



Comparison of three headspace sampling techniques for gas chromatographic analysis of cocoa masses from different varieties and origins

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ABSTRACT

Volatile organic compounds (VOCs) have a pivotal role in defining cocoa and derivatives quality and providing insights into botanical background, geographical origin, postharvest practices and industrialization. This study aimed to assess the performance of three headspace sampling techniques—solid phase microextraction (HS-SPME), headspace sorption extraction (HSSE) and dynamic headspace (DHS)—for gas chromatographic-mass spectrometric analysis of VOCs in cocoa masses and the potential for distinguishing geographical origin by applying chemometrics. Cocoa masses were elaborated from eight cacao varieties (CATIE-R1, CATIE-R4, CATIE-R6, CC-137, CCN-51, ICS-95, PMCT-58 and TSH-565) grown in two estates located in South Caribbean and Northern region of Costa Rica. A total of 139 VOCs were annotated: 101 with HS-SPME, 97 with DHS and 73 with HSSE, with predominance of alcohols, esters, ketones, pyrazines and monoterpenoids. Of these, 55 VOCs were commonly detected with the three techniques. DHS enabled higher relative peak areas than HS-SPME and HSSE, although yielding higher variability. Chemometric analysis showed that all three techniques effectively differentiated sample origin, mainly due to VOCs such as 2-heptanol, 2-heptanone, isovaleric acid, isobutyric acid, and acetophenone. This study outlines the relevance of selecting the appropriated sampling technique for VOC analysis in cocoa masses and their potential application for traceability.

1. Introduction

Cacao beans are a globally traded commodity, serving as the raw material for elaborating valuable products such as cocoa mass, cocoa powder, cocoa butter, chocolates, among others (Codex Alimentarius, 2022a, 2022b, 2022c, 2022d). Cocoa mass—also called cocoa liquor—is obtained from the roasted or not cacao beans that are broken, winnowed and grinded (Cacao of Excellence, 2023; Codex Alimentarius, 2022d; End and Dand, 2023), and it is mainly used to obtain those products.

The chemical composition of cacao beans and derivatives is determined by an interrelation of factors as genetics, climate, soil, agricultural practices (Febrianto and Zhu, 2020; Hegmann et al., 2020; Jaimez

et al., 2022; Kadow, 2020; Perez et al., 2020), post-harvest and industrial processes (Aprotosoai et al., 2016; Escobar et al., 2021; Herrera-Rocha et al., 2021, 2024; Llano et al., 2024; Mayorga-Gross et al., 2016; Papalexandratou et al., 2019). Therefore, chemical analysis as volatile organic compounds (VOCs) profiling can provide information for traceability purposes (Escobar et al., 2021; Kadow, 2020; Liberto et al., 2025; Marseglia et al., 2020; Tuentner et al., 2020). This knowledge can also be useful for quality assessment, as several VOCs correlate with aroma properties thus influencing consumers perception (Becerra et al., 2023; Herrera-Rocha et al., 2023; Perez et al., 2020; Putri et al., 2024).

Gas chromatography coupled with mass spectrometry (GC-MS) is the most widely used analytical method for the determination of VOCs. This

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method requires previous extraction of VOCs from the sample, a challenging step for complex matrixes as cocoa derivatives, due to the diversity of compounds with differing concentrations and properties such as polarity, volatility and molecular weights (Herrera-Rocha et al., 2023; Liberto et al., 2017). To achieve this, headspace sampling techniques are often applied. Examples include static headspace solid phase micro-extraction (HS-SPME), stir bar sorptive extraction (SBSE), headspace sorptive extraction (HSSE), and dynamic headspace (DHS) sampling, each differing in selectivity, sensitivity, repeatability, among other performance parameters. Therefore, the choice of a headspace technique primarily depends on the analytical aim and the nature of the sample (Dias et al., 2023; Diez-Simon et al., 2020; Li et al., 2020; Paiva et al., 2021; Ríos-Reina et al., 2018; Ruvalcaba et al., 2020; Segura-Borrego et al., 2020).

