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A study of volatile compounds in hop by headspace sampling and comprehensive two-dimensional gas chromatography☆

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ABSTRACT

As a main ingredient in beer production, hop (*Humulus lupulus* L.) and its essential oils, resins and polyphenols, significantly influence the aroma and bitter taste of beer. This study investigates the volatile organic compound (VOC) profile of ten previously uncharacterised hop cultivars (Super Pride, Centennial, Pacific Jade™, Superdelic™, Nectaron™, Rakau™, Pacifica™, Motueka™, Nelson Sauvin™ and Simcoe®) using headspace solid-phase microextraction (HS-SPME), combined with comprehensive two-dimensional gas chromatography (GC×GC) and mass spectrometry (MS). After SPME conditions were carefully selected the developed method enabled the tentative identification of 136 VOCs across ten hop cultivars, providing insights into their complex chemical composition. Major aspects of this work include the development of a recommended HS-SPME sampling method, and an investigation of the precision of the GC×GC–MS methodology. A detailed analysis of the identified VOCs and compound classes is performed. Consequently, key compounds, classes and markers are determined. This contributes to our understanding of hop VOC profile and offers opportunities for the development of methods for hop characterisation and quality control.

1. Introduction

Solid phase microextraction (SPME) is one of the most important sample preparation techniques applied to complex samples for the analysis of volatile organic compounds (VOCs) nowadays. For solid samples, this technique relies on the equilibrium of VOCs between the sample and headspace, and then sorption of the volatile compounds from the headspace by a polymeric phase supported on a fibre. Most commonly, the sorbed VOCs are subsequently desorbed in the injector port, separated using gas chromatography (GC) and analysed/identified by mass spectrometry (MS). Advanced chemical methodologies are evolving to handle the complexity of small molecules in both natural extracts, and synthetic tracers [1]. Since its first publication in 1991 by Arthur and Pawliszyn [2], SPME has undergone extensive refinement, including the use of different fibre materials [3,4], diverse geometries [5], a better understanding of the theory [6,7], automated systems [8,9] and an exceptionally diverse array of analytical applications. One of the most relevant demonstrations of its importance in sample analysis can be recognised by a Web of Science search, with >17.5 thousand studies reporting the use of SPME, both for a myriad of sampling applications, and as a subject of method development. SPME has many advantages, such as its simplicity in the sample preparation step, alignment with the principles of green chemistry as a solvent-free technique, the requirement of a relatively small amount of sample, and its versatility with respect to matrix composition [10]. However, reported limitations include the considerable cost of the fibres and fibre longevity and robustness, and limitations in use for quantitative analysis and sample extraction capabilities [10]. As a sample preparation tool, SPME can be used in headspace (HS) and direct immersion modes for direct sampling, or with protective coverings designed to exclude unwanted matrix materials from interfering with the sorption and analysis steps [10,11]. A wide range of matrices across numerous research fields have been investigated using SPME, e.g., environmental science [12,13], bioanalysis [14–16], and food analysis [17–20] among others.

Food samples particularly benefit from SPME methodology, and its potential for hop applications is no exception. More specifically, hop—*Humulus lupulus*, a commercially valuable plant from the *Cannabaceae* family—is widely used as a key bittering, flavouring and

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preserving raw material in beer production. Secondary metabolites and bioactive compounds from this family can significantly enhance immunological function and physical performance [21]. In hop specifically, these complex chemical constituents—primarily essential oils, resins, and polyphenols [22,23]—define the final product's characteristics, including its aroma, bitterness, foam stability, and antiseptic properties. Given the advantages of SPME, this technique has proved effective for the determination of volatile compounds in hop, with a range of prior studies using it in conjunction with one-dimensional GC (1DGC) [24,25].

Hop are composed of non-volatile bitter acids and volatile essential oils which are both found in lupulin [26,27]. Although hop essential oils only make up a small proportion of dried strobili (0.5-3.0 % *v/w*), their terpenes and terpenoids are among the most important products of the plant, responsible for the flavour in beer [26]. Fig. 1 shows the chemical structures of typical representatives of terpenes and terpenoids, one of the main groups of chemical compounds in hop [28]. According to several studies on the composition and odour characteristics of hop essential oils, the monoterpene hydrocarbon (MTH) myrcene and the sesquiterpene hydrocarbons (STHs) α-humulene, and β-caryophyllene, dominate the VOC composition [28–31]. The diverse chemical components in hop create different aromas. For example, geraniol, geranyl acetate, geranyl isobutyrate and linalool provide a floral aroma, while δ-cadinene, γ-cadinene, limonene and α-muurolene contribute to citrus-piney aromas [32]. The complex chemical composition of *Humulus lupulus* L. has been studied over the past centuries [26]. However, complete identification of all characteristic aroma compounds remains ongoing, due to analytical challenges. In 1DGC-MS, many overlapping peaks are apparent, and very low abundance peaks are barely detectable. These circumstances complicate the identification of the volatile components, and the sensitivity and selectivity of the analytical method are impaired.

Therefore, hop analysis can benefit from the separation advantages of comprehensive two-dimensional GC (GC×GC), coupled with MS for identification [33]. Prior research has applied GC×GC to profile the total composition of various samples, revealing a significant proportion of their heterogeneity. This demonstrates the advantages of GC×GC, as compounds that coelute on the first column (^{1}D) are often resolved using the second dimension column (^{2}D), resulting in greater peak separation capacity. According to the literature there are only a limited number of studies using SPME in combination with GC×GC for hop analysis. Studies such as the one by Yan et al. [34] have employed hydrodistillation sample preparation, using a Clevenger apparatus before determination of hop extracts by GC×GC to chemotype and understand the composition of different hop varieties. To the best of our knowledge, only one study has utilised hop and HS-SPME-GC×GC [20], reporting the advantages of using GC×GC for hop analysis over 1DGC, chemotyping different hop cultivars, and characterising their classes by statistical strategies.

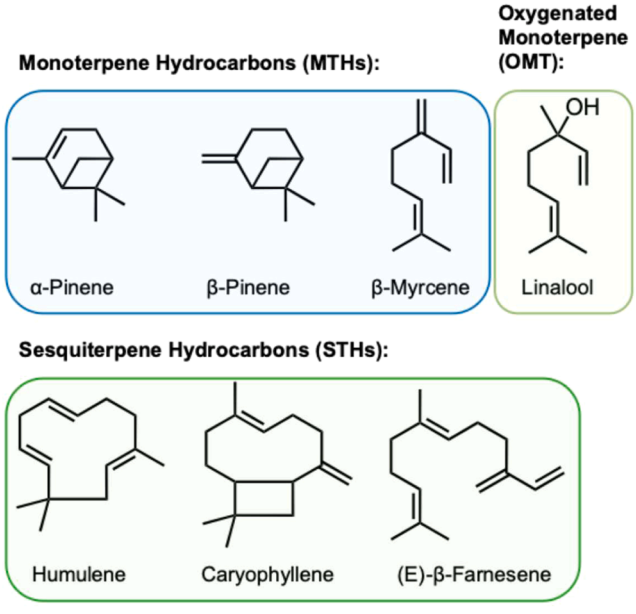

Fig. 1. Typical representatives of terpenes and terpenoids, one of the main groups of chemical components in hop, categorised into their characteristic classes (MTHs, STHs, OMT). The classes are colour coded according to a recurring colour scheme used in the present work [28].

The aim of this study is to investigate the SPME extraction and equilibrium of compounds in detail, based on the chemical classes identified through GC×GC, and to propose recommended SPME conditions for the analysis of hop pellets. For this application, these conditions will establish the principles of this sample preparation technique, with variables such as fibre type, hop mass, extraction temperature and time analysed. Additionally, this study aims to characterise the VOC composition of newly reported hop varieties, contributing to their chemotyping and enhancing the understanding of their chemical profiles.

2. Materials and methods

2.1. Reagents, chemicals, and solutions

For the HS-SPME-GC×GC analysis of hop, reagents (all from Sigma-Aldrich Pty. Ltd., Macquarie Park, Australia) with a purity of ≥98% were used, namely: *n*-hexane, 2-octanol, undecane as internal standards (I.S.), a series of alkanes from C_8 to C_{21}, humulene, β-myrcene, β-linalool, geraniol and geranyl acetate.

