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Extraction kinetics of tea aroma compounds as a function brewing temperature, leaf size and water hardness

José A. Sánchez-López[1,2] | Sine Yener[3,4] | Samo Smrke[1] | Tilmann D. Märk[5] | Günther Bonn[4] | Ralf Zimmermann[2,6] | Franco Biasioli[3] | Chahan Yeretzian[1]

[1]Institute of Chemistry and Biotechnology, Zurich University of Applied Sciences (ZHAW), Wädenswil, Switzerland

[2]Joint Mass Spectrometry Centre, Chair of Analytical Chemistry, Institute of Chemistry, University of Rostock, Rostock, Germany

[3]Department of Food Quality and Nutrition, Research and Innovation Centre, Fondazione Edmund Mach, San Michele all'Adige, Italy

[4]Institute of Analytical Chemistry & Radiochemistry, Leopold Franzens University Innsbruck, Innsbruck, Austria

[5]Institute of Ion Physics and Applied Physics, Leopold-Franzens University of Innsbruck, Innsbruck, Austria

[6]Joint Mass Spectrometry Centre, Cooperation Group Comprehensive Molecular Analytics/CMA, Helmholtz Zentrum München, Neuherberg, Germany

Correspondence
Chahan Yeretzian, Institute of Chemistry and Biological Chemistry, Zurich University of Applied Sciences (ZHAW), 8820 Wädenswil, Switzerland.
Email: yere@zhaw.ch

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Abstract

Changes in the headspace volatile profile of tea infusion during brewing were determined by analysing the aliquots taken every 30 s using proton transfer reaction time-of-flight mass spectrometry in conjunction with principal component analysis and hierarchical cluster analysis. The effect of three different brewing temperatures (60, 70 and 80°C), two leaf sizes (broken and full leaves) and two water mineralizations (soft and hard), on the concentration of volatile compounds in the headspace of tea, was studied as a function of infusion time. An increase in brewing temperature resulted in increased volatile content in the headspace, reflecting an increase in extraction efficiency. Such differences on extraction efficiency were more pronounced with increasing brewing times. Leaf size had also a big impact on the extraction of volatile compounds, but mainly during the early phase of brewing. Water mineralization had a low but noticeable impact on the volatile content. Furthermore, clusters of samples prepared with different combinations of brewing parameters but resulting in analogous volatile profiles could be identified using hierarchical clustering analysis.

KEYWORDS
PTR-MS, tea aroma, tea infusion, volatile extraction

1 | INTRODUCTION

Tea, one of the world most popular beverages, is obtained from the hot water infusion of *Camellia sinensis* leaves. Tea leaves are divided into several main classes, such as green tea (unfermented), oolong tea (semi-fermented) and black tea (fermented), depending on the way they have been processed. Green tea is mainly produced and consumed in Asia; however, its sensory characteristics and health-promoting properties[1-3] have contributed to its growing popularity and increasing consumption in the Western world as well.

Green tea brewing is considered an art in some cultures, and recommendations on brewing temperature, brewing time, water-to-tea ratio or the number of repeated infusions (re-steeping) depend on the tea variety, region and individual preferences. Extensive research has also been performed on the extraction kinetics of

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This article is part of the special issue of the *Flavour and Fragrance Journal* entitled "On-line monitoring of odorants using direct-injection mass spectrometry" edited by Jean-Luc Le Quere health-related green tea compounds such as flavanols, flavonols, catechins or caffeine as a function of brewing parameters.[4-8] In the case of volatile aroma compounds, previous studies have focused on the analysis of either the dry leaves or the final infusion, with the aim of characterizing the aroma profile of different tea varieties,[9-13] discrimination of tea according to variety,[14] grade,[15] fermentation[16] or origin,[17] and on the impact of some parameters such as the temperature on the final volatile composition of the infusion.[18] To the best of our knowledge, no work has been performed on the extraction dynamics of tea volatiles and how different brewing parameters can influence the volatile profile of the tea infusion over the brewing time.

In this work, we investigated the effect of three different extraction parameters—water temperature, leaf size and water mineralization—on the composition of the infusion as a function of extraction times. This was performed by analysing the headspace (HS) of aliquots taken all along the brewing time. The concentration of volatile organic compounds (VOC) in the HS of tea samples was measured by proton transfer reaction time-of-flight mass spectrometry (PTR-ToF-MS). The use of this fast and highly sensitive direct injection mass spectrometry technique allowed for the direct sampling and monitoring of the HS of tea infusions without the need for any pre-concentration step.[19,20] PTR-ToF-MS allows not only the recording of the overall mass spectral profile of each sample. Due to its high mass resolution, mass spectral ion peaks can also be tentatively assigned to specific compounds.[21] Identification was done based on an in-house library. It includes results from former studies and compounds identified in previous experiments as well as data on a large number of standards.

In the following, we will discuss the impact of the three brewing parameters on the extraction dynamics of VOC. In a first part, we will focus on the whole volatile profile (fingerprinting) by using principal component analysis (PCA) and hierarchical clustering analysis (HCA). In a second part, we will have a closer look on one aroma compounds of green tea infusions—dimethylsulphide.[17]

2 | MATERIALS AND METHODS

2.1 | Tea samples

Full leaves of Gunpowder Chinese green tea from the province Zhejiang of the highest quality grade (3505AAA), from last harvest before experimentations (2016), were selected for these experiments. They were purchased in a local specialty shop (Schwarzenbach Kolonialwaren; www.schwarzenbach.ch/) and stored in its original bag at room temperature before analysis. Gunpowder tea leaves are withered, steamed, rolled and then dried. Rolling renders the leaves less susceptible to physical damage and breakage allowing to obtain a homogenize batch of intact full leaves. Furthermore, rolled full leaves allow retaining more of their flavour and aroma, providing a strong and aromatic tea.

A portion of the full leaves was chopped (broke) with a kitchen blender in order to obtain broken leaves of a few millimetres average size. Three grams of tea leaves (either full or broken) were infused in 150 mL water at three different temperatures (60, 70 and 80°C) using two commercial waters of different hardness: Aqua Panna (AP—solid residue: 139 mg L^{-1}; HCO_3^-: 103 mg L^{-1}; SO_4^{2-}: 22 mg L^{-1}; NO_3^-: 2.9 mg L^{-1}; Cl^-: 8.5 mg L^{-1}; Na^+: 6.4 mg L^{-1}; Ca^{2+}: 32 mg L^{-1}; Mg^{2+}: 6.2 mg L^{-1}. Total hardness: 105 mg L^{-1} of $CaCO_3$) and San Benedetto (SB—solid residue: 265 mg L^{-1}; HCO_3^-: 313 mg L^{-1}; SO_4^{2-}: 3.7 mg L^{-1}; NO_3^-: 9 mg L^{-1}; Cl^-: 2.2 mg L^{-1}; Na^+: 6 mg L^{-1}; Ca^{2+}: 50.3 mg L^{-1}; Mg^{2+}: 30.8 mg L^{-1}. Total hardness: 252 mg L^{-1} of $CaCO_3$). Samples were gently agitated during infusion with a magnetic stirrer. Each infusion was performed in triplicate. In summary, 180 measurements were performed including 3 temperatures × 2 leaf sizes × 2 waters × 5 time points × 3 replicates.

