing volatile release from tea infusions were also tested.

MATERIALS AND METHODS

Preparation of Black Tea Infusions. All infusions were prepared using bottled mineral water (Highland Spring, Blackford, United Kingdom). This water contained an average mineral composition and represented medium water hardness within the United Kingdom. Typical analysis at source (mg/L) was as follows: calcium (32.0), magnesium (8.0), potassium (0.5), sodium (4.5), bicarbonate (133.0), chloride (5.0), sulfate (7.0), nitrate (<2.0), fluoride (<0.1), iron (<0.01), and aluminum (<0.01); total dissolved solids at 180 °C (136 mg/L), pH 7.8.

Dry black tea (2.8 g; LYL640 blend, Unilever R&D, Colworth, United Kingdom) was transferred into a prewarmed vacuum-insulated flask (Thermos, Rolling Meadows, 500 mL). Boiled mineral water (280 mL) of the desired temperature was added to the dry tea, and the thermos was immediately sealed and inverted once. After 3 min, the thermos was inverted five times and then left for a further minute. At 4 min, the tea infusion was inverted one final time before the leaves were removed by filtering through double-layered muslin (Moorlands Cheesemakers, Bruton, United Kingdom).

Headspace Sampling. Solid-phase microextraction (SPME) of the volatiles released from tea infusions was carried out using a 2 cm stable flex fiber, coated with 50/30 μm poly(divinylbenzene) (DVB)/carboxen/poly(dimethylsiloxane) (PDMS) (Supelco, Bellefonte, PA) and conditioned as recommended by the manufacturer.

The tea infusion (200 mL) was prepared using 100 °C water according to the standard tea preparation method described above (i.e., 1 g tea per 100 g water), transferred to a 250 mL glass bottle (Fisher Scientific, Loughborough, United Kingdom), and sealed with a screwcap. The SPME fiber was exposed to the tea infusion headspace through a small hole in the bottle screwcap for 5 min and then transferred to the GC injector.

GC-EI/APCI-MS Analysis of Tea Headspace to Identify Volatile Components. Volatile compounds from the SPME fiber were analyzed by GC (Thermo Finnigan 8000, Hemel Hempstead, United Kingdom) fitted with a BP5 column (SGE, Milton Keynes, United Kingdom; 30 m × 0.25 mm i.d.; film thickness, 1.0 μm). The injector was operated in splitless mode (240 °C; 1 min) with helium as the carrier gas (head pressure, 20 psi). The oven temperature program was as follows: 45 °C for 2 min, 10 °C/min to 230 °C, and hold for 5 min. The exit of the column was split using an outlet capillary column splitter (SGE), with 0.1 mm i.d. deactivated fused silica tubing (SGE) leading to the EI-MS source. The EI-MS was operated in full-scan mode over the *m*/*z* range of 40−250 (scan time, 0.45 s; interscan delay, 0.05 s). The remaining flow was carried through a heated transfer line (0.32 mm i.d. deactivated fused silica tubing; Supelco) into the source of the APCI-MS (Micromass, Manchester, United Kingdom) with a corona pin voltage of 4 kV.

Gas-phase APCI-MS was operated in full-scan mode over the *m*/*z* range of 40−250 (scan time, 0.45 s; interscan delay, 0.05 s). The chromatographic separations of tea headspace were carried out in duplicate at six different cone voltages (12, 15, 18, 21, 24, and 27 V), thus altering the fragmentation patterns of the individual volatiles. Eluting compounds were identified using retention time and mass spectral library matching (NIST 1998, Ringoes, NJ) of the unknown compounds against authentic standards (purity in brackets), sourced as below.

From Aldrich (Poole, United Kingdom) came dimethyl sulfide (99%), pentanal (97%), *E*-2-hexenal (98%), hexanal (98%), benzaldehyde (98%), *E*-2-octenal (94%), 6-methyl-5-hepten-2-one (99%), *E*,*E*-2,4-heptadienal (90%), *E*-2-heptenal (97%), heptanal (95%), 2-heptanone (98%), phenylacetaldehyde (90%), and linalool (97%). From Fluka (Buchs, Switzerland) came 2-methyl propanal (99%), 2-methyl butanal (90%), 3-methyl butanal (98%), and methyl salicylate (99%). From Acros Organics (Geel, Belgium) came *Z*-4-heptenal (96%). β-Damascenone (98%) and β-ionone (97%) were a gift from Firmenich SA (Geneva, Switzerland).

Standard Tea Headspace Sampling Procedure. The apparatus used to analyze the headspace of freshly infused tea is shown schematically in **Figure 1**.

The tea infusion was poured through a double layer of muslin into a polypropylene funnel connected to plastic tubing (20 cm, 5 mm i.d.). The tea infusion flowed along the tubing, through a hole in the neck of a borosilicate glass bottle (1 L, Fisher Scientific) with the base removed, and into a mug. The APCI-MS probe entered through a 1 mm hole in the bottle screwtop. Headspace was sampled at 25 mL/min into the ionization source through deactivated fused silica tubing (1 m × 0.53 mm i.d.) surrounded by a heated (160 °C) transfer line to prevent condensation of water or volatiles. A typical sampling time of headspace was 4−5 min.