Pelletised hop samples type 90 of the following hop cultivars were examined in this investigation: Super Pride and Centennial (Hop Products Australia, North Hobart, Australia), Pacific Jade™, Superdelic™ and Nectaron™ (New Zealand Hops Ltd., Appleby, New Zealand), Rakau™, Pacifica™, Motueka™, (Clayton Hops NZ, Brightwater, New Zealand) and Simcoe® (Yakima Chief Ranches, Zillah, WA, USA). Cascade (Home Make It, Clayton, Australia) and Saaz (Hop Products Australia, North Hobart, Australia) hop were used to establish SPME operating parameters, subsequent to our earlier study [19], given that these are well established varieties in the literature.

2.2. Sample preparation operating parameters

The SPME extraction procedure focused on selecting the most suitable fibre for extraction, and determining the ideal hop sample mass and SPME parameters. A design of experiment (DoE) approach was employed to select the best temperature, equilibrium time, and extraction time of the HS-SPME process.

Pelletised hop was used for extraction and ground immediately after removal from the freezer, where it had been stored in vacuum-sealed aluminium packaging until analysis. The resulting powder was then aliquoted into a 20 mL clear glass SPME vial, which was fitted with a screw top. The first experiments used a mass of 200 mg, a heating temperature of 50 °C, an equilibrium time of 10 min and an extraction time of 30 min. The SPME fibres were obtained from Supelco (Supelco, Bellefonte, PA): 100 μm PDMS, 85 μm PA, 60 μm PEG, 65 μm DVB/PDMS, 85 μm CAR/PDMS, and 50/30 μm DVB/CAR/PDMS. Fibre selection was performed by 1DGC–MS analysis of all six fibres, with assessment of the number of peaks, the total area and saturated peaks. The four most suitable fibres were selected and then used for further analysis by GC×GC–MS.

The best total 'mass response', an objective measurement parameter for the method development process, was reported for the seven classes of compounds, characterised by the compound with the most significant presence in each class, as reported by Yan et al. [34]. The objective function was chosen to be the chromatographic area divided by the area

of the internal standard for the compound representing each class; i.e. isoamyl isobutanoate (esters – Est), β-myrcene (MTH), β-linalool (oxygenated monoterpenes – OMT), caryophyllene (STH), humulene epoxide II (oxygenated sesquiterpenes – OST), 2-undecanone (ketones – Ket) and perillene (furans – Fur).

The amount of ground hop was selected to be 25 mg, 50 mg, 100 mg, 250 mg, 500 mg and 1000 mg, in replicate analyses (n = 3), in accordance with various mass ranges reported in the literature [24,35]. The masses were analysed in a univariate condition, using the same conditions for extraction as before, selecting the most suitable mass for the DoE.

The DoE applied was based on the Box-Behnken Design (BBD) [36] with three factors, considering the most important parameters described for SPME in the literature and their potential application to hop. Compared to other DoE response surface methodologies, the BBD is highly efficient for three factors. It requires only 15 experiments including three replicates of the centre point, while avoiding extreme factor combinations [36]. Thus, the selected factors and their levels were: (a) extraction temperature: 50 to 70 °C [25], (b) equilibration/incubation time: 10 to 30 min [35], and (c) extraction/sorption time: 30 to 50 min [37]. The design included 15 experiments, as shown in Table 1, where the last three lines represent the replicates of experiments for the central point. The same compounds evaluated in the univariate study of hop quantity were analysed using the BBD, resulting in seven experimental designs representing each class.

2.3. Chromatographic and mass spectrometry instrument conditions

This study employed two GC systems: 1DGC–MS and GC×GC–MS. For 1DGC, the instrument was an Agilent 7890a (Agilent Technologies, Mulgrave, Australia) hyphenated with an Agilent 7000 (Agilent Technologies, Mulgrave, Australia) triple quadrupole mass spectrometer system. The chromatographic configuration was based on literature [20], with manual injection, a fibre desorption time of 3 min, a split ratio of 50:1, a carrier gas flow of 1.0 mL min^{-1}, an injection temperature of 250 °C and a column comprising a DB-5 ms UI phase (Agilent Technologies; 30 m x 0.25 mm x 0.25 μm). The oven started at 50 °C (hold for 3 min), then increased at a rate of 3 °C min^{-1} to 210 °C, followed by a rate of 10 °C min^{-1} to 240 °C and maintained for 3 min. The MS conditions were set to an ionisation energy of 70 eV, a scan range of *m/z* 35–350, an ion source temperature of 250 °C, a quadrupole temperature of 150 °C and a data acquisition rate of 22.8 Hz.

The GC×GC–MS system was configured as an Agilent 7890A GC coupled with a 5975C single quadrupole mass spectrometer (mass selective detector, MSD; Agilent Technologies) and a SSM1800 solid-state modulator (SSM) system from J&X (J&X Technologies, Nanjing, China). The GC×GC system was equipped with a DB-5 ms UI (Agilent; 30 m × 0.25 mm × 0.25 μm) as the ^{1}D column, and a SUPELCOWAX™10 (Supelco; 1.5 m × 0.1 mm × 0.1 μm) as the ^{2}D column. Fused silica capillaries were used as the modulator column (1 m × 0.15 mm) and the transfer line to the MSD (0.45 m × 0.1 mm). All columns were joined by glass press fit connections (Restek Corp., Bellefonte, PA). The GC×GC–MS oven temperature program was the same as that used in 1DGC–MS analysis and specific MS settings were as follows: MS source temperature 230 °C, quadrupole temperature 150 °C, threshold 80, scan rate 12,500 (u/s) and 22.8 scans s^{-1}. The solid-state modulator configuration and conditions was based on the literature [20]; the modulator inlet oven followed the GC temperature program, while the outlet oven was set 20 °C above the GC oven temperature. The trap temperature program was: −50 °C (8 min), rising at a rate of 2 °C · min^{-1} to −20 °C (37 min), while the modulation period was 6 s and desorption time was 1 s.

Table 1
Experimental conditions and corresponding codes for BBD, studying the extraction variables: temperature (°C), equilibration (min), and extraction time (min) for 500 mg of Cascade hop using HS-SPME-GC×GC–MS.

Experiment	Variables of extraction		
	Temperature (°C)	Equilibration time (min)	Extraction time (min)
1	50 (−1)	10 (−1)	40 (0)
2	70 (+1)	10 (−1)	40 (0)
3	50 (−1)	30 (+1)	40 (0)
4	70 (+1)	30 (+1)	40 (0)
5	50 (−1)	20 (0)	30 (−1)
6	70 (+1)	20 (0)	30 (−1)
7	50 (−1)	20 (0)	50 (+1)
8	70 (+1)	20 (0)	50 (+1)
9	60 (0)	10 (−1)	30 (−1)
10	60 (0)	30 (+1)	30 (−1)
11	60 (0)	10 (−1)	50 (+1)
12	60 (0)	30 (+1)	50 (+1)
13	60 (0)	20 (0)	40 (0)
14	60 (0)	20 (0)	40 (0)
15	60 (0)	20 (0)	40 (0)

The retention index (RI) values were calculated according to the linear temperature programmed van den Dool and Kratz equation (Eq. (1)) [47] with reference to adjusted retention times and a series of linear alkanes (C8-C21). Two alkane mixtures (C8-C15, and C16-C21) were sampled by SPME and separately injected into the GC.

$$I_x = 100n + \frac{100(t'_x - t'_n)}{(t'_{n+1} - t'_n)} \quad (1)$$

Where: '*Ix*' is the retention index of the solute, '*n*' is the number of carbons in the alkane immediately preceding the solute, $n + 1$ is that immediately after the solute, and t are the respective retention times.

2.4. Data treatment and software

GC–MS and GC×GC–MS data processing and chromatographic analysis were performed using Agilent MassHunter Workstation Qualitative Analysis software version 10.0 (Agilent Technologies) and J&X Canvas W1.5.14.30115 software (J&X Electronic Technology, Nanjing, China). MS identification was performed based on the NIST 11 mass spectrometry library.

Tentative identification of the VOC was carried out by correlation of experimental and literature/reference data, including MS R. Match score (>700) and linear RI, which were calculated using the alkane standard mixtures. During SPME method development, data treatment employed tests of normality (David's test), removal of outliers (Dixon's test), homoscedasticity (Cochran or Barlett depending on the outlier's removal) and one-way Anova.