HS-SPME has been the most applied technique in cocoa applications (Becerra et al., 2023; Braga et al., 2018; Britto de Andrade et al., 2021; Cemin et al., 2024; Herrera-Rocha et al., 2023; Lemarcq et al., 2022; Marseglia et al., 2020; Tuentner et al., 2020). Alternative techniques have been relatively less exploited (Chaudhary et al., 2025), for instance SBSE in cocoa powder and fresh cocoa pulp (Bickel Haase et al., 2021; Quel-al-Vásconez et al., 2022), and DHS in cocoa mass and chocolate (Crafack et al., 2014; Cucu et al., 2021; Owusu et al., 2012; Waehrens et al., 2016). To the best of our knowledge, up to date no studies have been identified regarding the application of HSSE for the analysis of cacao derivatives. Furthermore, although HS-based sampling techniques such as HS-SPME, HSSE and DHS are well-established, their comparative performance when applied to complex matrices such as cocoa mass remains insufficiently explored under harmonized experimental conditions. In particular, cocoa mass represents a challenging high-fat matrix in which extraction efficiency, selectivity, and reproducibility may vary significantly depending on the technique used. Therefore, a systematic comparison can provide useful insights into the practical applicability, strengths, and limitations of each method.

For this reason, the objective of this study is to provide a comparative assessment of HS-SPME, HSSE, and DHS coupled to GC-MS for the analysis of VOCs in cocoa masses obtained from eight cacao varieties and two geographical origins in Costa Rica under standardized conditions. The experimental design was fully harmonized, with all extraction techniques assessed using the same analytical platform, identical sample sets, and controlled conditions, thereby minimizing external variability and enabling a more reliable comparison of their intrinsic analytical performance. The study aims to elucidate the relative strengths, limitations, and complementarity of each technique in terms of VOCs coverage, and to assess the potential of the resulting VOC profiles to differentiate cocoa masses by geographical origin for future traceability applications.

2. Materials and methods

2.1. Samples

Eleven cocoa mass samples were produced from the fruits harvested from eight cacao clones (*Theobroma cacao* L.): CATIE-R1, CATIE-R4, CATIE-R6, CC-137, PMCT-58, CCN-51, ICS-95 and TSH-565 (Table 1). Seven samples originated from estate C (South Caribbean region) and four from estate O (Northern region). Moreover, three clones (CATIE-R1, CCN-51 and ICS-95) were represented in both geographical origins, whereas CATIE-R4, CATIE-R6, CC-137 and PMCT-58 were exclusive to estate C and TSH-565 was exclusive to estate O. Further details regarding each clone are available in the *International Cocoa Germplasm Database* <https://icgd.reading.ac.uk> (University of Reading, n.d.) and the Catalogue of cacao clones selected by CATIE for commercial plantings (Phillips-Mora et al., 2012). The mature fruits were harvested in two different estates of Costa Rica: a) International Cacao Collection at CATIE (IC3) and Clonal Mother Garden located in Turrialba (South Caribbean region) codified as 'C'; and b)

Table 1

Geographical origins of estates where different cacao varieties were harvested for making the cocoa masses.

Estate	Geographical origin of the estate	Cacao variety	Sample code
C	Turrialba ¹ (South Caribbean region)	CATIE-R1	CR1_C
		CATIE-R4	CR4_C
		CATIE-R6	CR6_C
		CC-137	CC1_C
		CCN-51	CCN_C
		ICS-95	ICS_C
		PMCT-58	PMC_C
O	Sarapiquí ² (Northern region)	CATIE-R1	CR1_O
		CCN-51	CCN_O
		ICS-95	ICS_O
		TSH-565	TSH_O

¹International Cacao Collection at CATIE (IC3) and Clonal Mother Garden located in Turrialba, Costa Rica (South Caribbean region) and ²Sarapiquí, Costa Rica (Northern region).

Sarapiquí (Northern region) codified as 'O', in July of 2022. To ensure strict geographical traceability, all fruits were directly harvested and labeled on-site by the authors.