Table 1. "Marker" Ions Chosen to Monitor Volatile Release from Tea Infusions by APCI-MS[a]

ion (*m*/*z*)	cone voltage (V)	compounds monitored by the APCI ion (molecular weight)	% of total peak area
63	18	dimethyl sulfide (62)	100
73	18	2-methyl propanal (72)	96
87	15	2-methyl butanal (86)	51
		3-methyl butanal (86)	33
		pentanal (86)	17
99	18	*E*-2-hexenal (98)	91
		unidentified compound	8
101	15	hexanal (100)	98
107	21	benzaldehyde (106)	95
109	21	*E*-2-octenal (126)	63
		6-methyl-5-hepten–2-one (126)	22
		unidentified compound	6
111	21	*E*,*E*-2,4-heptadienal (110)	49
		(*E*,*Z*-2,4-heptadienal) tentative (110)	43
		unidentified compound	6
113	15	*E*-2-heptenal (112),	49
		Z-4-heptenal (112)	28
		unidentified compound	13
115	15	heptanal (114),	79
		heptanone (114)	19
121	15	phenylacetaldehyde (120)	98
137	15	linalool (154)	78
		a mono terpene	15
153	21	methyl salicylate (152)	66
		unidentified compound	13
		unidentified compound	12
191	15	β-damascenone (190),	55
		β-ionone (192)	7
		unidentified compound	10
		unidentified compound	16
193	18	β-ionone (192)	87
		unidentified compound	12

[a] The identities of the compounds found at particular *m*/*z* and cone voltage values were obtained via GC-APCI-EI-MS. Named compounds were identified using authentic standards and comparing GC retention times and mass spectra. Note that only those peak areas contributing to ≥5% of total peak area have been included in this table (hence, contributions do not always tally to exactly 100%). Two replicate GC-EI/APCI-MS chromatograms for each ion/cone voltage combination were used to determine percentage peak area contributions.

The APCI system was calibrated by injecting (syringe pump) known concentrations of volatile compounds in hexane or cyclohexane solutions at 1.5 μL/min into the heated make up gas entering the APCI-MS (10 L/min) and measuring peak height. From the calibration curve, the quantity in any sample could be calculated by proportion taking into account the flow rate.

Data Processing. Raw data were processed using in-house software to smooth the data (moving five point average) and to extract a data point every 3 s. The data points were expressed as the intensity of the ion count (Imax) or as the cumulative counts (cumulative ion count; equivalent to area under the curve) during the sampling period (4−5 min).

Reproducibility of the Headspace Sampling Technique. Reproducibility of the tea infusion headspace sampling procedure was determined by carrying out 25 analyses of tea infusions prepared according to the standard technique. Values of cumulative ion count and I_{max} were determined for each of the ions of interest, and the corresponding % coefficient of variation (%CV = SD × 100/mean) values were calculated.

Effect of Preparation Water Temperature on Release of Volatiles from Tea Infusions. *Immediate Analysis of Tea Infusions.* Tea infusions were prepared according to the standard method using water of seven different temperatures (40, 50, 60, 70, 80, 90, and 100 °C). Five replicate infusions were prepared using water of each temperature and analyzed in random order. As soon as brewing was complete, infusions were poured into the mug via the sampling apparatus (**Figure 1**). The scan file was set to simultaneously monitor the intensity of 15 selected ions (**Table 1**), each at specified cone voltages (dwell time, 0.1 s; interscan delay, 0.02 s).

Analysis of Tea Infusions following 30 min of Incubation at 60 °C. Infusions were prepared as previously described using the same seven infusion water temperatures as above. As soon as brewing was complete, infusions were filtered and poured into 250 mL screwtop glass bottles (Fisher Scientific) and sealed immediately. Bottles were held in a water bath set at 60 °C for 30 min, before being poured into the mug through the sampling apparatus.

Effect of Infusion and Incubation Temperatures. Infusions were prepared as described previously using water at 40, 70, and 100 °C. As soon as brewing was complete, infusions were filtered and poured into 250 mL screwtop glass bottles and sealed immediately. Bottles of infusion were held in water baths set at 40, 60, and 80 °C for 30 min before being poured into the mug via the sampling apparatus. This experiment conformed to a full-factorial design; infusions made using each water temperature were incubated at each incubation temperature, resulting in nine possible combinations (five replicates of each).

Statistical Analysis. In order to explore the influence of both infusion and incubation temperatures on the volatile release, principal components analysis (PCA) was applied. The software used for PCA was Matlab v7.1 (The MathWorks, Inc, Natick, MA) in combination with the PLS toolbox v3.5.2 (Eigenvector Research, Inc., Wenatchee, WA).

RESULTS AND DISCUSSION

To determine which compounds in tea headspace could be reliably identified and monitored using APCI-MS analysis, volatiles from the headspace were collected on a SPME fiber and subjected to GC analysis by EI and APCI-MS detectors. The GC-EI/APCI-MS procedure provided a means to link the ions monitored by the APCI technique to the presence of specific compounds in the headspace of tea infusions. This procedure resulted in two chromatograms, both of which were very similar in both the compounds detected and their relative intensities, as noted by previous workers (*16*).