Statistica 10 software (StatSoft, Tulsa, OK) was utilised for experimental design. The DoE included an Anova test, a Pareto chart and the response surface methodology. Statistical data handling was carried out using Excel® (Microsoft, USA).

OriginPro® 2021b (OriginLab® Corporation, Northampton, MA) was utilised for the graphical representation of the analytical data. Chemical molecular structures were created using ChemDraw® Professional 22.2.0 (PerkinElmer®, Waltham, MA). Furthermore, Microsoft® Excel® (Microsoft Corporation, Redmond, WA) and Microsoft® PowerPoint® (Microsoft Corporation, Redmond, WA) were employed for data analysis and illustration.

3. Results and discussion

3.1. Selection of an appropriate SPME method

The initial step in selecting the acceptable SPME conditions involved selecting a suitable fibre type. In this study, six different fibre types were analysed using 1DGC–MS (Table 2). The present method uses SPME to assess the extraction/qualification of hop headspace composition, and in particular reports various compounds based on the SPME extraction

Table 2
Comparative study of SPME fibre types and determination of compounds in Cascade hop by HS-SPME-GC–MS.

	1	2	3	4	5	6
	100 µm PDMS	85 µm PA	60 µm PEG	65 µm PDMS/ DVB	85 µm CAR/ PDMS	50/30 µm DVB/ CAR/ PDMS
No. of peaks	185	127	161	196	205	240
Total area	7.34 × 10^8	2.00 × 10^8	7.51 × 10^7	8.48 × 10^8	1.60 × 10^9	1.78 × 10^9
No. of saturated peaks*	2	6	1	5	11	9

* Saturated peaks are those that saturate the response of the detector under the sampling conditions used. This was observed for the Cascade sample during method development, and not for other samples. Saturation affects the total area reported for the samples.

recovery as expressed by the GC–MS and GC×GC–MS relative responses. Actual hop compositions of VOC will vary according to specific chemical properties of the compounds and the extraction fibre, temperature and sorption equilibria. The best performance can be observed for the 85 µm CAR/PDMS and the 50/30 µm DVB/CAR/PDMS fibres, which show the highest number of peaks and the largest total chromatographic area. The presence of Carboxen, combined with the bipolar and adsorptive properties of these fibres, likely contributed to the enhanced extraction of hop compounds. According to literature [10], the 50/30 µm DVB/CAR/PDMS is recommended for volatile and semi-volatile compounds with a molecular mass range of 40–275, whereas the 85 µm CAR/PDMS is more suitable for low molecular mass compounds (30–225). However, the increase in sensitivity also led to a higher occurrence of saturated peaks, when using the two fibres with the best performance. To determine the most suitable SPME fibre for volatile extraction in GC×GC, the four fibres that were assessed to be top-performing in 1DGC–MS—100 µm PDMS, 65 µm DVB/PDMS, 85 µm CAR/PDMS, and 50/30 µm DVB/CAR/PDMS —were further examined.

Fig. 2 displays the HS-SPME-GC×GC–MS chromatograms of Cascade hop obtained using the four different selected SPME fibres. It can be observed that the 100 µm PDMS fibre exhibited the fewest peaks among the four tested fibres, the 85 µm CAR/PDMS displayed a greater number of peaks, especially within the first 15 min. However, this fibre also showed greater peak broadening (apparent tailing in the first dimension) and longer retention times. In contrast the 50/30 µm DVB/CAR/PDMS and 65 µm DVB/PDMS fibres demonstrated the best performance, with similar distribution of compounds. Considering the number of 'saturated peaks' detected in the GC×GC–MS analysis and the overall chromatographic performance, the 65 µm DVB/PDMS was selected.

After the selection of the fibre, seven key representative classes were selected based on the study of Yan et al. (2018) [34] to characterise the best response relative to those classes, starting by the detection of the effective hop mass. The results for β-myrcene are presented in Fig. 3, while the other compounds can be seen in Figs. S1 to S6. In Fig. 3a, the chromatographic peak areas were normalised using the response of the internal standard 2-octanol. The peak area ceased to increase at the point at which the response stabilised, indicated by a horizontal trend in the graph. This reflects the finite sorption capacity of the fibre, as the sample mass increases, the amount of extracted analytes represents a progressively smaller fraction of the total. Among the compound classes, Est, MTH, and OMT were the last to reach this plateau condition, whereas ketones reached it first. The optimal sample mass was chosen as the minimum amount of hop required to ensure a representative extraction for all compounds. Based on these findings, a mass of 500 mg was selected for subsequent experiments.

To further select suitable extraction parameters, a multivariate optimisation approach using the BBD was applied. The factors equilibration time, sorption time, and temperature were selected based on literature. According to Kovačevič and Kač (2001) [25], temperatures between 25 and 80 °C were evaluated for humulene and β-myrcene in GC–MS, with apparent thermal equilibrium observed between 50 and 70 °C. Consequently, this temperature range was selected. Regarding a suitable equilibration time, Su and Yin (2021) [35] applied 10 min, and for the present study, times between 10 and 30 min were tested as a suitable range. The selection of an appropriate sorption time and fibre exposure duration was based on the work of Van Opstaele et al. (2012) [37], who used values between 30 and 50 min. To enhance the robustness of the DoE models, pure error and bidirectional interactions were incorporated into the experimental design.

An ANOVA table was constructed to evaluate the efficiency of the DoE models, with determination coefficient (R^2) values and lack-of-fit p-values presented in Table 3. The models exhibited satisfactory performance, with lack-of-fit p-values exceeding 0.05 and R^2 values approaching 1. The lowest R^2 value (0.8770) was observed for the ketone 2-undecanone; however, it still provided a reliable fit within the model framework.

Subsequently, Pareto charts were constructed (Fig. 3b) to identify the significant factors for each analyte. The extraction temperature was the most significant factor for all compounds, whereas time was less influential. Apart from humulene epoxide II, the temperature exhibited a negative effect, where an increase in temperature reduced the SPME efficiency. The sorption process is inherently exothermic. Thus,

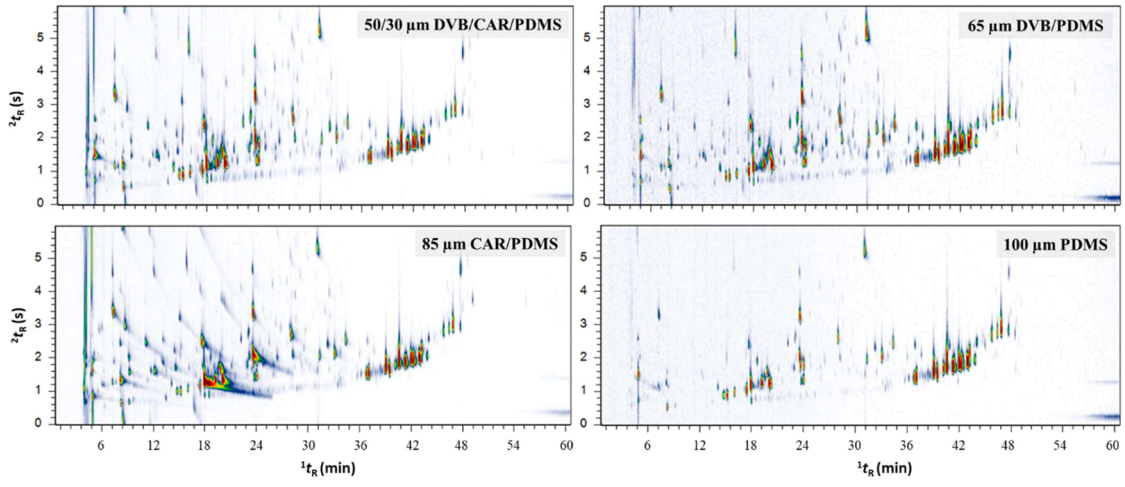

Fig. 2. Chromatograms for Cascade hop analysis using HS-SPME-GC×GC–MS with four different SPME fibres.