The seeds were extracted from the fruits and followed a standardized and micro-fermentation procedure described by Seguine et al. (2014) followed by the solar drying protocol detailed by Phillips-Mora et al. (2012). The cacao beans were roasted under clone-specific temperature-time conditions in an oven (9110-0218, Binder) in function of the cacao clone: CATIE-R1, CATIE-R4 and CATIE-R6 were roasted at 122 °C for 23 min, CC-137 and CCN-51 at 115 °C for 21 min, ICS-95 at 111 °C for 21 min, TSH-565 at 118 °C for 26 min, and PMCT-58 at 149 °C for 13 min. Then, the cocoa beans were cracked, and shells were separated in a winnower (Capco), ground (Grindomix GM-200, Retsch), and finally refined and conched (ECGC-125 grinder, Cocoa-town) for 24 h to obtain the cocoa masses. Prior to analysis, the cocoa masses were grinded in a blade grinder at room temperature, vacuum-packed and stored under refrigeration until analysis. All the post-harvest and transformation steps were done at the *Centro Agronómico Tropical de Investigación y Enseñanza* (CATIE), Costa Rica.

2.2. Chemicals and supplies

4-Methyl-2-pentanol (Merck, Darmstadt, Germany) was used as internal standard (IS) with a final concentration of 0.79 mg/mL in a 0.5% hydroalcoholic solution. An alkane analytical standard mixture C₁₀-C₄₀ of 50 mg/L in n-hexane (Supelco®, Merck KGaA, Darmstadt, Germany) was used for the calculation of linear retention indices (LRI). For HS-SPME technique, a 2 cm SPME fiber coated with 50/30 µm of divinylbenzene/Carboxen on polydimethylsiloxane fiber (DVB/CAR/PDMS) (Supelco®, Merck KGaA, Darmstadt, Germany) was used. Twister® of 10 mm long coated with 0.5 mm polydimethylsiloxane (PDMS) layer (24 µL of PDMS) (Gerstel, Müllheim an der Ruhr, Germany) were employed for HSSE analysis, and Tenax TA™ adsorbent packed liners (Gerstel, Müllheim an der Ruhr, Germany) for DHS sampling.

2.3. Sampling methods

For the three extraction methods, a total of 2.0 g of cocoa sample were placed in 20 mL glass vials and spiked with 10 µL of the IS solution prior to extraction. The vials were then placed in a thermostated autosampler tray at 20 °C, except for HSSE analysis, as explained below. No further homogenization step was applied. However, due to the nature of headspace sampling techniques, the internal standard is expected to volatilize and equilibrate within the headspace during the incubation step, enabling its effective interaction with the sample matrix. All extraction techniques were performed under identical incubation and

extraction conditions (60 °C and 40 min) to ensure strict methodological comparability across HS-SPME, HSSE, and DHS. This experimental design was intentionally selected to minimize variability arising from technique-specific optimization and to allow a direct evaluation of the intrinsic performance and extraction behavior of each headspace approach under standardized conditions. Although each technique may exhibit different optimal operating conditions, the application of uniform parameters provides a controlled experimental framework that facilitates unbiased comparison and avoids confounding effects associated with differential optimization strategies. Moreover, the selected sorptive phases for each extraction method correspond to those most commonly employed and validated in previous studies for VOC analysis of food matrices (Cordero et al., 2009; Jelén et al., 2012; Jelén and Wieczorek, 2025). All samples were analyzed in duplicate, and blanks were run between samples.

2.3.1. Headspace solid phase microextraction (HS-SPME)

The procedure was based in Van Durme et al. (2016) methodology with some modifications. The sample with the IS was incubated at 60 °C for 10 min shaking at a speed of 250 rpm. Afterwards, the VOCs extraction was performed by exposing the SPME fiber to the headspace of the vial at 60 °C for 40 min. Then, for desorption of compounds, the fiber was inserted and kept into the injector at 250°C for 3 min in splitless mode.