APCI cone voltage was used to distinguish between compounds that can produce the same *m*/*z* values following their ionization. **Figure 2** illustrates the effect of cone voltage on the assignment of ions to the presence of a particular compound. At cone voltage 15, the ion at *m*/*z* 121 can be almost entirely attributed to the presence of the protonated molecular ion of phenylacetaldehyde (11.98 min on the APCI-MS trace, MW 120). At cone voltage 27, however, methyl salicylate (MW 152) also produces an ion with *m*/*z* 121 due to the loss of methanol (14.76 min on the APCI-MS trace). Therefore, for the detection of phenylacetaldehyde in this system, the ion at *m*/*z* 121 was monitored using a cone voltage of 15 V.

Inspection of the other compounds, identified by retention times and the EI-MS library, showed three different levels of confidence in assigning ions to the presence of specific compounds in tea headspace. **Figure 3** shows ion chromatograms obtained from the APCI detector following the GC-EI/APCI-MS procedure. Trace A shows clearly that the ion at *m*/*z* 101 is almost entirely due to hexanal (retention time 6.89 min) with a very minor contribution from the compound at retention time 8.07 min. Six such compounds were identified in this category where a single ion accounted for over 90% of the signal at that *m*/*z* value (**Table 1**). Trace B shows that ions with *m*/*z* 87 are found at three different retention times, corresponding to 2- and 3-methyl butanal and pentanal. A further five ions were assigned to a set of isobaric or isomeric components (**Table 1**). Trace C is the ion chromatogram at *m*/*z* 139, which contains over 12 peaks including 2,6-nonadienal, an important aroma in tea. However, it is not possible to monitor 2,6-nonadienal with any degree of certainty due to the contributions from the other 11 compounds, and this shows the limitations of the technique.