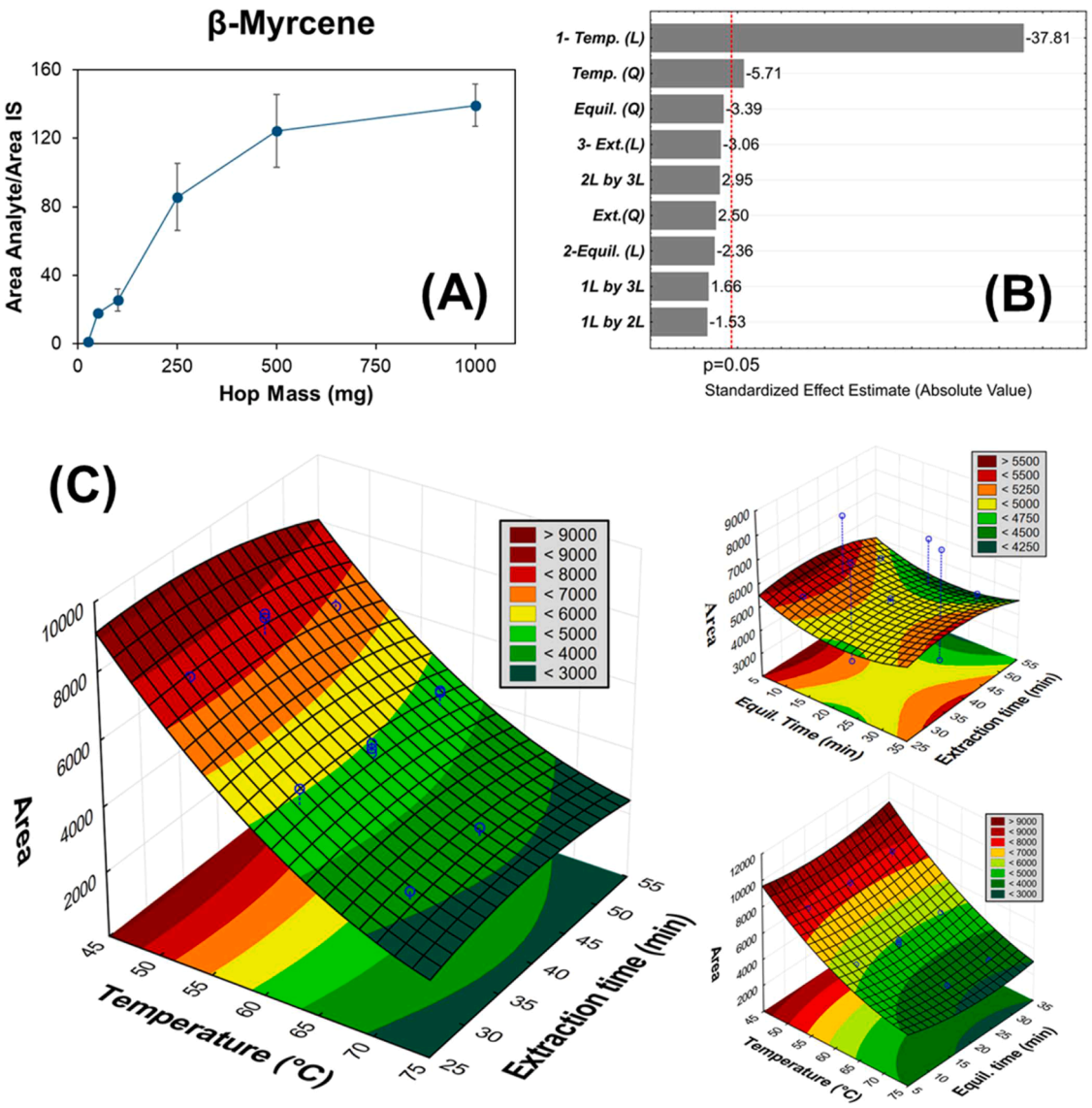

Fig. 3. Varied extraction conditions for β-myrcene in Cascade hop by HS-SPME-GC×GC–MS (a) mass study curves for volatile compounds, (b) Pareto charts showing the significance of factors in the extraction process, and (c) response surface plots for the most significant factors affecting compound extraction. For (b): 1. Temp. = Temperature of extraction (min), 2. Equil. = Equilibration time (min), 3. Ext. = Extraction time (min), L = Linear and Q= Quadratic.

according to Le Chatelier's principle, increasing the extraction temperature typically reduces the analyte amount at equilibrium. However, the observed increase in extraction yield at higher temperatures, especially for large molecules such as oxygenated sesquiterpenes (humulene epoxide II), indicates that the system was operating under kinetic control rather than reaching thermodynamic equilibrium.

However, the equilibrium and extraction time showed low significance, suggesting that the extraction system had already reached equilibrium. To further analyse the data, response surface methodology (RSM) was applied to assess the most significant interactions (Fig. 3c). The results confirmed that temperature played the most critical role in compound extraction, where lower temperatures significantly increased the extraction yields across six of the seven studied classes. Based on these findings, a temperature of 50 °C was selected as the best condition, since it was indicated for six of the seven compound classes. Additionally, the shortest equilibrium time (10 min) and sorption time (30 min) were chosen to improve analytical efficiency. Following the development of an optimised sampling method via HS-SPME with suitable extraction times, the precision of the method for instrumental analysis was evaluated.

3.2. Precision of the GC×GC–MS method

The precision of the established HS-SPME-GC×GC–MS method was investigated by examining its repeatability (intra-day) and (long-term) reproducibility (inter-day). In particular, the GC×GC system was re-installed to the same configuration with new columns after being used for other studies; the aim was to exactly reproduce 2D retention positions. To investigate long-term reproducibility, the Saaz cultivar was chosen to ensure comparability between measurements taken during the earlier experimental design of method conditions (DoE measurements) and later studies (ca. six months later) which applied the method to hop samples in the same instrument.

The extraction was performed using the established HS-SPME method above. For each GC×GC–MS measurement, the five highest peaks were considered in this section. Apart from α-bergamotene and (*E*)-β-farnesene the other compounds chosen (β-myrcene, caryophyllene, humulene) are major terpenes in hop [38–40].

3.2.1. Repeatability and reproducibility of the GC×GC–MS method

The repeatability of the GC×GC–MS was determined by

Table 3
Details of the BBD experimental design for seven compounds from different chemical classes in Cascade hop, including the reported and experimentally determined retention indices (RI), determination coefficients (R^2), and lack-of-fit p-values as assessed by ANOVA.

No.	Compound	Class [a]	Lit. RI [b]	Exp. RI [c]	R^2	Lack of Fit p-value
1	β-myrcene	MTH	991 ±2	988	0.9850	0.1276
2	isoamyl isobutyrate	Est	1015 ±3	1014	0.9919	0.4209
3	perillene	Fur	1101 ±2	1098	0.9704	0.4153
4	β-linalool	OMT	1099 ±2	1100	0.9819	0.1972
5	2-undecanone	Ket	1294 ±2	1292	0.8770	0.1789
6	caryophyllene	STH	1419 ±3	1426	0.9496	0.1243
7	humulene epoxide II	OST	1606 ±2	1613	0.9835	0.4900

a. MTH - Monoterpene hydrocarbons; Est - Esters; Fur - Furans; OMT - Oxygenated monoterpenes; Ket - Ketones; STH - Sesquiterpene hydrocarbons; OST - Oxygenated sesquiterpenes.
b. Literature retention index (RI) for a 5% phenyl-95% methyl polysiloxane column phase with data from the NIST11 library ± standard deviation.
c. Retention index calculated using the van den Dool and Kratz equation.

quadruplicate measurements conducted within one day (Supporting Information Table S1). To investigate the reproducibility, an analysis of three replicates (respectively four replicates on day one) was carried out on three consecutive days (Supporting Information Table S2). The analytical results are expressed by their relative standard deviation (% RSD), for the mean retention times in ^{1}D and ^{2}D, the peak areas and heights. The intra- and inter-day RSD values for the ^{1}D retention time revealed no variability, except for α-bergamotene which exhibited an RSD of 0.1% in reproducibility. ^{2}D retention time repeatability values range from 0.5% to 0.8%, while humulene showed an RSD of 2.5%, and reproducibility values ranged from 2.0% to 2.5%. The RSD values for peak area intra-day precision ranged from 7.4% to 30.7% and the inter-day precision values from 11.1% to 22.3%. For peak heights, intra-day precision RSD values ranged from 3.8% to 25.9% and inter-day precision from 7.3% to 15.3%. The increased RSD of the peak areas and heights can be explained by inconsistent integration during data analysis with Canvas. Consequently, the peak integration of the hop samples (see Section 2.4) was reviewed and manually corrected, if necessary. Manual integration was utilised in Canvas for peak consolidation in cases where automated peak detection resulted in fragmented signals or an incomplete area of a peak.