Highlights

- Extraction kinetics of VOCs, using PTR-ToF-MS, as a function of water temperature, mineralization and leaf size
- Increase in brewing temperature (60, 70, 80°C) resulted in increased extraction efficiency
- Leaf size had a big impact on extraction, mainly during the early phase of brewing; finer grind resulted in faster extraction
- Water mineralization had a low but noticeable impact

2.2 | Analysis of tea volatiles by PTR-ToF-MS

The VOC of the tea infusions were analysed by PTR-ToF-MS using direct injection HS analysis. During the first five min of each tea infusion, 1 mL aliquots were taken by a micropipette every 30 s, filtered and transferred into 22-mL glass vials (Supelco). The vials were placed into a cooling tray at 4°C until measurement. The sampling order was randomized to prevent memory effects.

The HS measurements were performed by using a commercial PTR-ToF-MS 8000 instrument (Ionicon Analytik GmbH). The instrumental conditions in the drift tube were set as following: drift voltage 550 V, drift temperature 110°C, drift pressure 2.33 mbar affording an E/N value (electric field strength/gas number density) of 140 Townsend (Td, 1 Td = 10^{-21} V m^2). All vials containing samples as well as blank (hot water) were incubated at 37°C for 30 min before HS analysis. The HS mixture was directly injected into the PTR-MS drift tube with a flow rate of 40 sscm via a PEEK tube. Sample injection was performed with a multipurpose autosampler (Gerstel GmbH). The sampling order was randomized, and one sample was analysed every 5 min. Each sample was measured for 30 s, over the time window from 0 to 30 s of the release curve. Considering that vials were incubated before HS analysis, the signal intensity was essentially constant over the 0-30 s time window, at an acquisition rate of one spectrum per s. Conclusions are not affected whether average intensity or the integral over the selected time window was used for data analysis. Here we chose to work with the average intensity over the 0-30 s time window.

2.3 | Data processing and analysis

2.3.1 | Treatment of mass spectrometric data

Raw mass spectrometric data were corrected for dead time and calibrated, and peaks were extracted according to the strategy and procedure as described elsewhere,[22] leading to a mass accuracy ≥0.001 Th which allowed sum formula determination. After background subtraction and peak detection, peak area extraction was performed by using a modified Gaussian to fit the data.[23] The volatile concentrations in ppbv for each detected peak were calculated from the amount of ions signal, according to the formulas described by Lindinger et al,[19] assuming a constant reaction rate coefficient ($k_R = 2\times10^{-9}$ cm^3 s^{-1}).

2.3.2 | Selection of mass peaks

The direct injection HS analysis of tea infusions resulted in the monitoring of 447 mass peaks in the range 15-250 m/z. After eliminating the interfering ions (O_2^+, NO^+ and water clusters) and their isotopologues, 430 mass peaks remained for further analysis. The signals belonging to blank vials were subtracted from the whole dataset, and only signals higher in intensity than 0.1 ppb were included. After this step, 88 PTR-ToF-MS mass peaks were left for further analysis, for each sample.

After mass peak selection and extraction, tentative peak identification was performed using an in-house library developed by the authors where the peak annotations were done automatically with the scripts developed under R programming language.[24] This finally leads to 34 compounds that could be identified with high confidence. Their concentrations (ppb) in gunpowder tea infusions after 5 min of brewing are listed in Table 1, for all the different brewing conditions investigated here. Table 1 also includes information on statistically significant differences, according to ANOVA ($P < .01$).

2.3.3 | Statistical analyses

Principal component analysis (PCA) was performed on mean centred and unit variance scaled data considering 88 mass peaks and 180 individual measurements. To identify conditions leading to similar volatile profiles, both the raw intensity and the intensity normalized to the intensity at 5 min (last time point) were subjected to hierarchical cluster analysis (HCA) using Ward's minimum variance method and half-squared Euclidean distances. Significant differences between HS concentrations were calculated using ANOVA and Tukey's test ($P < .01$). Statistical analyses were performed using the scripts and packages developed under R programming language.[24]

3 | RESULTS AND DISCUSSION

3.1 | Principal component analysis of the tea brewing process

Principal component analysis of the 180 different measurement points allowed visualization of the different parameters influencing the aroma profile during tea preparation (Figure 1). Time and temperature both showed a clear and consistent impact on the extraction of VOCs. The first principal component (PC1) separated samples according to the brewing time. All PC1 loadings were positive (data not shown), thereby indicating that an increase in extraction time resulted in higher concentration of VOC (higher extraction yield). The same effect was observed with increasing temperature, separating samples extracted at different temperatures along the first principal component PC1 axis. Analogous to the impact of brewing time, higher PC1 scores correspond to higher temperature and higher extraction yield. The second principal component shows some differentiation for leave size, and, although PC2 carried only 2.5% of the variance, it allowed differentiation between infusions prepared from full leaves and those from broken leaves.

A PCA with all samples (left, large frame of Figure 1) provides an overall insight and information about the evolution of the volatile profile. Yet, in order to better visualize how the different brewing parameter affects the volatile profile over time, data were split and presented as four individual PCAs, each for a specific brewing time: 0.5, 2, 3.5 and 5 min (right frames, Figure 1).

30 s: After 30 s of extraction, only infusions from full and broken leaves can be separated on the PCA, resulting in a separation along the first component (PC1) on the score plot. At this early stage of the extraction process, broken leaves allow for a higher extraction efficiency. In contrast, no separation for brewing temperature or mineral content is observed.

2 min: As brewing time increases (2 min), the effect of temperature becomes more evident. Samples with the same leaf size but prepared at different temperatures are starting to separate in the space of the two first components. Samples infused at the same temperature but with different leaf sizes could also be distinguished. Hence, while separation by leaf size is still visible (as for 30 s), we start observing in addition a separation by temperature. On the other hand, some infusions from different leaves sizes at different temperatures (ie broken leaves at 60°C and full leaves at 80°C) did not show separation on the PCA. By observing individual leaf size and temperature combinations, samples infused with different waters were partially separated by the second component. This was the only infusion time point at which the effect of water mineralization could be seen on the PCA.