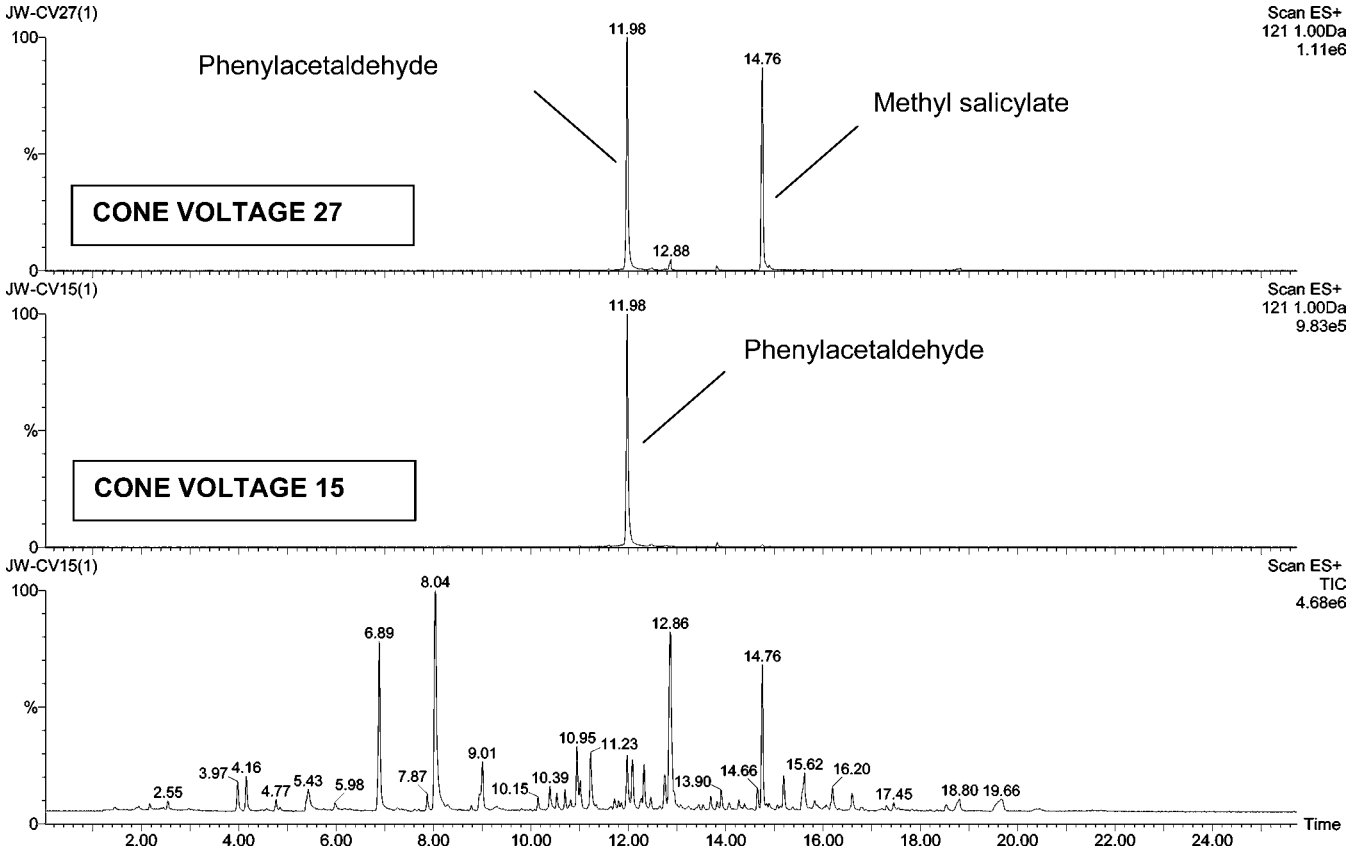

Figure 2. Chromatograms obtained using the GC-EI/APCI-MS procedure. The lower trace shows the TIC from the EI detector, and the upper traces show the intensity of ion 121 from the APCI detector when analyzed at cone voltages 15 and 27.

Many other ions showed similar behavior with too many compounds contributing to the ion mass for assignation of a compound (or a group of related compounds) to be made.

Examination of all of the ion chromatograms led to the selection of 15 ions that represented individual compounds or groups of compounds (**Table 1**). Six compounds of the tea headspace could be assigned to one ion mass and therefore be monitored and quantified with certainty. For two ion masses, *m*/*z* 111 and 113, the compounds involved were stereoisomers (heptadienal and heptenal, respectively), while for three ions, *m*/*z* 87, 109, and 115, the compounds were isobaric (2- and 3-methyl butanal, pentanal; 2-octenal, 6-methyl-5-hepten-2-one; heptanone, and heptanal, respectively). For the remaining four ions, some key volatile compounds from tea were present but were associated with some unknown compounds.

Reproducibility of the Headspace Sampling Technique. The reproducibility of the cumulative ion count and I_{max} for each of the 15 monitored ions as well as the total ion count (TIC) was calculated from analysis of 25 replicate tea infusions. Both cumulative ion count and I_{max} values yielded acceptable %CV values for all ions (most <5%) indicating a reproducible infusion and analytical technique. Irrespective of the data form, the highest values of %CV (about 8%CV) were obtained for ion *m*/*z* 191, which corresponds mainly to β-damascenone. The signal intensity for this ion was lower than the other compounds, and the lower signal:noise ratio for this compound may account for the greater variation.

Reality of the System. The system described is a compromise between one that tries to represent a realistic system and one that attempts to maintain a reasonable level of control. The real-life situation of volatile release from a mug of tea infusion is uncontrolled, particularly with regards to the impact of air movement on the transfer and distribution of volatiles from the infusion. Protecting the mug from the influence of the atmosphere has resulted in a technique that is both reliable and reproducible. It is, however, accepted that this environment does not completely represent the real-life situation, but it does allow comparisons to be made between different infusion preparation methods and tea blends.

Effect of Preparation Water Temperature on Release of Volatiles from Tea Infusions. The effect of water temperature on the release of volatiles into the headspace of the final beverage was studied using tea infusions prepared from 40 to 100 °C in steps of 10 °C. **Figure 4** shows the effect of temperature on the release of the isobaric aldehydes (2- and 3-methyl butanal and pentanal; *m*/*z* 87) and the ion at *m*/*z* 191 (mainly β-damascenone) illustrating two very different types of behavior. All other compounds showed a behavior intermediate between these extremes. Mean values of cumulative ion count were plotted and normalized to 100% release, allowing direct comparisons to be made between the compounds. For the aldehyde compounds represented by *m*/*z* 87, temperature had a linear effect on release into the headspace [the same pattern was also seen for *m*/*z* 73 (2-methylpropanal)], whereas for β-damascenone the relationship was better fitted by an exponential function; this was not due to the limit of detectability being approached.

Statistical analysis [one-way analysis of variance (ANOVA)] showed that infusion water temperature had a significant effect ($P = 0.05$) on the release of all volatile compounds monitored in tea headspace, release increasing as a function of increasing infusion water temperature. Posthoc testing in the form of Tukey's honestly significant difference (Tukey's HSD) test showed that, for the majority of compounds, raising the temperature in steps of 10 °C resulted in a significantly different level of release. This result agrees with the data obtained from