3.2.2. *(Long-term) reproducibility of the GC×GC–MS retention times*

The (long-term) reproducibility of the retention times was evaluated by comparing the measurements acquired on the third day (Day 3) and the results obtained approximately six months prior (during the DoE study), subsequent to the establishment and validation of the analytical method in the same laboratory environment, with the same column set phases and dimensions, and nominal conditions. Table 4 shows the average retention times in ^{1}D and ^{2}D (^1t_R, ^2t_R) of the triplicate determinations. Additional measurements were performed at a slightly increased flow rate of 1.1 mL min^{-1}. This was intended to compensate for shifts in the GC×GC 2D plot relative to the previous experimental studies (i.e. conducted six months earlier), such that closely similar retentions were obtained, when an equivalent column set was installed in the GC. The measurements of the third day showed increased and non-reproducible retention times in both dimensions compared to the DoE measurements. As an increased flow rates typically result in decreased retention times, the elevated flow rate was implemented to realign the third day results more closely with the earlier DoE data.

Table 4
Reproducibility of the retention times (^1t_R, ^2t_R) of the GC×GC–MS analyses expressed by average retention times of the triple determinations.

Peak	Measurement	^1t_R [min]	^2t_R [s]
1. β-myrcene	Day 3	19.66	1.97
	DoE	17.29	1.08
	1.1 mL min^{-1}	19.06	1.84
2. caryophyllene	Day 3	40.36	2.12
	DoE	38.26	1.38
	1.1 mL min^{-1}	39.76	1.95
3. α-bergamotene	Day 3	40.72	1.96
	DoE	38.66	1.25
	1.1 mL min^{-1}	40.06	1.85
4. (*E*)-β-farnesene	Day 3	41.36	2.23
	DoE	39.36	1.44
	1.1 mL min^{-1}	40.76	2.10
5. humulene	Day 3	41.86	2.31
	DoE	39.76	1.57
	1.1 mL min^{-1}	41.22	2.14

Furthermore, the variance of the retention times, shown in the Table, highlight the importance of R. Match score and RI data for the identification of VOCs. Since retention times can vary significantly even within the same laboratory, they provide only a preliminary basis for identification. Given that the identification in this study was performed by means of chromatographic data, the NIST11 database and R. Match score, and that the individually identified retention times for the various analytes were reproducible, further adjustment of the flow rate to return retention time data to the DoE results was deemed unnecessary.

3.3. *Chemotyping of different hop genotypes using HS-SPME-GC×GC–MS*

The composition of hop headspace is very complex [22]. The use of advanced analytical platforms to understand the complex chemical profiles of plant-derived materials and their resulting biological differences is essential [41]. Based on that and in the results, the separation of individual VOCs was incomplete for hop analysis using 1DGC, which lacks sufficient peak capacity. In 1DGC–MS analysis, many overlapping peaks are evident, with impaired resolution. This complicates the identification of the volatile components, and the sensitivity and selectivity of the analytical method are compromised. The GC×GC analysis offers significantly higher resolution, through the addition of a second separation dimension; most of the peaks no longer overlap [42]. Compositions of the varieties Super Pride (SP), Centennial (CEN), Pacific Jade™ (PJ), Superdelic™ (SUP), Nectaron™ (NEC), Rakau™ (RAK), Pacifica™ (PAK), Motueka™ (MOT), Nelson Sauvin™ (NS) and Simcoe® (SIM) were investigated as part of this work using the established HS-SPME-GC×GC–MS method. Hop cultivar selection primarily focused on varieties originating from Australia and New Zealand to enhance the potential for correlations between hop composition and lineage for planned phylogenetic analysis using hierarchical cluster analysis and principal component analysis. Moreover, varieties whose VOC profiles have been previously reported in the literature (e.g., CEN), as well as those less extensively investigated new hop varieties (e.g., SUP, NEC), were examined using GC×GC–MS with the aim of improving the characterisation and confirming the applicability of the established method. GC×GC–MS provides a more detailed profile of hop aroma compounds even for previously studied varieties due to its superior separation, resolution, and sensitivity compared to conventional 1DGC [42–46] .

The distinct VOCs were tentatively identified through GC×GC–MS data analysis performed with J&X Canvas W1.5.14.30115 and NIST 11. The results are summarised in Supporting Information Table S3. To confirm the tentative identities of the compounds, an R. Match score of >700 was applied. In addition, retention indices (RIs) (with a deviation of ±20) were calculated using alkane standards and the van den Dool and Kratz equation and applied as a criterion for identification [47,48].

Of particular interest for the investigation of hop compositions are the ^{1}D and ^{2}D retention times listed in Table S3, as well as the chemical class affiliation of the components. In addition, the table includes compound identification numbers (No.), compound name (compound), CAS number (CAS), molecular formula (formula), retention indices (RIs) obtained from literature and this experiment, as well as the relative GC×GC chromatographic area of each species in % (as obtained using the SPME method).

With a total of >90 tentatively identified VOCs, most of the compounds in the cultivars Super Pride (90), Pacific Jade™ (92) and Rakau™ (92) were elucidated. The number of compounds found in the other seven hop varieties range between 70 and 89 (Centennial (82), Superdelic™ (89), Nectaron™ (84), Pacifica™ (73), Motueka™ (70), Nelson Sauvin™ (85), Simcoe® (88)), highlighting that significantly more compounds were identified in all ten varieties using the GC×GC method compared to a 1DGC method. In the literature, fewer than 70 hop components per hop cultivar have reportedly been identified using GC–MS [38–40].

A total of 136 volatile chemical compounds were tentatively identified in the ten hop cultivars. The classification of VOCs depicts the number of compounds assigned to each class. The predominant classes observed in this work include esters (Est, 48), monoterpene hydrocarbons (MTH, 18) and sesquiterpene hydrocarbons (STH, 40). In addition, the following number of identified compounds can be assigned to the respective classes, arranged in ascending order according to the retention time of the first representative of each respective class: 3 Ald, 3 HC, 2 Oth, 8 Ket, 8 OMT, 3 Alc and 3 OST.

Since the number of VOCs assigned to a class does not automatically correspond to the largest area percentage, the expression of the importance of the respective class in the hop samples can be assessed more precisely based on the area. Table 5 shows the total area percentage of the identifiable VOCs in the ten different samples. It becomes clear that 100% of the sample could not be identified using the criteria described for data analysis. The discrepancies in the total identifiable area percentages across samples—particularly those with lower identification rates—may be attributed to the inherent limitations of the quadrupole mass analyser in a GC×GC setup. The high data acquisition speeds required for narrow second dimension peaks can lead to mass spectral skewing and reduced sensitivity, which hinders the accurate library matching of trace-level hop constituents. Nevertheless, in most samples, >90% of the volatile components were assigned. Wong *et al.* discussed the value of GC×GC to report more of the total composition of samples [49].

The three most abundant compounds with the largest area percentage across all samples are the key terpenes β-myrcene, and the isomers caryophyllene and humulene (see Supporting Information Table S3). A dominance of the three terpenes is also noted in the literature [38–40]. However, the two hop varieties Super Pride and Motueka™ deviate from this observation. In both samples, caryophyllene and humulene show a significantly lower abundance compared to all other samples. Cultivation- and genetic-induced shifts in terpene metabolism may explain the comparatively reduced content of caryophyllene and humulene. These changes may favour the production of bitter compounds or fruity monoterpenes [50]. Besides β-myrcene (17.7%), the VOCs β-eudesmene (20.3%) and α-selinene (20.7%) are particularly strongly represented in Super Pride. In Motueka™, β-myrcene (58.5%), (*E*)-β-farnesene (13.3%) and α-selinene (5.1%) are most abundant.