3.5 min: After 3.5 min of infusion, the different temperatures within one leaf size were separated along the first principal

TABLE 1 Concentration (ppb) of tentatively identified mass peaks in the headspace of gunpowder tea infusions after 5 min of brewing in different conditions. Data followed by different letters are significantly different according to ANOVA ($P < .01$)

Measured m/z	Theoretical m/z	Sum formula	Tentative identification	Broken leaves			
				Soft water			Hard water
				60°C	70°C	80°C	60°C
33.0327	33.033	CH_4OH^+	Methanol	40.79 ± 7.34^{a}	45.37 ± 4.76^{ab}	44.76 ± 0.69^{ab}	48.76 ± 1.62^{bc}
45.0334	45.034	$C_2H_4OH^+$	Acetaldehyde	77.39 ± 6.61^{ab}	111.59 ± 5.56^{cd}	151.61 ± 1.21^{f}	96.45 ± 9.47^{c}
49.0108	49.011	CH_4SH^+	Methanethiol	0.82 ± 0.21^{bce}	0.91 ± 0.36^{de}	1.15 ± 0.32^{e}	0.39 ± 0.08^{ac}
55.054	55.054	$C_4H_7^+$	1,3-Butadiene	12.18 ± 0.75^{bc}	15.16 ± 1.01^{e}	16.84 ± 1.68^{ef}	12.28 ± 0.71^{bc}
63.026	63.026	$C_2H_6SH^+$	Dimethyl sulfide	22.00 ± 3.23^{a}	28.32 ± 2.41^{bd}	34.22 ± 1.63^{ef}	25.32 ± 2.55^{abc}
69.0327	69.034	$C_4H_4OH^+$	Furan fragment	1.44 ± 0.20^{ab}	1.68 ± 0.19^{bcd}	1.54 ± 0.08^{ac}	1.51 ± 0.11^{ac}
69.0696	69.07	$C_5H_8H^+$	Isoprene	14.49 ± 1.54^{ab}	17.06 ± 1.1^{bd}	19.03 ± 2.77^{d}	14.55 ± 0.75^{ab}
71.0488	71.049	$C_4H_6OH^+$	Butenal	0.86 ± 0.06^{ac}	1.08 ± 0.01^{de}	1.30 ± 0.04^{fg}	0.91 ± 0.07^{ac}
73.0644	73.065	$C_4H_8OH^+$	Methylpropanal	25.34 ± 2.82^{ab}	30.35 ± 1.91^{cde}	32.27 ± 0.87^{de}	27.56 ± 2.07^{bc}
75.0436	75.044	$C_3H_6O_2H^+$	Propionic acid	1.68 ± 0.05^{a}	2.03 ± 0.23^{a}	1.74 ± 0.16^{a}	1.77 ± 0.10^{a}
81.0694	81.07	$C_6H_8H^+$	Terpene fragment (Cyclohexadiene)	2.97 ± 0.41^{ac}	3.60 ± 0.70^{cd}	3.62 ± 0.33^{cd}	2.95 ± 0.17^{ac}
83.0485	83.049	$C_5H_7O^+$	Methylfuran	2.69 ± 0.22^{a}	3.12 ± 0.24^{bcd}	3.11 ± 0.20^{bcd}	2.78 ± 0.29^{ab}
83.0853	83.086	$C_6H_{20}H^+$	Cyclohexene (Terpene fragment)	5.43 ± 0.28^{b}	6.73 ± 0.21^{df}	7.45 ± 0.94^{ef}	5.48 ± 0.46^{bc}
85.0643	85.065	$C_5H_8OH^+$	Pentenal Pentenone	2.01 ± 0.14^{a}	3.06 ± 0.26^{b}	4.17 ± 0.10^{cd}	2.22 ± 0.22^{a}
87.0432	87.044	$C_4H_6O_2H^+$	Butanedione	1.07 ± 0.03^{ab}	1.34 ± 0.07^{cd}	1.4 ± 0.09^{de}	1.24 ± 0.09^{bcd}
87.0799	87.08	$C_5H_{20}OH^+$	Pentenol	9.51 ± 0.92^{ac}	11.41 ± 0.52^{def}	11.89 ± 0.33^{ef}	10.22 ± 0.74^{bcd}
95.0851	95.086	$C_7H_{20}H^+$	Methylcyclohexadiene (Terpene fragment)	1.14 ± 0.11^{bc}	1.45 ± 0.17^{e}	1.59 ± 0.08^{ef}	1.16 ± 0.10^{bc}
96.0815	96.081	$C_6H_9NH^+$	Ethylpyrrole	0.64 ± 0.09^{ac}	0.76 ± 0.04^{cd}	0.72 ± 0.04^{bcd}	0.67 ± 0.09^{ad}
97.0641	97.065	$C_6H_8OH^+$	Hexadienal Ethylfuran	4.55 ± 0.57^{ac}	5.45 ± 0.65^{ce}	5.15 ± 0.27^{bce}	4.79 ± 0.48^{acd}
99.08	99.08	$C_6H_{20}OH^+$	Hexenal Methylpentenone	2.02 ± 0.20^{ab}	2.33 ± 0.14^{cf}	2.43 ± 0.06^{def}	2.15 ± 0.19^{bcd}
101.0953	101.096	$C_6H_{22}OH^+$	Hexenol	0.95 ± 0.15^{ab}	1.21 ± 0.13^{deg}	1.28 ± 0.02^{fg}	1.05 ± 0.08^{bd}
107.0481	107.049	$C_7H_6OH^+$	Benzaldehyde	1.11 ± 0.02^{ac}	1.16 ± 0.05^{ac}	1.19 ± 0.14^{ac}	1.23 ± 0.11^{ac}
109.1005	109.101	$C_8H_{22}H^+$	Cyclooctadiene	1.36 ± 0.05^{ac}	1.48 ± 0.11^{ac}	1.48 ± 0.19^{ac}	1.38 ± 0.08^{ac}
111.0798	111.08	$C_7H_{20}OH^+$	Heptadienal	0.97 ± 0.08^{ab}	1.35 ± 0.18^{bcd}	1.48 ± 0.18^{cd}	0.98 ± 0.14^{ab}
113.0955	113.096	$C_7H_{22}OH^+$	Heptenal	0.25 ± 0.04^{ab}	0.31 ± 0.03^{bd}	0.36 ± 0.03^{d}	0.25 ± 0.04^{ab}
115.1113	115.112	$C_7H_{24}OH^+$	Heptanone	0.60 ± 0.05^{ab}	0.71 ± 0.06^{bcdf}	0.73 ± 0.04^{cdf}	0.65 ± 0.06^{ad}
121.1006	121.101	$C_9H_{22}H^+$	Methylethylbenzene	0.33 ± 0.06^{ab}	0.38 ± 0.08^{ac}	0.4 ± 0.02^{bc}	0.36 ± 0.06^{ac}
123.1163	123.117	$C_9H_{24}H^+$	Santene	0.74 ± 0.08^{ac}	0.91 ± 0.13^{ef}	0.91 ± 0.06^{ef}	0.74 ± 0.05^{ac}
127.1112	127.112	$C_8H_{24}OH^+$	Octenone Methylheptenone	0.48 ± 0.01^{ac}	0.52 ± 0.08^{bc}	0.54 ± 0.06^{bc}	0.46 ± 0.03^{ac}
131.1059	131.107	$C_7H_{24}O_2H^+$	Heptanoic acid hexyl formate	0.15 ± 0.03^{ab}	0.16 ± 0.04^{ab}	0.14 ± 0.01^{ab}	0.16 ± 0.02^{ab}
135.1158	135.117	$C_{20}H_{24}H^+$	Methylpropylbenzene	0.17 ± 0.02^{bcde}	0.18 ± 0.03^{cde}	0.19 ± 0.00^{ef}	0.17 ± 0.02^{ade}
137.1317	137.133	$C_{20}H_{26}H^+$	Various monoterpenes	0.31 ± 0.04^{ac}	0.36 ± 0.07^{bce}	0.36 ± 0.04^{ce}	0.31 ± 0.01^{ac}
139.1109	139.112	$C_9H_{24}OH^+$	Nonadienal	1.09 ± 0.09^{ac}	1.30 ± 0.21^{ce}	1.35 ± 0.10^{def}	1.07 ± 0.08^{ac}
141.1263	141.127	$C_9H_{26}OH^+$	Nonenal	0.28 ± 0.02^{ac}	0.31 ± 0.03^{ce}	0.31 ± 0.01^{cd}	0.28 ± 0.02^{ac}

component axis. Different leaf sizes within one temperature could be differentiated as well, although the differences between sizes at 80°C became minimal.