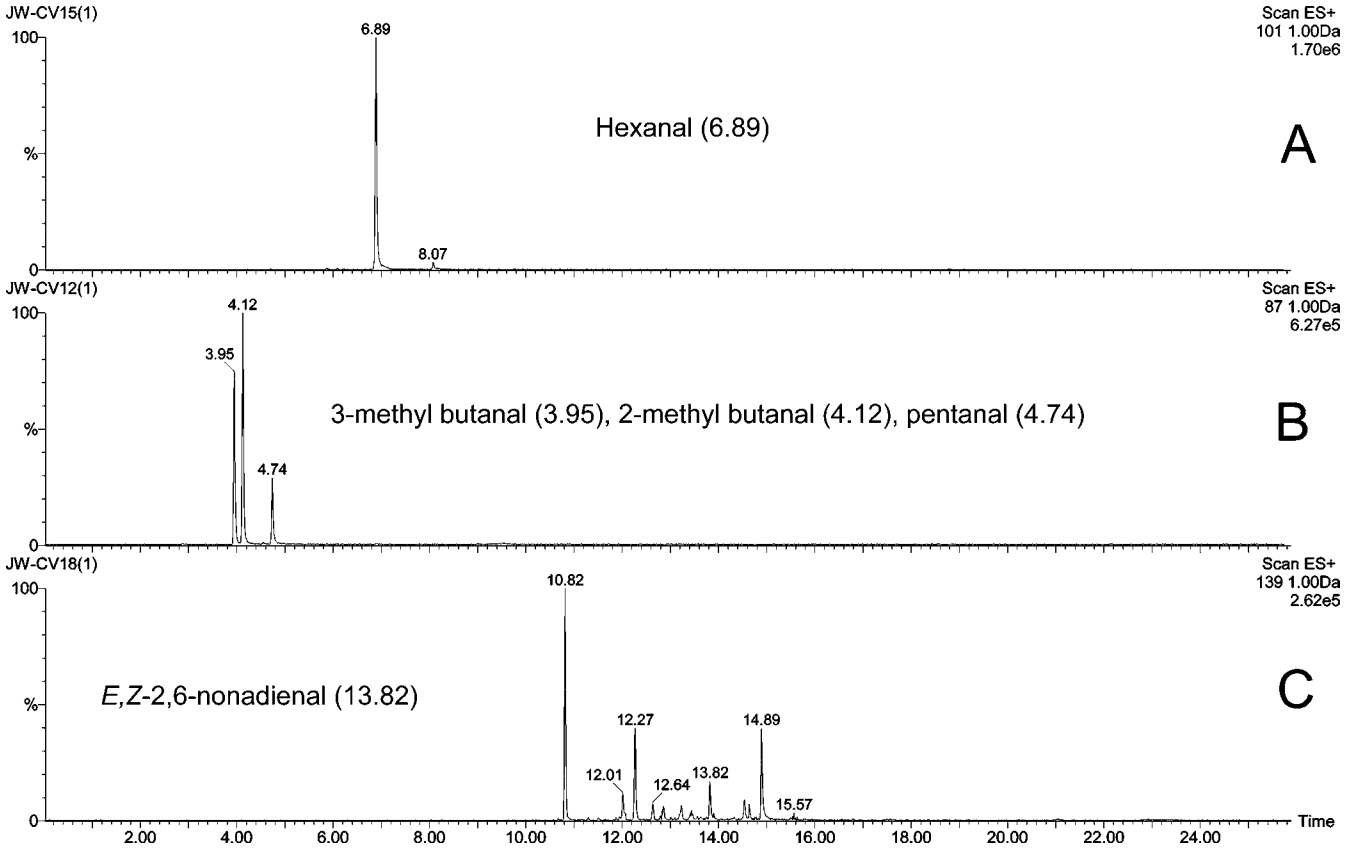

Figure 3. Ion traces showing (**A**) allocation of one compound to an ion; *m*/*z* 101 hexanal (**B**) allocation of groups of similar compounds to an ion; *m*/*z* 87 2- and 3-methyl butanals and pentanal and (**C**) where the many contributions to an ion mass make allocation impossible; *m*/*z* 139, contributions from over 12 compounds.

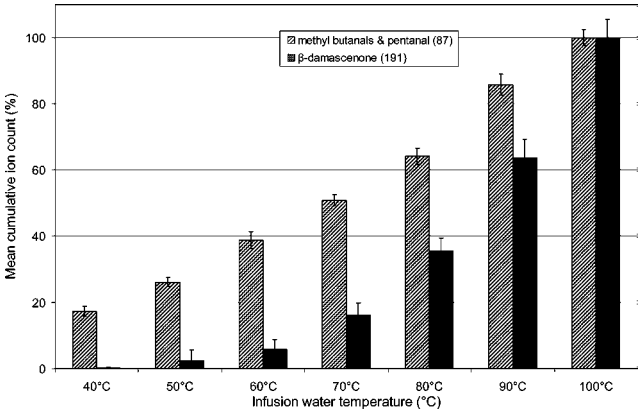

Figure 4. Effect of infusion water temperature on the release of different compounds from tea infusion headspace.

tea infused at 20 and 95 °C, which showed that extraction from tea leaf into the aqueous phase was very dependent on temperature although only data for three compounds were presented (*11*).

These results report the overall effect of infusion water temperature on volatile release into the headspace from tea infusions. However, from these data, it is not clear whether preparing tea using hotter water results in a greater extraction of volatile compounds from the dry tea leaf (i.e., greater concentration of volatiles in solution) or whether release levels observed are solely a function of the final temperature of infusion within the mug.

Vapor pressure of compounds increases as a function of temperature and subsequently causes an increase in the value of Henry's law constants and so gas−liquid partition coefficients. Solubility of volatile compounds also changes with temperature, again resulting in a change in the value of Henry's law constants. These basic physical laws may explain why the infusions prepared using hotter infusion water resulted in greater levels of volatile release into the headspace.