2.3.2. Headspace sorptive extraction (HSSE)

After placing the sample with the IS in the vial, a Twister® was included inside a glass insert and then the vial was tightly capped. The capped vial was heated in a thermostatic bath following the same extraction parameters previously described for the HS-SPME procedure (40 min at 60 °C). Afterwards, the Twister® was removed with tweezers when the vial reached room temperature, rinsed with Milli-Q grade water, and dried with a lint-free tissue. The stir bar was put into a glass tube of 60 mm in length, 6 mm o.d. and 4 mm i.d., and then desorbed in the Thermal Desorption Unit (TDU) (TDS2, Gerstel, Müllheim and der Ruhr, Germany) in splitless mode. The desorption temperature program was the following: 35 °C for 1 min, ramped at 60 °C/min to 250 °C held for 5 min. The VOCs were cryo-focused on a CIS-4 PTV injector (Gerstel, Müllheim and der Ruhr, Germany) at -35 °C for total desorption time and then raised at 10 °C/s to 260 °C and held for 4 min. Solvent vent mode was employed for transferring of sample to analytical column.

2.3.3. Dynamic headspace sampling (DHS)

The vial with the sample and the IS were incubated in the DHS unit at 60 °C during 2 min and agitated at 250 rpm. Next, purging of the sample headspace was done with 50 mL/min of N₂ during 40 min and the VOCs were trapped on a pre-conditioned Tenax TA™ adsorbent at 30°C. Finally, desorption and injection steps were done with the same procedure for the HSSE analysis previously described.

2.4. Gas Chromatography- mass spectrometry analysis (GC-MS)

Gas chromatography analysis was performed using an Agilent 8890 GC System (Santa Clara, United States) coupled to a mass spectrometer (Agilent, 5977B GC/MSD) and equipped with a CP-WAX 57 CB column of 50 m x 0.25 mm and 0.2 µm film thickness (Agilent CP97723, Santa Clara, United States) for compound separation. Helium was used as carrier gas with a controlled flow of 1 mL/min. The oven temperature program was as follows: 40 °C for 5 min of hold time, then raised to 140 °C at 2 °C/min and held for 5 min, and afterwards raised to 220°C at a rate of 10 °C/min (held for 5 min), based on Hamdouche et al. (2019), with the modification of the final temperature as the authors used 250 °C. Electronic ionization was applied at 70 eV, ion source and transfer line temperature were set at 230 °C and 250 °C, respectively. Full scans were recorded in a *m/z* range of 29–350. To monitor instrumental performance throughout the analytical sequence, a quality control (QC)

sample was analyzed after every ten sample injections. The QC samples were used to verify chromatographic stability and analytical repeatability during the GC-MS analyses. Moreover, all samples were analyzed in randomized order to minimize potential instrumental drift throughout the analytical sequence.

2.5. Identification of volatile compounds

The acquired chromatographic data were converted to AIA format by MSD ChemStation F.01.01.2317© (Agilent Technologies, Inc., Santa Clara, United States). Then, data was deconvoluted with software PARADiSe v6.0.1 based on the PARAFAC2 algorithm, and following the steps previously described in the literature (Johnsen et al., 2017; PARADiSe, 2023; Quintanilla-Casas et al., 2023).

Volatile compounds were identified based on a combination of mass spectral information (match factor ≥ 90%), linear retention indices (LRI), and authentic reference standards when available. Identification reliability was classified into three confidence levels: A, mass spectrum and LRI agreed with authentic standards; B, mass spectrum agreed with spectral databases and LRI agreed with literature values; and C, mass spectrum agreed with the spectral database. Annotated VOCs were classified into chemical groups with ClassyFire web application (Djombou Feunang et al., 2016) and Human Metabolome Database (<https://hmdb.ca/>, Wishart et al., 2022).

2.6. Statistical analysis

Relative peak areas (VOC peak area/IS peak area) of annotated compounds were used for the statistical analysis. The normalized areas of the replicates were reported as averages with their corresponding standard deviations. Principal component analysis (PCA) was applied to the experimental data of each headspace sampling technique. In all cases, pre-processing included an autoscaling step. This analysis was done with PLS_Toolbox 9.2.1 (Eigenvector Research Inc., USA) under the MATLAB R2022a environment.