5 min: At the end of the infusion (5 min), the first component allowed only separation of infusions from the same leaf size at different temperatures, and it was the second component that allowed

		Full leaves					
		Soft water			**Hard water**		
70°C	**80°C**	**60°C**	**70°C**	**80°C**	**60°C**	**70°C**	**80°C**
47.54 ± 4.27^{ab}	55.50 ± 4.14^{cd}	48.52 ± 1.71^{bc}	51.20 ± 1.89^{b}	55.53 ± 0.63^{cd}	47.14 ± 1.17^{ab}	55.90 ± 2.62^{d}	56.83 ± 1.60^{d}
130.50 ± 6.39^{e}	174.99 ± 5.69^{g}	68.35 ± 2.64^{a}	93.69 ± 8.09^{bc}	129.78 ± 9.36^{de}	75.60 ± 8.88^{ab}	108.59 ± 10.26^{c}	151.26 ± 14.03^{f}
0.59 ± 0.14^{acd}	0.74 ± 0.28^{bce}	0.49 ± 0.08^{acd}	0.58 ± 0.07^{acd}	0.83 ± 0.33^{ce}	0.24 ± 0.04^{a}	0.36 ± 0.05^{ab}	0.53 ± 0.05^{acd}
14.70 ± 1.35^{de}	17.92 ± 0.52^{f}	9.44 ± 0.54^{a}	12.04 ± 0.57^{bc}	15.61 ± 0.55^{e}	10.65 ± 0.98^{ab}	12.96 ± 0.53^{cd}	16.85 ± 1.27^{ef}
30.59 ± 0.77^{cde}	38.43 ± 1.14^{fg}	21.00 ± 1.69^{a}	28.12 ± 2.71^{bd}	34.35 ± 3.63^{eg}	24.31 ± 1.20^{ab}	31.31 ± 2.72^{de}	39.81 ± 3.45^{g}
1.63 ± 0.03^{ad}	1.69 ± 0.07^{bcd}	1.40 ± 0.13^{a}	1.65 ± 0.10^{ad}	1.73 ± 0.11^{cd}	1.46 ± 0.13^{ac}	1.72 ± 0.07^{cd}	1.85 ± 0.09^{d}
16.8 ± 1.75^{bd}	18.79 ± 1.51^{d}	11.74 ± 0.76^{a}	14.19 ± 0.89^{ab}	17.27 ± 1.54^{bd}	13.03 ± 1.57^{a}	15.08 ± 0.73^{abc}	18.35 ± 1.68^{cd}
1.15 ± 0.14^{ef}	1.35 ± 0.05^{g}	0.76 ± 0.09^{a}	0.95 ± 0.03^{bcd}	1.27 ± 0.09^{fg}	0.78 ± 0.08^{ab}	0.98 ± 0.07^{cd}	1.37 ± 0.08^{g}
31.03 ± 1.04^{cde}	34.59 ± 0.79^{e}	22.81 ± 1.42^{a}	28.53 ± 2.43^{bd}	33.74 ± 1.93^{e}	24.13 ± 3.09^{ab}	30.23 ± 2.11^{cde}	34.15 ± 1.89^{ef}
1.76 ± 0.13^{a}	1.98 ± 0.16^{a}	1.80 ± 0.33^{a}	1.86 ± 0.23^{a}	2.09 ± 0.26^{a}	1.66 ± 0.10^{a}	1.91 ± 0.19^{a}	2.00 ± 0.15^{a}
3.38 ± 0.26^{bcd}	4.12 ± 0.53^{d}	2.64 ± 0.21^{ab}	3.04 ± 0.11^{ac}	3.45 ± 0.16^{cd}	2.32 ± 0.10^{a}	3.08 ± 0.15^{bc}	3.65 ± 0.27^{cd}
3.16 ± 0.08^{bcd}	3.27 ± 0.18^{cd}	2.61 ± 0.01^{a}	2.98 ± 0.17^{ac}	3.31 ± 0.16^{cd}	2.65 ± 0.21^{a}	3.20 ± 0.11^{cd}	3.48 ± 0.10^{d}
6.58 ± 0.61^{cde}	7.79 ± 0.54^{f}	4.03 ± 0.12^{a}	5.16 ± 0.32^{ab}	6.74 ± 0.51^{df}	4.81 ± 0.58^{ab}	5.79 ± 0.22^{bd}	7.20 ± 0.61^{ef}
3.14 ± 0.11^{b}	4.83 ± 0.12^{d}	1.85 ± 0.07^{a}	2.84 ± 0.13^{b}	4.19 ± 0.19^{c}	1.94 ± 0.17^{a}	3.14 ± 0.23^{b}	4.95 ± 0.14^{d}
1.38 ± 0.11^{de}	1.57 ± 0.12^{e}	1.01 ± 0.08^{a}	1.14 ± 0.02^{ac}	1.32 ± 0.11^{cd}	0.95 ± 0.15^{a}	1.16 ± 0.03^{ac}	1.33 ± 0.14^{cd}
11.35 ± 0.62^{def}	12.51 ± 0.34^{f}	8.31 ± 0.54^{a}	10.14 ± 0.81^{bcd}	12.14 ± 0.62^{ef}	8.74 ± 1.26^{ab}	10.78 ± 0.57^{ce}	12.13 ± 0.48^{ef}