To test whether vapor pressure could explain the behaviors seen in **Figure 4**, the vapor pressures of the compounds were estimated using the Mpbpwin v1.41 program within the EPI suite software (U.S. Environmental Protection Agency, Washington, DC) using the Antoine (*20*) and modified grain methods (*21*). **Figure 5** shows the results using two compounds that exhibit the two types of behavior. β-Damascenone showed a good correlation between volatile release and vapor pressure with infusion water temperature (**Figure 5a**), whereas no such relationship was observed for dimethyl sulfide (**Figure 5b**). There are several explanations for this observation. One is that the estimation of vapor pressure from a software package makes assumptions about the ideality of the solutions and ignores interactions between solution components. It is well-known that interactions with nonvolatiles can affect the phase partitioning of volatile compounds (*22*, *23*), and polyphenolic compounds may account for up to 48.5% of the solids present in a cup of tea (*24*). It is almost inevitable that increasing the infusion water temperature also increases the amount of nonvolatile compounds extracted from the leaf. However, it is difficult to explain why some volatiles are affected and others are not on the basis of interactions. An alternative explanation is that the two types of behavior noted are due to different limiting factors in the release mechanisms. To investigate this concept further and decouple the effects of water temperature on extraction and on the partition of volatile compounds, tea samples were infused at

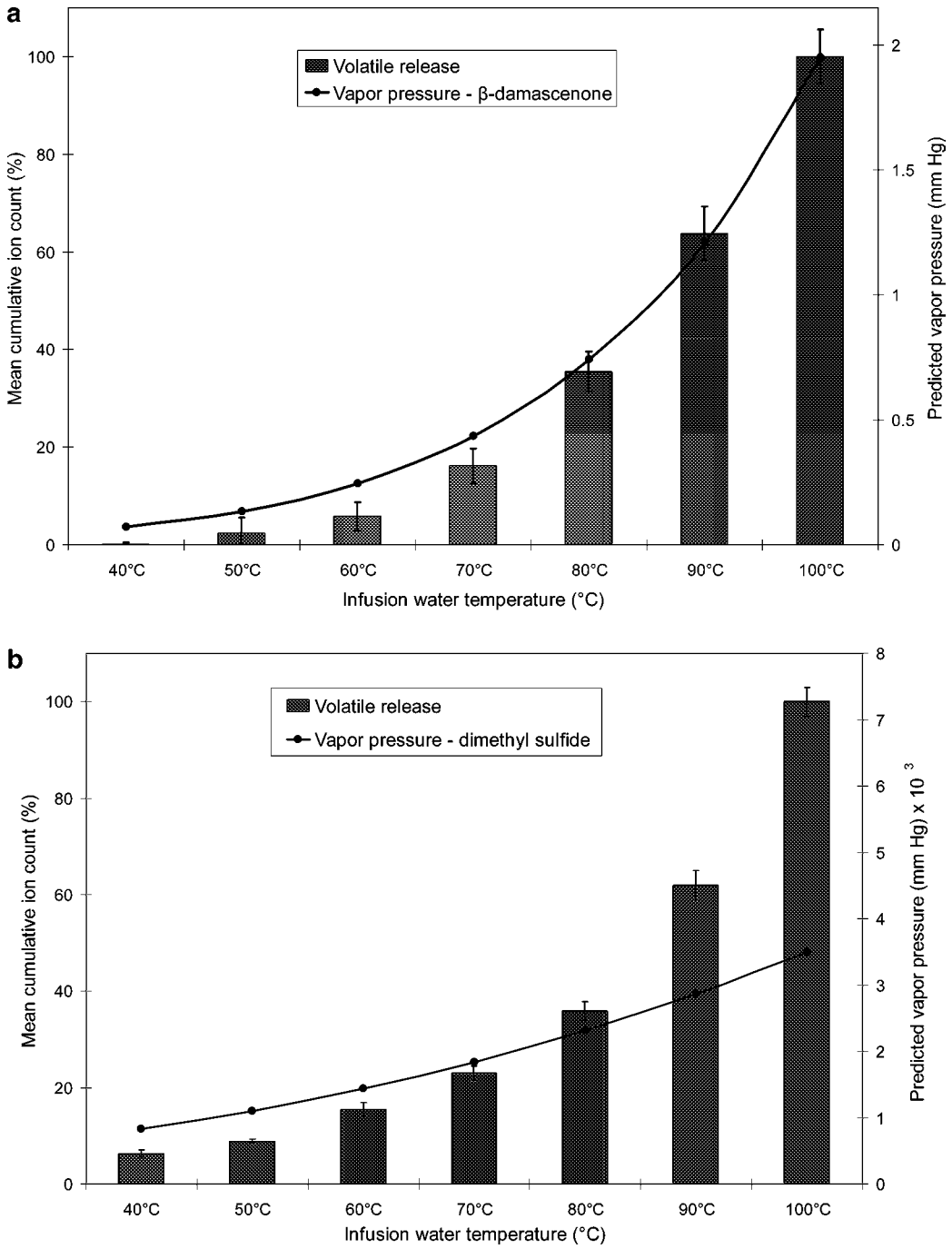

Figure 5. Effect of infusion water temperature on the release of β-damascenone (**a**) and dimethyl sulfide (**b**) from tea infusions showing good and poor correlation, respectively, with predicted values of vapor pressure.

different temperatures and then brought to a common temperature for analysis of volatile release as described below.