Referring back to the three components that generally have the highest abundance, Motueka™ contains the largest area of β-myrcene (58.5%), followed by Centennial (49.4%) and Simcoe® (46.8%). The largest area percentage of caryophyllene is found in Superdelic™ (14.8%), Pacifica™ (13.4%) and Centennial (13.2%). Moreover, the highest abundance of humulene is observed in Nelson Sauvin™ (32.2%), followed by Pacifica™ (30.3%) and Superdelic™ (30.0%). The MTH β-myrcene lends the hop variety citrusy, resinous-pine aroma, while the STHs caryophyllene and humulene produce spicy and woody aromas [51].

Fig. 4 enables a closer examination of the relative percentages of the respective classes to the total area, regarding all ten hop samples. The classes of STHs, MTHs and Ests account for the largest area percentage in the compositional profile of most hop samples. Although the class of Ests comprises the largest number of compounds (48), it is underrepresented in area compared to the STHs and MTHs. In Pacifica™, the area share of Ests (0.4%) is the lowest, whereas Super Pride contains the most esters by area (11.3%). The class of MTHs is the second most abundant. A particularly large share of its total area can be found in Motueka™ (60.1%), Centennial (50.5%) and Simcoe® (48.3%). The significant abundance of MTHs is primarily attributed to the concentration of β-myrcene, the characteristic VOC for this class. The largest class in terms of area percentage, STHs, is predominantly characterised by the isomers caryophyllene and humulene and is most abundant in Superdelic™ (68.1%), Super Pride (53.3%) and Rakau™ (52.5%). The area percentages of Kets and OMTs are significantly lower, but relatively large compared to all other identified classes. Kets are most abundant in Super Pride (4.2%) and Simcoe® (1.7%), while the largest area percentage of OMTs can be found in Nectaron™ (2.1%), Simcoe® (2.0%) and Motueka™ (1.5%).

Correlations between the relative percentages of the respective classes to the total area and the potential aroma composition from literature reports of compounds and the hop cultivars can be observed (see Supporting Information Fig. S7). Previous studies have shown that MTHs are predominantly responsible for a citrusy, fruity aroma and STHs contribute to a woody, earthy and herbal or spicy aroma [39]. For example, Super Pride, which has low MTHs, is less fruity and citrusy

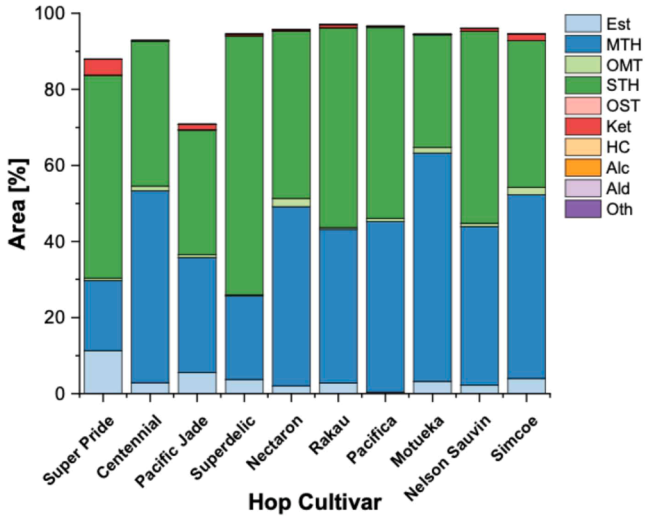

Fig. 4. Chemical composition of the ten hop cultivars under investigation based on the area percentages of identified component classes, as detailed in Supporting Information Table S3.

Table 5
Total area percentage* of the identified VOCs in the ten different samples.

Hop cultivar	SP	CEN	PJ	SUP	NEC	RAK	PAC	MOT	NS	SIM
Total area [%]	88.0	92.9	70.9	94.6	95.8	97.1	96.7	94.5	96.1	94.6

* Based on TIC data, some of the total peak area cannot be identified according to the criteria in the text.

compared to the other cultivars, while Superdelic™, having the highest percentage of STHs exhibits the woody, earthy, and herbal aroma [52, 53]. However, since an aroma component cannot always be distinctly assigned to a single class but rather to multiple classes, only a broad assessment can be made here.

Fig. 4 shows that the hop varieties Nelson Sauvin™ and Rakau™ are most similar in respect to the area distribution of the different classes. This similarity likely arises from the comparable area percentage of characteristic representatives within the respective classes: β-myrcene, caryophyllene, and humulene, as outlined in Supporting Information Table S3.

The distribution of the classes over the 2D GC×GC plot was examined in more detail using the apex plot (Fig. 5). The figure demonstrates that a distinction between different classes can be made based on their retention time windows – i.e. positions. Compounds of a class are identified with the same colour/symbol and accordingly grouped in dashed geometric shapes indicating their respective classes. Est are found across the entire retention period in the first dimension, with notable accumulation in the second dimension occurring between ca. ^2t_R = 1.25 s - 1.75 s. Kets are also found over a wide range of the retention times, predominantly after ca. ^2t_R = 1.80 s in the second dimension. MTHs eluted relatively early in ^{1}D (^1t_R = 17.07 min - 25.27 min) and were detected in ^{2}D between ca. ^2t_R = 1.00 - 3.00 s. In the first dimension, the retention time window of OMTs is comparatively large. In ^{2}D, however, this class eluted last compared to all other classes (^2t_R = 1.79 - 4.45 s). Since the ^{2}D separation is based on polarity, ^{2}D retention time extends from less polar to polar, and polar molecules therefore are found at late ^{2}D retention times. The most polar group is the OMTs. However, polar Ests are also present in the different hop samples. The large group of STHs, characterised by 40 compounds, covers a retention range from ^1t_R = 37.07 - 48.97 min and from ^2t_R = 1.13 s - 2.76 s. OMTs is the least volatile class with the highest boiling point and are polar molecules. Therefore, this class eluted last in both dimensions.

In addition to examining the classes and their distribution over the 2D plot, marker components of the individual hop varieties were determined. The marker components are VOCs that occurred exclusively in one of the cultivars examined (Table 6). For Super Pride, evidently the largest number of marker compounds were identified, which in this work are unique to this hop cultivar.

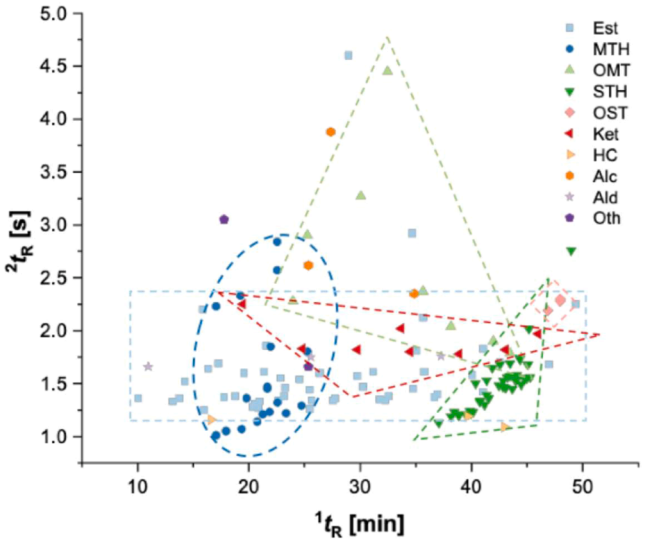

Fig. 5. Apex plot illustrating the chemical composition of the ten hop cultivars under investigation based on the identified retention times in first (^1t_R) and second dimension (^2t_R) (see Supporting Information Table S3). The identified chemical compounds are categorised by class and colour coded.

Table 6
Proposed marker compounds determined for the hop cultivars under investigation. Marker compounds are VOCs that were identified exclusively in one sample. No hop-cultivar-specific marker compounds were determined for the hop varieties Centennial, Pacific Jade™, Nectaron™, or Nelson Sauvin™.

Hop Cultivar	Marker VOC (Peak Number)
Super Pride	amyl isobutyrate (37), 2-nonanol (49), benzyl isobutyrate (70), methyl undecenoate (92)
Superdelic™	γ-selinene (127)
Rakau™	*cis*-α-bergamotene (90)
Pacifica™	*cis*-β-ocimene (35)
Motueka™	ipsdienol (56), *trans*-α-bergamotene (90), β-bisabolene (117)
Simcoe®	*trans*-calamenene (122)

4. Conclusions

In this work, an approach was developed to analyse the complex VOC profile of hop cultivars. Using HS-SPME coupled with GC×GC–MS, 136 VOCs were identified in ten hop varieties, primarily originating from Australia and New Zealand. Detailed analysis revealed Est, MTHs and STHs as the predominant classes, accounting for the largest area percentages. β-myrcene, caryophyllene and humulene were found to be the most abundant volatiles across all samples. Apex plot analysis showed distinct elution patterns for the different VOC classes. Furthermore, marker compounds were identified, particularly in the cultivar Super Pride.