1.39 ± 0.07^{de}	1.70 ± 0.06^{f}	0.95 ± 0.07^{ab}	1.19 ± 0.01^{cd}	1.54 ± 0.14^{ef}	0.90 ± 0.06^{a}	1.22 ± 0.05^{cd}	1.52 ± 0.06^{ef}
0.73 ± 0.03^{cde}	0.75 ± 0.01^{cd}	0.57 ± 0.06^{a}	0.64 ± 0.07^{ac}	0.75 ± 0.09^{cd}	0.58 ± 0.09^{ab}	0.72 ± 0.04^{cd}	0.79 ± 0.03^{d}
5.38 ± 0.18^{ce}	5.41 ± 0.16^{ce}	4.06 ± 0.22^{a}	4.78 ± 0.39^{acd}	5.52 ± 0.32^{de}	4.27 ± 0.53^{ab}	5.25 ± 0.41^{ce}	5.75 ± 0.17^{e}
2.31 ± 0.02^{bcf}	2.56 ± 0.13^{f}	1.84 ± 0.09^{a}	2.03 ± 0.19^{ac}	2.41 ± 0.08^{def}	1.78 ± 0.14^{a}	2.26 ± 0.15^{bce}	2.55 ± 0.07^{ef}
1.18 ± 0.06^{cdef}	1.39 ± 0.07^{g}	0.75 ± 0.05^{a}	1.00 ± 0.07^{bc}	1.24 ± 0.03^{deg}	0.88 ± 0.09^{ab}	1.07 ± 0.06^{be}	1.27 ± 0.15^{eg}
1.31 ± 0.19^{c}	1.28 ± 0.06^{bc}	0.99 ± 0.18^{ab}	1.01 ± 0.05^{ac}	1.09 ± 0.06^{a}	1.03 ± 0.24^{ac}	0.96 ± 0.03^{ac}	1.19 ± 0.25^{ac}
1.52 ± 0.22^{acd}	1.62 ± 0.14^{c}	1.23 ± 0.11^{a}	1.36 ± 0.03^{ac}	1.45 ± 0.12^{ac}	1.25 ± 0.15^{ab}	1.49 ± 0.14^{ac}	1.57 ± 0.28^{bc}
1.44 ± 0.35^{cd}	1.77 ± 0.22^{d}	0.75 ± 0.14^{a}	1.05 ± 0.10^{ac}	1.41 ± 0.19^{bcd}	0.77 ± 0.06^{a}	1.15 ± 0.08^{ac}	1.63 ± 0.35^{d}
0.32 ± 0.04^{bd}	0.38 ± 0.06^{d}	0.22 ± 0.01^{a}	0.26 ± 0.01^{ab}	0.34 ± 0.03^{cd}	0.22 ± 0.02^{a}	0.27 ± 0.03^{abc}	0.36 ± 0.02^{d}
0.67 ± 0.02^{bcde}	0.81 ± 0.05^{f}	0.54 ± 0.03^{a}	0.61 ± 0.05^{ac}	0.75 ± 0.03^{df}	0.55 ± 0.05^{a}	0.64 ± 0.04^{ad}	0.78 ± 0.10^{ef}
0.43 ± 0.05^{bc}	0.46 ± 0.06^{c}	0.34 ± 0.01^{ab}	0.36 ± 0.03^{ac}	0.42 ± 0.02^{bcd}	0.29 ± 0.01^{a}	0.36 ± 0.05^{ac}	0.43 ± 0.03^{bc}
0.89 ± 0.03^{def}	1.02 ± 0.07^{f}	0.65 ± 0.02^{ab}	0.75 ± 0.03^{bcd}	0.94 ± 0.04^{ef}	0.61 ± 0.05^{a}	0.81 ± 0.04^{ce}	0.97 ± 0.05^{f}
0.55 ± 0.10^{bc}	0.57 ± 0.07^{c}	0.37 ± 0.03^{a}	0.43 ± 0.03^{ab}	0.51 ± 0.06^{bc}	0.43 ± 0.08^{ab}	0.50 ± 0.02^{ac}	0.56 ± 0.07^{bc}
0.14 ± 0.01^{a}	0.17 ± 0.01^{ab}	0.16 ± 0.01^{ab}	0.18 ± 0.04^{ab}	0.18 ± 0.01^{ab}	0.15 ± 0.02^{ab}	0.18 ± 0.02^{ab}	0.19 ± 0.00^{b}
0.18 ± 0.01^{cdf}	0.22 ± 0.00^{f}	0.14 ± 0.02^{ab}	0.15 ± 0.01^{ac}	0.17 ± 0.01^{ade}	0.13 ± 0.01^{a}	0.15 ± 0.02^{ad}	0.18 ± 0.01^{df}
0.36 ± 0.05^{ce}	0.44 ± 0.04^{e}	0.27 ± 0.05^{ab}	0.33 ± 0.03^{acd}	0.34 ± 0.02^{acd}	0.26 ± 0.00^{a}	0.34 ± 0.02^{acd}	0.41 ± 0.03^{de}
1.29 ± 0.08^{ce}	1.56 ± 0.13^{f}	0.97 ± 0.06^{ab}	1.13 ± 0.04^{acd}	1.34 ± 0.02^{def}	0.89 ± 0.06^{a}	1.20 ± 0.09^{bce}	1.42 ± 0.16^{ef}
0.28 ± 0.02^{acd}	0.36 ± 0.04^{e}	0.25 ± 0.02^{a}	0.29 ± 0.01^{acd}	0.32 ± 0.01^{ce}	0.26 ± 0.00^{ab}	0.30 ± 0.02^{bcd}	0.34 ± 0.03^{de}