Decoupling the Effect of Temperature on Extraction and Partition of Volatile Compounds. Tea infusions were prepared at different temperatures and placed in sealed glass bottles at 60 °C for 30 min prior to analysis to ensure that all samples were the same temperature during analysis, irrespective of the temperature of infusion water used. **Figure 6** shows the effect of infusion water temperature on release of *E*-2-hexenal, immediately after infusion (i.e., at the infusion water temperature) and at the standard temperature of 60 °C.

While an increase in *E*-2-hexenal release as a function of infusion water temperature was evident in both experiments, the increase was far greater when infusions were analyzed immediately. It is clear that the reason for the increased levels of release when using higher temperature infusion water was at least partly due to the temperature of infusion within the mug. With an increase in vapor pressure, there was an increase in Henry's law constant and a corresponding increase in the air−water partition coefficient values leading to a greater release into the headspace.

While one-way ANOVA testing showed that infusion water temperature exhibited a significant effect ($P = 0.05$) on the release of all monitored compounds, relative differences in release behavior between compounds appeared to be more marked when all infusions were analyzed at a constant temperature.

In the final part of the experiment, infusions were prepared using one of three infusion water temperatures (40, 70, or 100 °C) and then held in sealed glass bottles in waterbaths at one

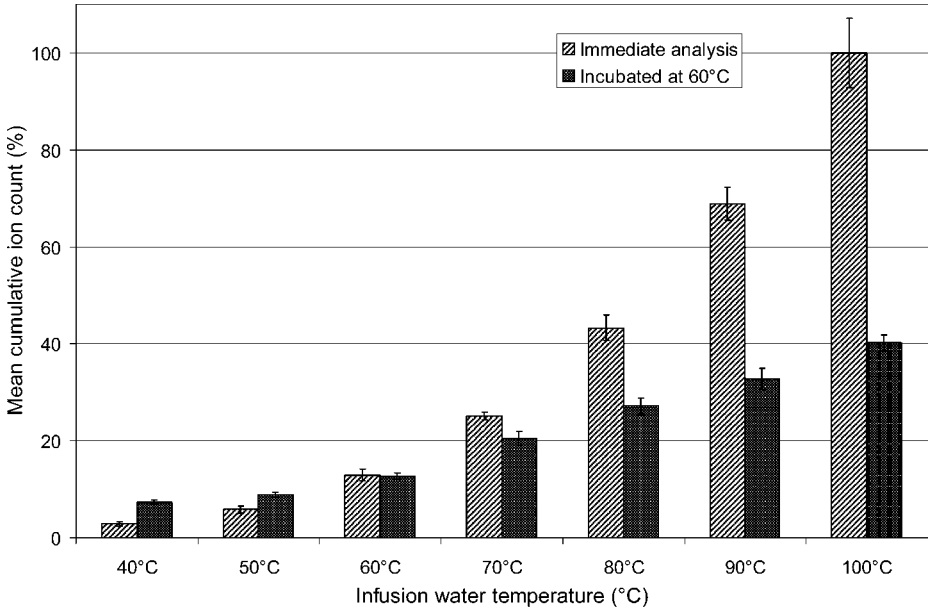

Figure 6. Effect of infusion water temperature on the release of *E*-2-hexenal (ion 99) when analyzed immediately and after incubation at 60 °C.