While a reproducible method for hop VOC analysis was successfully established, subsequent chemotyping, including hierarchical cluster analysis and principal component analysis (data not shown), did not show the expected correlation between hop composition and lineage. This suggests limitations in the current dataset, likely due to insufficient ancestral similarity among the hop samples examined. For this reason, future studies with more closely related lineages could potentially establish such correlations, as the potential for a comprehensive investigation by GC×GC–MS, suitable data analysis, and chemotyping has been demonstrated.

Ethical statement

No ethical issues were involved in this study. Experiments with living animals were not performed in this study.

CRediT authorship contribution statement

Julia Hillebrand: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis. **Glaucimar A.P. Resende:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Conceptualization. **Michelle S.S. Amaral:** Writing – review & editing, Investigation. **Philip Marriott:** Writing – review & editing, Writing – original draft, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2026.467195.

Data availability

Data will be made available on request.

References

[1] Z. Mou, Y. Zhu, L. Zhang, M. Ma, Z. Li, Y. Guo, J. Zheng, Z. Zhao, K. Zhang, X. Chen, Z. Li, AquaF" building blocks for water-compatible S $_N$ 2 ^{18} F-fluorination of small-molecule radiotracers, J. Am. Chem. Soc. 146 (2024) 17517–17529, https://doi.org/10.1021/jacs.4c05854.

[2] C.L. Arthur, J. Pawliszyn, Solid phase microextraction with thermal desorption using fused silica optical fibers, Anal. Chem. 62 (1990) 2145–2148, https://doi.org/10.1021/ac00218a019.

[3] M. Ntorkou, C.K. Zacharis, Sorbent-based microextraction combined with GCMS–: a valuable tool in bioanalysis, Chemosens 13 (2025) 71, https://doi.org/10.3390/chemosensors13020071.

[4] F. Ghaemi, A. Amiri, R. Yunus, Methods for coating solid-phase microextraction fibers with carbon nanotubes, TrAC - Trends Anal. Chem. 59 (2014) 133–143, https://doi.org/10.1016/j.trac.2014.04.011.

[5] H. Piri-Moghadam, M.N. Alam, J. Pawliszyn, Review of geometries and coating materials in solid phase microextraction: opportunities, limitations, and future perspectives, Anal. Chim. Acta 984 (2017) 42–65, https://doi.org/10.1016/j.aca.2017.05.035.

[6] L. Kudlejova, S. Risticevic, D. Vuckovic, Solid-phase microextraction method development, in: handbook of solid phase microextraction, Elsevier (2012) 201–249, https://doi.org/10.1016/B978-0-12-416017-0.00007-3.

[7] J. Pawliszyn, Theory of solid-phase microextraction, in: handbook of solid phase microextraction, Elsevier (2012) 13–59, https://doi.org/10.1016/B978-0-12-416017-0.00002-4.

[8] H.L. Lord, E.A. Pfannkoch, Sample preparation automation for GC injection. Comprehensive Sampling and Sample Preparation, Elsevier, 2012, pp. 597–612, https://doi.org/10.1016/B978-0-12-381373-2.00061-2.

[9] R. Eisert, J. Pawliszyn, Design of automated solid-phase microextraction for trace analysis of organic compounds in aqueous samples, J. Chromatogr. A 776 (1997) 293–303, https://doi.org/10.1016/S0021-9673(97)00332-4.

[10] Y. Nolvachai, M.S.S. Amaral, R. Herron, P.J. Marriott, Solid phase microextraction for quantitative analysis – Expectations beyond design? Green Anal. Chem. 4 (2023) 100048 https://doi.org/10.1016/j.greeac.2022.100048.

[11] V. Jalili, A. Barkhordari, A. Ghiasvand, A comprehensive look at solid-phase microextraction technique: a review of reviews, Microchem. J. 152 (2020) 104319, https://doi.org/10.1016/j.microc.2019.104319.

[12] C. Ribeiro, A.R. Ribeiro, A.S. Maia, V.M.F. Gonçalves, M.E. Tiritan, New trends in sample preparation techniques for environmental analysis, Crit. Rev. Anal. Chem. 44 (2014) 142–185, https://doi.org/10.1080/10408347.2013.833850.

[13] W. Alahmad, S.I. Kaya, A. Cetinkaya, P. Varanusupakul, S.A. Ozkan, Green chemistry methods for food analysis: overview of sample preparation and determination, Adv. Sample Prep. 5 (2023) 100053, https://doi.org/10.1016/j.sampre.2023.100053.

[14] H. Kataoka, A. Ishizaki, K. Saito, Recent progress in solid-phase microextraction and its pharmaceutical and biomedical applications, Anal. Methods 8 (2016) 5773–5788, https://doi.org/10.1039/C6AY00380J.

[15] X. Feng, Y. Kuang, L. Gan, S. Zhou, J. Zheng, G. Ouyang, Solid phase microextraction for the bioanalysis of emerging organic pollutants, TrAC Trends Anal. Chem. 177 (2024) 117786, https://doi.org/10.1016/j.trac.2024.117786.

[16] D. Vuckovic, X. Zhang, E. Cudjoe, J. Pawliszyn, Solid-phase microextraction in bioanalysis: new devices and directions, J. Chromatogr. A 1217 (2010) 4041–4060, https://doi.org/10.1016/j.chroma.2009.11.061.

[17] J. Aspromonte, S. Mascrez, D. Eggermont, G. Purcaro, Solid-phase microextraction coupled to comprehensive multidimensional gas chromatography for food analysis, Anal. Bioanal. Chem. 416 (2024) 2221–2246, https://doi.org/10.1007/s00216-023-05048-0.

[18] A.C. Paiva, L.W. Hantao, Exploring a public database to evaluate consumer preference and aroma profile of lager beers by comprehensive two-dimensional gas chromatography and partial least squares regression discriminant analysis, J. Chromatogr. A 1630 (2020) 461529, https://doi.org/10.1016/j.chroma.2020.461529.

[19] D. Eggermont, N.D. Spadafora, J. Aspromonte, R. Pellegrino, G. Purcaro, Exploring different high-capacity tools and extraction modes to characterize the aroma of brewed coffee, Anal. Bioanal. Chem. 415 (2023) 4501–4510, https://doi.org/10.1007/s00216-023-04654-2.

[20] G.A.P. Resende, M.S.S. Amaral, B.G. Botelho, P.J. Marriott, Comprehensive two-dimensional gas chromatography–mass spectrometry as a tool for the untargeted study of hop and their metabolites, Metabolites 14 (2024) 237, https://doi.org/10.3390/metabo14040237.

[21] F. Hassan, C. Liu, M. Mehboob, R.M. Bilal, M.A. Arain, F. Siddique, F. Chen, Y. Li, J. Zhang, P. Shi, B. Lv, Q. Lin, Potential of dietary hemp and cannabinoids to modulate immune response to enhance health and performance in animals: opportunities and challenges, Front. Immunol. 14 (2023), https://doi.org/10.3389/fimmu.2023.1285052.

[22] C. Almaguer, C. Schönberger, M. Gastl, E.K. Arendt, T. Becker, Humulus lupulus - a story that begs to be told. A review, J. Inst. Brew. 120 (2014) 289–314, https://doi.org/10.1002/jib.160.

[23] Z. Tong, L. Jia-Jiong, F. Xiong, W. Ping-Ping, C. Hai-Ming, C. Chun, Key structural characteristics enhancing digestion resistance in rice starch-sugarcane polyphenols complexes, Food Chem. 505 (2026) 148040, https://doi.org/10.1016/j.foodchem.2026.148040.

[24] Z. Liu, L. Wang, Y. Liu, Rapid differentiation of Chinese hop varieties (Humulus lupulus) using volatile fingerprinting by HS-SPME-GC–MS combined with multivariate statistical analysis, J. Sci. Food Agric. 98 (2018) 3758–3766, https://doi.org/10.1002/jsfa.8889.