separation based on leaf size. It appears that, while leaf size is separated along PC1 for short extraction times, it evolves with time to separate after 5 min along PC2 (loading: 7.5%). In contrast, separation for temperature is along PC1, irrespective of extraction time. A closer look at the PTR-ToF-MS profiles reveals that PC1 essentially separates for overall intensity of HS volatiles, whereas PC2 reflects

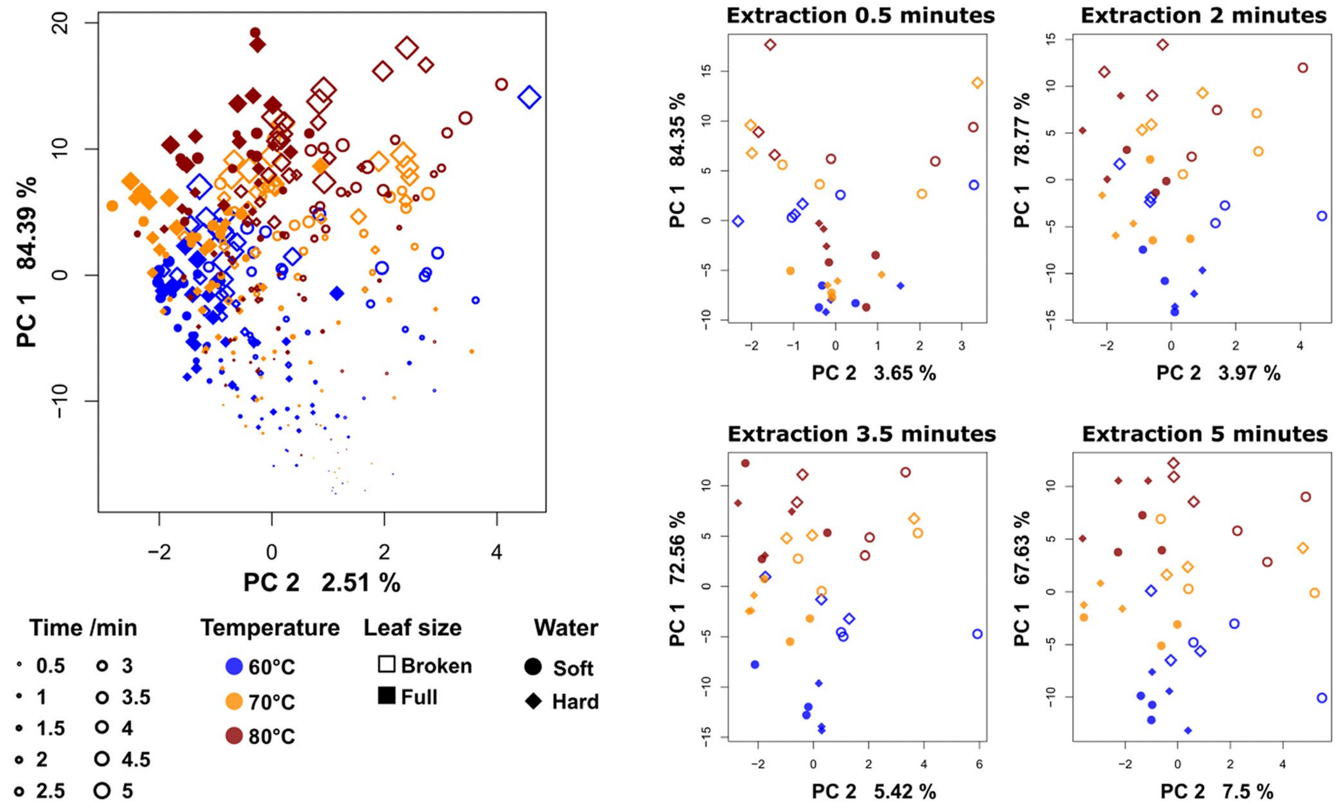

FIGURE 1 PCA score plots of the (left) 180 analysed samples (3 temperatures × 2 leaf sizes × 2 waters × 5 time points × 3 replicates) and (right) individual PCA score plots at four different infusion times (0.5, 2, 3.5 and 5 min)

changes in the composition of the volatile profile at comparable overall intensity.

Reviewing the PCAs for the individual extraction times, it appears that an extraction time of two min led to the finest differentiation of VOC profiles for changing extraction conditions; all three parameters, extraction temperature, leaf size and mineral content of the water, led to some degree of separation along the principle components axes. Indeed, two min extraction time is essentially the only time at which the mineral content of the water seems to have an impact. At shorter times, the main separation is by leafsize along PC1 and no differentiation with extraction temperature is observed. At longer extraction times than 2 min, the separation by temperature along PC1 is the dominate effect together with a small separation for leave size along PC2.

3.2 | Identification of extraction conditions resulting in similar volatile profile

In addition to differences in extraction rate and profile as a function of different extractions conditions, we also examined which set of different extraction conditions lead to similar profiles. Hence, to gain some deeper insight into the infusion and extraction process, samples were grouped by hierarchical cluster analysis (HCA) using two different approaches. The first approach entailed the clustering of all the time points measured for each of the conditions, using the mean value of the three replicates performed.

All PCA and HCA were performed on the bases of the all the 88 m/z signals, after having removed signal from the blank and setting a threshold of 1ppb. Using R, we compared the m/z of those peaks with an in-house library of compounds. This resulted in 34 compounds being tentatively identified and 54 that remained unknown peaks. HCA was hence repeated using only the 34 tentatively identified compounds (Table 1). Both HCA resulted in the same five main clusters (Table 2). The analysis shown in Figure 2 was performed on the basis of the 88 m/z dataset.

This grouping of samples according to their volatile content allowed the identification of which infusion conditions resulted in similar HS composition. Cluster 1 contained 13 samples, all but one belonging to infusions from full leaves. In this cluster, we found samples that had been infused at 60°C (from 0.5 to 1.5 min), at 70°C (0.5 and 1 min) and at 80°C (0.5 min). These results reflect the effect of temperature on the extraction of aroma compounds from the leaves. At the lower temperature of 60°C, the extraction is slow, with minor differences in the aroma profile during the first 1.5 min of infusion. At the other extreme, the infusion at 80°C reached in only 30 s extraction time the same volatile composition as an infusion at 60°C after 1.5 min or at 70°C after 1 minute. With the exception of the sample infused at 60°C in hard water for 30 s (B60SB_0.5), no other infusion of broken leaves was found in the first cluster, reflecting the differences in extraction speed/efficiency between full and broken leaves. Overall, this cluster can be characterized as one with an overall low extraction yield on volatiles. The first samples prepared from

TABLE 2 Results from hierarchical cluster analysis on the mean value of the three replicates analysed for each of the different infusions (3 temperatures × 2 leaf sizes × 2 waters × 10 time points). AP (Aqua Panna): Softer water; SB (San Benedetto) harder water

			Cluster				
Size	**Water**	**Temp. °C**	**1**	**2**	**3**	**4**	**5**
Full	AP	60	0.5/1/1.5	2/2.5/3/3.5	4/4.5/5		
		70	0.5/1	1.5/2	2.5/3	3.5/4/4.5/5	
		80	0.5	1/1.5	2	2.5/3	3.5/4/4.5/5
	SB	60	0.5/1/1.5	2/2.5/3/3.5	4/4.5	5	
		70	0.5/1	1.5/2	2.5	3/3.5/4/4.5/5	
		80	0.5	1/1.5		2/2.5/3/3.5	4.5/5
Broken	AP	60		0.5/1/1.5/2	2.5/3/3.5/4	4.5/5	
		70		0.5/1	1.5/2/2.5	3/3.5/4/4.5	5
		80		0.5	1/1.5	2/2.5/3	3.5/4/4.5/5
	SB	60	0.5	1/1.5/2	2.5/3	3.5/4/4.5/5	
		70		0.5/1	1.5	2/2.5/3	3.5/4/4.5/5
		80		0.5	1	1.5/2	3/3.5/4/4.5/5

broken leaves could be found in Cluster 2. If we check the infusion at 60°C, we find samples between 2 and 3.5 min in the second cluster for full leaves while for broken leaves the longest time is 2 min. Analysing all the clusters, we can identify a general trend. For the same size of leaf, the higher the temperature used for infusion, the lower the time needed to achieve similar extraction of VOCs. For a fixed temperature, aroma compounds are extracted faster from broken leaves, and therefore, less time is needed to obtain comparable composition than in infusions made from full leaves.