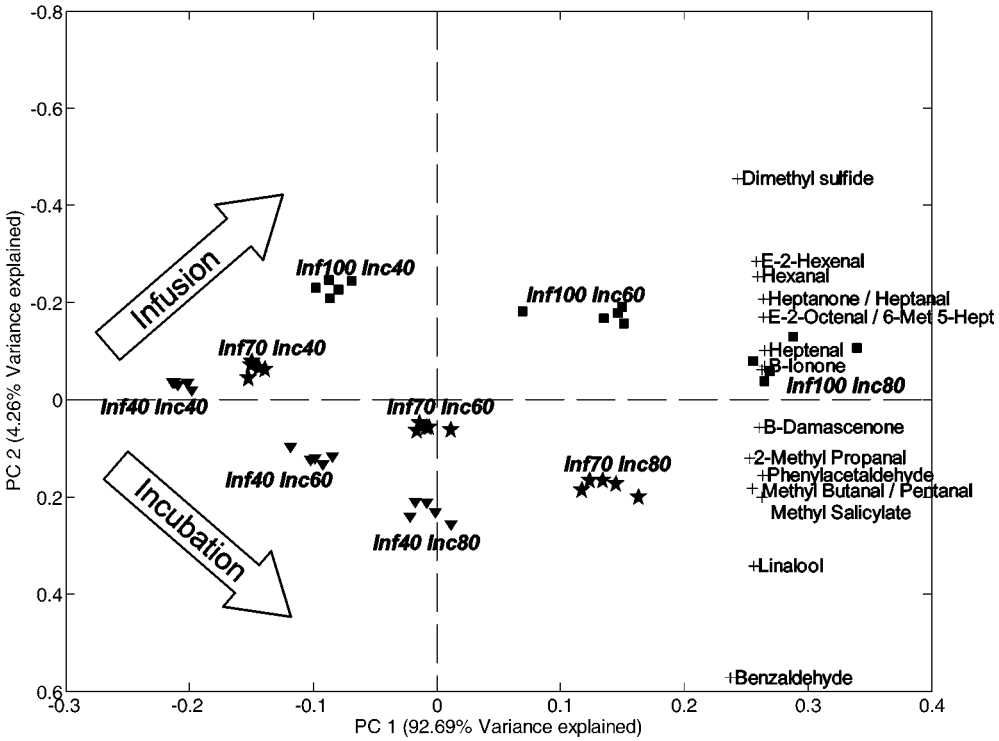

Figure 7. Biplot from PCA analysis of infusion and incubation data from tea samples infused at 40, 70, and 100 °C and then incubated at either 40, 60, or 80 °C. The infusion and incubation arrows show the direction of the effects, and the locations of the named compounds show the relative effects of infusion and incubation on their release. Five replicates of each treatment were prepared and analyzed.

of three incubation temperatures (40, 60, or 80 °C) for 30 min prior to analysis. This enabled the relative effect of release for both the infusion and the incubation temperatures to be determined.

General linear model univariate analysis (two-way ANOVA) confirmed that both the infusion and the incubation temperatures had significant ($P = 0.05$) effects on release of all monitored compounds from tea infusions. The individual data points (45 in all) were then analyzed using PCA. Prior to analysis, the data were autoscaled; that is, for each of the cumulative ion count values, the average for the respective ion was subtracted and divided by its standard deviation. This resulted in a data set where, for each ion, the average over all samples was 0 and the variance was 1. This preprocessing ensured that each ion had the same influence on the subsequent data analysis, irrespective of its absolute value or variation over different samples. This is a valid choice since the absolute values in the raw data depend as much on ionization efficiency as on concentration.

The PCA on the autoscaled data revealed that 97% of the total variance present in the data set could be explained with two principal components (PC) (**Figure 7**). Inspection of the biplot shows clusters of the five replicate measurements at each infusion (inf) and incubation (inc) temperature with clear trends as infusion temperature and incubation temperatures change. For instance, the three clusters of data for an incubation

temperature of 40 °C show that increasing the infusion temperature from 40 (▼) to 70 (★) to 100 °C (■) shows a linear change in release. The same linear pattern can be observed for incubation temperatures of 60 and 80 °C although there is now a clear incubation effect, working in the opposite direction to infusion. The arrows on the plot show the direction of the effects of infusion temperature and incubation temperature on volatile release. The individual volatile compounds are also included in the biplot, and their position is governed by the relative effect of infusion or incubation on their release. Dimethyl sulfide is the compound most affected by infusion temperature while benzaldehyde is the compound most affected by incubation. Compounds lying near the zero axis of PC2 are equally affected by both effects.

The interpretation of the biplot in **Figure 7** is that infusion and incubation temperatures are the major drivers of volatile release but that there is also a compound-dependent effect. The release of dimethyl sulfide as a function of infusion temperature did not correlate with vapor pressure (**Figure 5b**), but its release is very much influenced by infusion temperature (**Figure 7**). Inspection of the temperature data shows that between an infusion temperature of 70 and 100 °C, there is a large increase in release whereas the change between 40 and 70 °C is much smaller suggesting either that its release from the tea leaf is driven by different temperature effects to the other compounds or that it is generated from breakdown of other compounds during the infusion process. Benzaldehyde represents the opposite behavior as its release is not greatly affected by infusion temperature but is affected by incubation temperature. Possible explanations are that the extraction from tea leaves is not highly reliant on temperature whereas release during incubation in the absence of leaves is either due to partition, which is temperature-dependent, or that benzaldehyde is released from other components in the tea solution.

The literature contains detailed information on the release of nonvolatile compounds such as caffeine from tea leaf (*12*) and some data on the release of volatiles from dry tea leaf to the infusion (*11*). Increased amounts of the volatile compounds were found in the infusion as the temperature increased; however, the amounts of most of the volatiles recovered from the infusion were greater than the amounts extracted from dry tea, which led to two hypotheses; either solvent extraction of dry teas was inefficient or that other precursors of these tea volatiles exist that somehow release the measured compound on hydration. The data from Schuh and Schieberle (*11*) and from this study provide evidence that extraction of volatile compounds from leaf tea shows some anomalous behavior and the phenomenon needs further study to understand the origins of the behavior.

However, the technique developed in this paper was focused more on the effect of preparation on the composition of tea headspace rather than mechanisms of release. The on-line technique has limitations in that it can only follow the release of the "marker" compounds associated with the 15 ions monitored but, nonetheless, can deliver useful information about volatile release during tea preparation. The compounds studied cover a range of different physicochemical properties and include some sensorially significant tea compounds. The system is capable of differentiating the effects of infusion and incubation temperatures on aroma release in tea as well as identifying the compound-dependent nature of release. From these results, physical chemistry is the major driver for aroma release but there is a suggestion that some chemical changes also occur.

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