3. Results and discussion

3.1. Comparison of the sampling techniques

A total of 139 volatile compounds (VOCs) were detected in the cocoa masses, 55 of them were detected in common after sampling with the three techniques (Table 2, Table 3 and Supplementary Material, Tables S1, S2 and S3). To facilitate visualization of the overlap and the unique compounds extracted by each method, a Venn diagram has been included (Fig. 1). The highest number of VOCs (i.e. 101 VOCs) were determined after sampling with HS-SPME, followed by DHS with 97 VOCs and, notably fewer, by HSSE with 73 VOCs. The detected VOCs were classified into 8 main chemical groups: acids, alcohols, aldehydes, alkanes, esters, ketones, monoterpenoids and pyrazines. The predominant groups were alcohols, esters, ketones, monoterpenoids, and pyrazines (Fig. 2). An additional group named “others” comprised a diversity of compounds which contributed to the overall complexity of the samples; further details are provided in the Tables S1, S2 and S3. These results were expected as samples came from cocoa beans that had been fermented, dried and roasted. These are crucial transformation steps for the development of VOCs, some of which actively participate in the aroma (Otero Rojas, et al., 2022; Peña-Correa et al., 2022; Quelal et al., 2023; Rojas et al., 2022).

3.1.1. Alcohols

Alcohols represented the largest chemical group totaling 24 annotated compounds. The highest number was detected after sampling with DHS (20), followed by HS-SPME (18) and HSSE (15) (Fig. 2), and 13 of them were detected in common after sampling with the three techniques (Tables 2 and 3). In addition, it was also observed that DHS enabled the

Table 2
Means of the relative¹ peak area values and correspondent standard deviations of VOCs detected in common after sampling with three headspace techniques in different cocoa masses made from estate 'C' cocoa.