[25] M. Kovačevič, M. Kač, Solid-phase microextraction of hop volatiles. Potential use for determination and verification of hop varieties, J. Chromatogr. A 918 (2001) 159–167, https://doi.org/10.1016/S0021-9673(01)00719-1.

[26] H. Korpelainen, M. Pietiläinen, Hop (Humulus lupulus L.): traditional and present use, and future potential, Econ. Bot. 75 (2021) 302–322, https://doi.org/10.1007/s12231-021-09528-1.

[27] N. Rettberg, M. Biendl, L.A. Garbe, Hop aroma and hoppy beer flavor: chemical backgrounds and analytical tools—a review, J. Am. Soc. Brew. Chem. 76 (2018) 1–20, https://doi.org/10.1080/03610470.2017.1402574.

[28] M.K. Hrnčič, E. Španinger, I.J. Košir, Ž. Knez, U. Bren, Hop compounds: extraction techniques, chemical analyses, antioxidative, antimicrobial, and anticarcinogenic effects, Nutrients 11 (2019) 257, https://doi.org/10.3390/nu11020257.

[29] G. Eyres, J.P. Dufour, Hop essential oil: analysis, chemical composition and odor characteristics, in: beer in Health and Disease prevention, Elsevier (2009) 239–254, https://doi.org/10.1016/B978-0-12-373891-2.00022-5.

[30] P.L. López, G.K. Guerberoff, N.R. Grosso, R.H. Olmedo, Antioxidant-efficient indicator determinate by the relationship between β-myrcene/caryophyllene (α, β) on hop (Humulus lupulus) essential oils under an accelerated oxidation test, Ind. Crops Prod. 205 (2023) 117399, https://doi.org/10.1016/j.indcrop.2023.117399.

[31] S.T. Katsiotis, C.R. Langezaal, J.J.C. Scheffer, Composition of the essential oils from leaves of various *humulus lupulus* L. cultivars, Flavour Fragr. J. 5 (1990) 97–100, https://doi.org/10.1002/ffj.2730050208.

[32] G.B. Nickerson, E.L. Van Engel, Hop aroma component profile and the aroma unit, J. Am. Soc. Brew. Chem. 50 (1992) 77–81, https://doi.org/10.1094/ASBCJ-50-0077.

[33] L. Mondello, C. Cordero, H.-G. Janssen, R.E. Synovec, M. Zoccali, P.Q. Tranchida, Comprehensive two-dimensional gas chromatography–mass spectrometry, Nat. Rev. Methods Primers. 5 (2025) 7, https://doi.org/10.1038/s43586-024-00379-3.

[34] D. Yan, Y.F. Wong, L. Tedone, R.A. Shellie, P.J. Marriott, S.P. Whittock, A. Koutoulis, Chemotyping of new hop (Humulus lupulus L.) genotypes using comprehensive two-dimensional gas chromatography with quadrupole accurate mass time-of-flight mass spectrometry, J. Chromatogr. A 1536 (2018) 110–121, https://doi.org/10.1016/j.chroma.2017.08.020.

[35] X. Su, Y. Yin, Aroma characterization of regional Cascade and Chinook hops (Humulus lupulus L.), Food Chem. 364 (2021) 130410, https://doi.org/10.1016/j.foodchem.2021.130410.

[36] S.L.C. Ferreira, R.E. Bruns, H.S. Ferreira, G.D. Matos, J.M. David, G.C. Brandão, E. G.P. da Silva, L.A. Portugal, P.S. dos Reis, A.S. Souza, W.N.L. dos Santos, Box-Behnken design: an alternative for the optimization of analytical methods, Anal. Chim. Acta 597 (2007) 179–186, https://doi.org/10.1016/j.aca.2007.07.011.

[37] F. Van Opstaele, B. De Causmaecker, G. Aerts, L. De Cooman, Characterization of novel varietal floral hop aromas by headspace solid phase microextraction and gas chromatography–mass spectrometry/olfactometry, J. Agric. Food Chem. 60 (2012) 12270–12281, https://doi.org/10.1021/jf304421d.

[38] P. Nezi, V. Cicaloni, L. Tinti, L. Salvini, M. Iannone, S. Vitalini, S. Garzoli, Metabolomic and proteomic profile of dried hop inflorescences (Humulus lupulus L. cv. Chinook and cv. Cascade) by SPME-GC-MS and UPLC-MS-MS, Separations 9 (2022) 204, https://doi.org/10.3390/separations9080204.

[39] M.R. Nance, W.N. Setzer, Volatile components of aroma hops (Humulus lupulus L.) commonly used in beer brewing, J. Brew. Distill. 2 (2011) 16–22.

[40] A. Aberl, M. Coelhan, Determination of volatile compounds in different hop varieties by headspace-trap GC/MS—in comparison with conventional hop essential oil analysis, J. Agric. Food Chem. 60 (2012) 2785–2792, https://doi.org/10.1021/jf205002p.

[41] D. Yu-Hao, C. Chun, W. Ping-Ping, W. Chun, F. Xiong, Sugarcane extract enhances stress resilience and longevity in Caenorhabditis elegans: NMR structural elucidation and transcriptomic mechanisms, Food Res. Int. 223 (2026) 117863, https://doi.org/10.1016/j.foodres.2025.117863.

[42] P.J. Marriott, S.T. Chin, B. Maikhunthod, H.G. Schmarr, S. Bieri, Multidimensional gas chromatography, TrAC Trends Anal. Chem. 34 (2012) 1–21, https://doi.org/10.1016/j.trac.2011.10.013.

[43] J.-M.D. Dimandja, GC×GC,Anal. Chem. (2004) 167A–174A, https://doi.org/10.1021/ac041549+.

[44] L. Ramos, Comprehensive two dimensional gas chromatography. Comp. Anal. Chem., first ed, Elsevier, Amsterdam,, 2009. ISBN: 9780080932699 eBook.

[45] V. Purdy, B. Kebede, R. Beatson, K. Templeton, P. Silcock, G.T. Eyres, Differences in New Zealand hop cultivars based on their unique volatile compounds: an integrated fingerprinting and chemometrics approach, Foods 10 (2021) 414, https://doi.org/10.3390/foods10020414.

[46] M. Iannone, E. Ovidi, S. Vitalini, V. Laghezza Masci, A. Marianelli, M. Iriti, A. Tiezzi, S. Garzoli, From hops to craft beers: production process, VOCs profile characterization, total polyphenol and flavonoid content determination and antioxidant activity evaluation, Processes 10 (2022) 517, https://doi.org/10.3390/pr10030517.
[47] H. van Den Dool, P.D. Kratz, A generalization of the retention index system including linear temperature programmed gas—liquid partition chromatography, J. Chromatogr. A 11 (1963) 463–471, https://doi.org/10.1016/S0021-9673(01)80947-X.
[48] W. Lai, Temperature-programmed retention indices for GC and GC-MS analysis of coal- and petroleum-derived liquid fuels, Fuel 74 (1995) 1436–1451, https://doi.org/10.1016/0016-2361(95)00108-H.
[49] Y.F. Wong, P. Perlmutter, P.J. Marriott, Untargeted metabolic profiling of eucalyptus spp. Leaf oils using comprehensive two-dimensional gas chromatography with high resolution mass spectrometry: expanding the metabolic coverage, Metabolomics 13 (2017) 46, https://doi.org/10.1007/s11306-017-1173-3.
[50] R. Hall, M. Beale, O. Fiehn, N. Hardy, L. Sumner, R. Bino, Plant metabolomics: the missing link in functional genomics strategies, Plant Cell. 14 (2002) 1437–1440, https://doi.org/10.1105/tpc.140720.
[51] Chris, The science behind identifying hop aromas, Beer Maverick (2020). https://beermaverick.com/the-science-behind-identifying-hop-aromas/. accessed March 20, 2026.
[52] Beer Maverick, Super Pride Hop, (n.d.). https://beermaverick.com/hop/super-pride/(accessed March 20, 2026).
[53] NZ hops Ltd., Superdelic, (n.d.). https://cdn.shopify.com/s/files/1/0378/6630/7719/files/Superdelic_Variety_Sheet_English.pdf?v=1698953220 (accessed March 20, 2026).