The second approach for grouping the samples was to use the whole time-intensity profile for each of the infusion conditions and hence retain the full information on the time-dependent extraction dynamics. Thereby, the matrix used for HCA consisted of 36 observations (3 temperatures × 2 leaf sizes × 2 waters × 3 replicates) and 440 variables (88 m/z × 5 time points). This clustering gave information about which infusion conditions resulted in similar time-intensity profiles. As it is shown in Figure 2A, HCA resulted in clustering of the samples according to leaf size and infusion temperature, with minor exceptions. One of the replicates of full leaves infused at 80°C was grouped with samples infused at 70°C; broken leaves infused at 70 and 80°C were grouped together. No difference between the two waters was observed within the clusters. In Figure 2A, the total volatile intensity was a dominating driver for separation between samples and small differences in extraction dynamics and volatile composition may have been missed. To eliminate the differences in total volatile intensity when extracting with different infusion conditions, and hence to compare the way volatiles were extracted, the time-intensity profiles were normalized to the intensity at the end of the infusion for each m/z and a HCA was performed again (Figure 2B). In this case, and once the impact of the total volatile intensity is eliminated from the analysis, the only separation that remains is between broken and full leaves. It appears that breaking/milling leaves lead (in addition to an increased extraction efficiency) to a different profile of extracted volatiles. The fact that samples infused at different temperatures within one leaf size were clustered in different groups when the raw intensity was used but they all belonged to the same group when normalized intensity was considered indicates that a change in temperature resulted in different overall amounts of volatile compounds being extracted—higher extraction efficiency with higher temperatures—however, the VOCs had similar extracted volatile profiles ('more of the same'). Only change from full to broken leaf leads to different extraction profiles and, therefore, different dynamics of the extraction. In other words, breaking the leaves not only increased the extraction rate, it also changed the extraction dynamics of compounds.

3.3 | Effect of leaf size on volatile extraction

Leaf size was responsible for the first observed differences in volatile extraction—already after 30 s extraction, the VOC profiles of full vs broken leaves could be separated (Figure 1). This separation is mainly based on extraction efficiency of aroma compounds in the early phase of the brewing process, which is higher from broken leaves, compared to full leaves. It reflects the overall intensity of extracted VOC.

Tea leaf size produced a minimum effect on the equilibrium concentration of some soluble tea compounds (ie caffeine and theaflavin)[25] but it significantly affects their extraction kinetics.[26] The kinetics of tea infusion has traditionally been modelled by considering the tea leaf as a lamina where compounds are extracted from the two large surfaces,[5,27] but the effect of the edges becomes important when the leaf size decreases and the leaf particles start resembling spheres.[26] This implies that in an agitated system, the extraction of soluble compounds from the tea leaves will be faster for smaller leaf sizes as has also been observed in the case of polyphenols,[4,28] caffeine[4] or minerals like calcium or aluminium.[29]

When we take a closer look into the extraction dynamics of individual volatile compounds, the same impact of full vs broken leaves is observed as already discussed based on the PCA, especially at the beginning of the extraction. An example is given in Figure 3 for the mass peak at m/z 63.026 which can be tentatively identified as dimethylsulphide, a green tea aroma compound.[17,30,31] It can be clearly seen that the differences between both full and broken leaves were significant only at the beginning of extraction. While the average content of dimethyl sulphide seems still higher also at longer brewing times, after 1.5 min of infusion, those differences were no long significant.

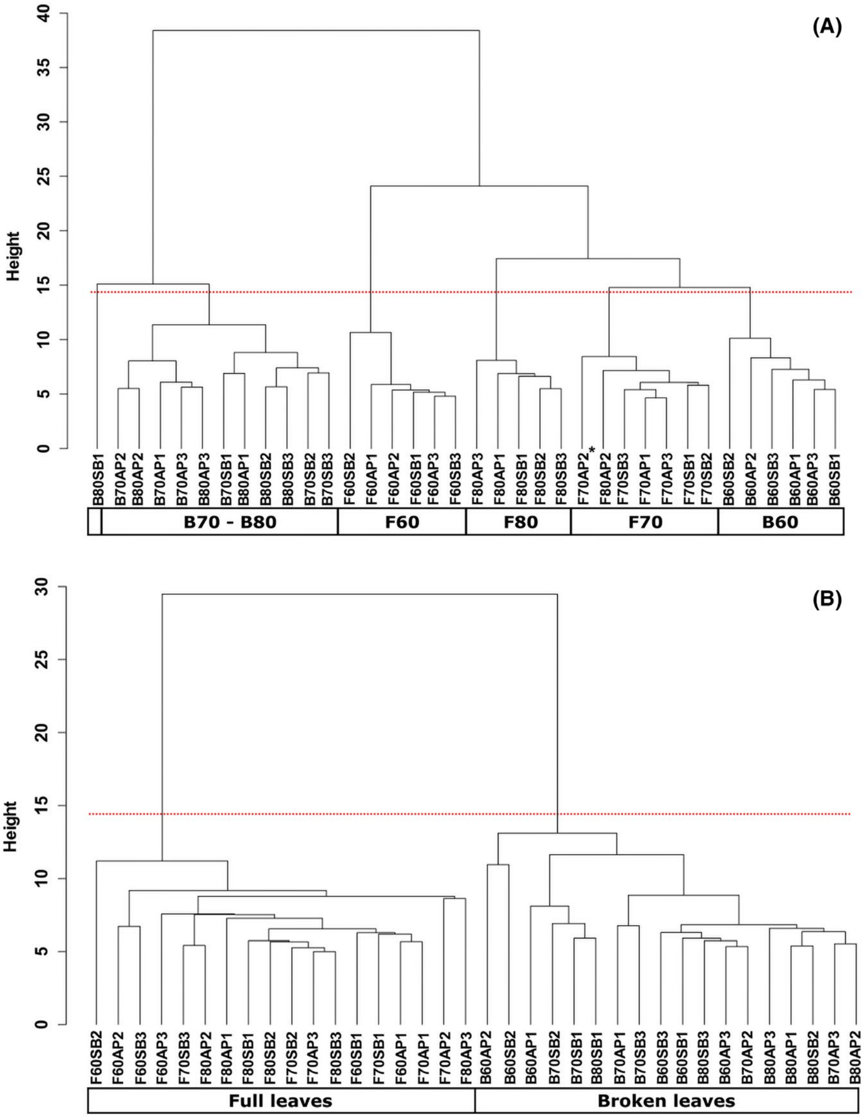

FIGURE 2 Hierarchical cluster analysis of the time-intensity profile for all samples (3 temperatures × 2 leaf sizes × 2 waters × 3 replicates) using (A) absolute intensity or (B) intensity normalized to the intensity at the last time point (5 min)

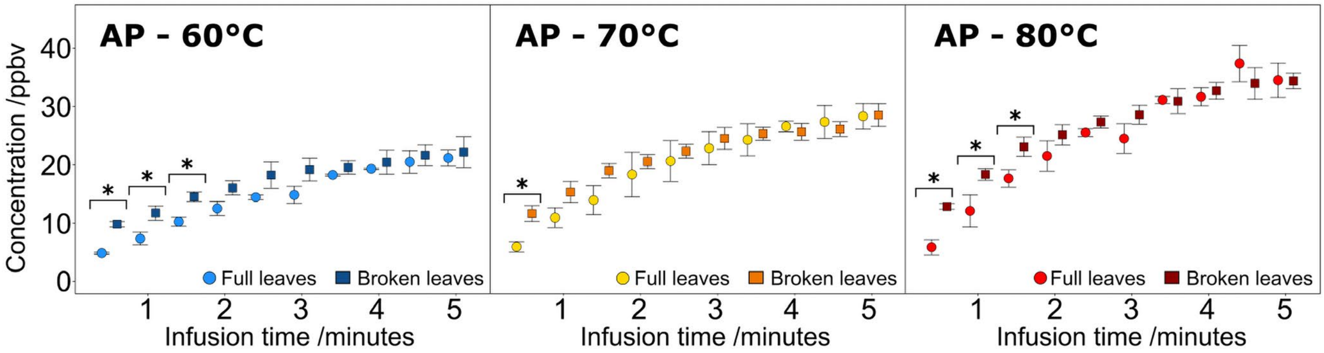

FIGURE 3 Effect of leaf size on dimethyl sulphide extraction at different temperatures. In each plot, the mean and the standard deviation of the three replicates is shown. Samples marked with an asterisk (*) are significantly different at that time point ($P < .01$). Points have been slightly moved along the *x*-axis to ease differentiation of samples and correspond to 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5 and 5 min