Compound	LRI	Technique	Mean relative peak areas						
			CC1_C	CCN_C	CR1_C	CR4_C	CR6_C	ICS_C	PMC_C
Acids									
Acetic acid (CAS: 64–19–7; LRI: 1425)	1425 ^a	HSSE	0.300 ± 0.106	11.052 ± 1.929	0.221 ± 0.175	0.218 ± 0.014	3.512 ± 0.152	0.246 ± 0.168	0.785 ± 0.449
		DHS	12.809 ± 0.265	26.592 ± 21.496	12.985 ± 0.958	24.031 ± 3.536	16.692 ± 8.600	18.485 ± 0.506	19.980 ± 0.667
		SPME	2.307 ± 0.665	24.463 ± 2.399	4.505 ± 0.272	6.191 ± 1.446	7.037 ± 0.267	3.506 ± 0.068	4.571 ± 0.185
Propanoic acid (Propionic acid) (CAS: 79–09–4; LRI: 1532)	1532 ^a	HSSE	0.026 ± 0.007	0.105 ± 0.007	0.018 ± 0.007	0.0140 ± 0.0001	0.036 ± 0.008	0.025 ± 0.001	0.047 ± 0.022
		DHS	0.360 ± 0.054	0.981 ± 0.544	0.177 ± 0.011	0.367 ± 0.119	0.429 ± 0.212	0.714 ± 0.054	0.557 ± 0.017
		SPME	0.027 ± 0.008	0.078 ± 0.007	0.034 ± 0.006	0.038 ± 0.012	0.034 ± 0.002	0.047 ± 0.001	0.0520 ± 0.0001
Butanoic acid (Butyric acid) (CAS: 107–92–6; LRI: 1629)	1629 ^a	HSSE	0.026 ± 0.001	0.089 ± 0.018	0.016 ± 0.005	0.022 ± 0.001	0.039 ± 0.011	0.044 ± 0.001	0.040 ± 0.012
		SPME	0.026 ± 0.003	0.065 ± 0.001	0.040 ± 0.003	0.049 ± 0.009	0.046 ± 0.002	0.046 ± 0.002	0.0539 ± 0.0001
		DHS	0.446 ± 0.194	0.489 ± 0.359	0.628 ± 0.302	0.314 ± 0.369	0.707 ± 0.26	0.723 ± 0.084	0.790 ± 0.046
3-Methylbutanoic acid (Isovaleric acid) (CAS: 503–74–2; LRI: 1672)	1672 ^a	HSSE	0.051 ± 0.008	0.268 ± 0.069	0.044 ± 0.012	0.0519 ± 0.0003	0.090 ± 0.016	0.092 ± 0.003	0.125 ± 0.037
		DHS	2.241 ± 0.195	7.053 ± 4.489	4.290 ± 0.678	6.128 ± 2.941	5.993 ± 2.832	6.546 ± 0.097	10.22 ± 0.937
		SPME	0.056 ± 0.017	0.241 ± 0.006	0.102 ± 0.016	0.128 ± 0.031	0.131 ± 0.014	0.1216 ± 0.0003	0.187 ± 0.017
2-Methylbutanoic acid (CAS: 116–53–0; LRI: 1675)	1675 ^a	HSSE	0.018 ± 0.003	0.064 ± 0.019	0.012 ± 0.003	0.0130 ± 0.0004	0.019 ± 0.004	0.037 ± 0.006	0.029 ± 0.008
		SPME	0.010 ± 0.004	0.0346 ± 0.0006	0.012 ± 0.002	0.017 ± 0.004	0.014 ± 0.001	0.0233 ± 0.0001	0.023 ± 0.003
		DHS	1.453 ± 0.906	1.022 ± 0.692	6.586 ± 8.009	2.335 ± 1.725	1.320 ± 0.684	1.821 ± 0.704	2.330 ± 0.200
Alcohols									
2-Pentanol (CAS: 6032–29–7; LRI: 1114)	1114 ^a	HSSE	0.025 ± 0.003	0.010 ± 0.001	0.014 ± 0.002	0.021 ± 0.004	0.064 ± 0.003	0.018 ± 0.003	0.031 ± 0.002
		DHS	0.108 ± 0.003	0.027 ± 0.013	0.040 ± 0.002	0.076 ± 0.018	0.095 ± 0.049	0.052 ± 0.001	0.130 ± 0.006
		SPME	0.278 ± 0.076	0.108 ± 0.024	0.147 ± 0.031	0.218 ± 0.053	0.310 ± 0.012	0.138 ± 0.004	0.332 ± 0.004
3-Methyl–1-butanol (Isoamyl alcohol) (CAS: 123–51–3; LRI: 1201)	1201 ^a	HSSE	0.174 ± 0.033	0.029 ± 0.002	0.193 ± 0.002	0.103 ± 0.008	0.103 ± 0.008	0.130 ± 0.007	0.192 ± 0.023
		DHS	0.564 ± 0.017	0.162 ± 0.060	0.608 ± 0.035	0.592 ± 0.173	0.303 ± 0.153	0.483 ± 0.01	0.803 ± 0.076
		SPME	0.266 ± 0.073	0.065 ± 0.014	0.322 ± 0.051	0.227 ± 0.052	0.162 ± 0.01	0.207 ± 0.002	0.306 ± 0.001