3.4 | Effect of water temperature on volatile extraction

Temperature had a greater effect on the extraction efficiency of volatiles from tea leaves than leaf size, but its effect was evident only after the first min of extraction as shown in the PCA. At short extraction times of below 1 min, the temperature (over the range from 60 to 80°C investigated here) did not reveal any differences, neither for overall intensity nor profile. The same interpretation holds for the time evolution of dimethyl sulphide (Figure 4). Differences amongst the three temperatures studied are significant from 1.5 min of brewing until the end. The two extreme temperatures (60 and 80°C) resulted in significantly different amounts of dimethyl sulphide for all time points in the case of broken leaves and for all but 0.5 min when infusions were prepared with full leaves. Increased extraction with temperature has been reported for soluble, non-volatile constituents of tea,[32] such as polyphenols or caffeine,[8,28,33] and for formation of tea foam in the case of black tea.[34] Wright and co-workers[18] also studied the effect of temperature on black tea volatiles by analysing the HS of the final infusions by atmospheric pressure chemical ionization mass spectrometry (APCI-MS). They also found that the amount of volatiles in the final infusions increased as a function of the temperature. In our case, as the volatile content was monitored over time, we were able to observe how those differences in extraction with temperature developed with time and became greater as the brewing time increased.

3.5 | Effect of water composition on volatile extraction

The last brewing parameter studied was water hardness. According to the PCAs, water composition had the smallest impact of all the parameters studied here with respect to volatile extraction. In fact, at an individual compound level, significant differences between the two types of water were found only for four compounds tentatively identified as methanol, acetaldehyde, dimethylsulphide and pentenal, and only in the case of broken leaves (Figure 4). For these compounds, infusion of broken leaves resulted in higher HS intensity when hard water was used. Hard water infusions have shown lower extractability of caffeine, theaflavins and other organic compounds from tea leaves than soft waters.[7,27,29] This effect has been attributed to the uptake of calcium by the leaves which can be complexed with pectin on the cell walls provoking their gelification and modifying the diffusion of organic compounds through the cell wall. That would have implied that infusions in soft water would have resulted in higher volatile intensities. In our case, the differences in calcium content were small (32.0 mg L^{-1} in soft water and 50.30 mg L^{-1} in hard water) which might have had low impact on the extractability of the compounds but still cannot explain the higher concentration of some volatiles when teas were prepared in hard water. Another possible explanation would be that the higher salt content in the hard water produced the salting-out of volatiles to the HS, but in this case, the same effect should have been observed in samples prepared from full leaves. Further research is needed to explain the effect of water composition on the extraction of volatile compounds from tea leaves.

4 | CONCLUSIONS

Changes in the volatile profile of tea infusions during brewing have been analysed for the first time with PTR-ToF-MS. The combination of a direct injection mass spectrometry technique with multivariate analysis has proven to be a useful tool to follow the tea brewing process and how it is affected by different brewing parameters. At the beginning of the extraction, the leaf shape is responsible of most of the observed differences in the volatile profile, but at longer extraction times, those differences become smaller. The

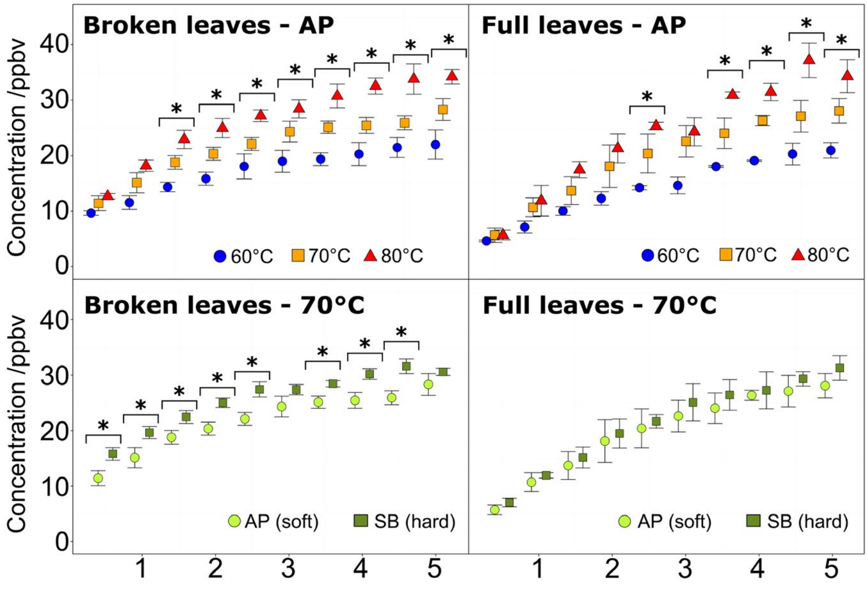

FIGURE 4 Effect of different infusion conditions on dimethyl sulphide extraction. In each plot, the mean and the standard deviation of the three replicates is shown. Samples marked with an asterisk (*) are significantly different at that time point ($P < .01$).Points have been slightly moved along the *x*-axis to ease differentiation of samples and correspond to 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5 and 5 min

opposite is found for temperature. At the beginning of the brewing, no differences could be observed between the three extraction temperatures 60, 70 and 80°C, but differences appeared, and increased, with time. The mineral content of the extraction water was the parameter that had the least impact on the volatile profile of tea infusions (within the parameter range investigated here)—differentiation between low and high mineral content was only observed at two min extraction time. Furthermore, we were able to classify samples according to their volatile profile and therefore determine which combinations of extraction parameters resulted in similar aroma. From an academic perspective, this approach will help obtaining a more detailed insight into the extraction process of tea flavour compounds. From an economic perspective, it can assist in new product developments (ie tea bag, capsules or instant tea) to optimize and recommend extraction parameters, achieve a similar profile to another product format or benchmark (eg market leader) or to approach the profile of a gold standard (ie loose-leaf tea). In summary, the study outlined here opens new perspectives towards a deeper understanding of the tea extraction process and can be used in new product development and in the improvement of existing products.

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CONFLICT OF INTEREST

The authors have no a conflict of interest in relation to this work.

ORCID

Samo Smrke https://orcid.org/0000-0002-7532-4371
Chahan Yeretzian https://orcid.org/0000-0002-4928-728X

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