1-Pentanol (CAS: 71–41–0; LRI: 1244)	1244 ^a	HSSE	0.036 ± 0.003	0.019 ± 0.001	0.0226 ± 0.0003	0.021 ± 0.001	0.022 ± 0.001	0.033 ± 0.001	0.039 ± 0.003
		DHS	0.114 ± 0.003	0.089 ± 0.014	0.128 ± 0.009	0.141 ± 0.042	0.074 ± 0.032	0.167 ± 0.001	0.167 ± 0.008
		SPME	0.053 ± 0.006	0.034 ± 0.004	0.043 ± 0.005	0.0485 ± 0.0001	0.036 ± 0.002	0.060 ± 0.001	0.0639 ± 0.0002
2-Heptanol (CAS: 543–49–7; LRI: 1316)	1316 ^a	HSSE	0.474 ± 0.058	0.515 ± 0.063	0.0480 ± 0.0001	0.066 ± 0.003	0.136 ± 0.012	0.436 ± 0.033	0.187 ± 0.036
		DHS	6.639 ± 0.097	4.374 ± 2.630	0.716 ± 0.115	1.041 ± 0.458	1.949 ± 1.007	7.393 ± 0.002	3.963 ± 0.102
		SPME	0.245 ± 0.036	0.544 ± 0.051	0.055 ± 0.004	0.083 ± 0.017	0.115 ± 0.01	0.311 ± 0.003	0.126 ± 0.015
3-Ethoxy–1-propanol (CAS: 111–35–3; LRI: 1372)	1372 ^{b,2}	HSSE	0.296 ± 0.042	0.015 ± 0.002	0.189 ± 0.009	0.105 ± 0.002	0.057 ± 0.006	0.199 ± 0.007	0.168 ± 0.025
		DHS	2.059 ± 0.124	0.090 ± 0.031	2.224 ± 0.318	1.280 ± 0.478	0.501 ± 0.26	2.313 ± 0.027	2.046 ± 0.244
		SPME	0.203 ± 0.013	0.022 ± 0.001	0.263 ± 0.008	0.122 ± 0.022	0.060 ± 0.008	0.1999 ± 0.0004	0.144 ± 0.005
2-Octanol (CAS: 123–96–6; LRI: 1414)	1414 ^a	HSSE	0.012 ± 0.002	0.019 ± 0.002	0.0028 ± 0.00001	0.0034 ± 0.0003	0.006 ± 0.001	0.016 ± 0.002	0.005 ± 0.001
		DHS	0.304 ± 0.012	0.174 ± 0.125	0.036 ± 0.006	0.045 ± 0.017	0.101 ± 0.054	0.367 ± 0.005	0.1474 ± 0.0001
		SPME	0.006 ± 0.001	0.0813 ± 0.0003	0.005 ± 0.001	0.0061 ± 0.0001	0.007 ± 0.001	0.0104 ± 0.0001	0.004 ± 0.001
2-Nonanol (CAS: 628–99–9; LRI: 1515)	1515 ^a	HSSE	0.119 ± 0.021	0.236 ± 0.041	0.008 ± 0.001	0.011 ± 0.002	0.024 ± 0.003	0.244 ± 0.032	0.031 ± 0.007
		DHS	1.422 ± 0.102	1.191 ± 1.044	0.173 ± 0.034	0.279 ± 0.116	0.481 ± 0.258	2.200 ± 0.076	0.609 ± 0.012
		SPME	0.042 ± 0.004	0.149 ± 0.047	0.017 ± 0.002	0.020 ± 0.003	0.020 ± 0.004	0.133 ± 0.004	0.018 ± 0.008
2,3-Butanediol, isomer 1 (LRI: 1554)	1554 ^c	HSSE	2.361 ± 0.521	10.26 ± 2.028	9.259 ± 0.376	9.219 ± 0.119	10.800 ± 1.408	9.989 ± 0.704	11.348 ± 3.098
		DHS	96.407 ± 3.918	105.260 ± 77.461	135.481 ± 22.150	130.027 ± 50.223	134.06 ± 74.56	159.200 ± 5.959	252.472 ± 0.735
		SPME	2.490 ± 0.541	7.456 ± 0.184	8.813 ± 0.268	7.303 ± 1.273	6.896 ± 0.473	5.372 ± 0.025	6.673 ± 0.114
2,3-Butanediol, isomer 2 (LRI: 1590)	1590 ^c	HSSE	1.871 ± 0.387	8.740 ± 1.469	2.670 ± 0.150	3.343 ± 0.038	2.855 ± 0.394	3.333 ± 0.248	3.988 ± 1.091
		DHS	68.740 ± 2.129	92.017 ± 63.78	88.900 ± 15.204	87.935 ± 34.815	74.632 ± 41.041	101.645 ± 2.944	156.991 ± 2.528
		SPME	1.892 ± 0.389	6.764 ± 0.278	4.116 ± 0.108	4.083 ± 0.839	3.022 ± 0.097	2.992 ± 0.048	3.641 ± 0.001
2-Furanmethanol (Furfuryl alcohol) (CAS: 98–00–0; LRI: 1661)	1661 ^a	HSSE	0.163 ± 0.039	0.384 ± 0.012	0.117 ± 0.052	0.0621 ± 0.0001	0.128 ± 0.028	0.078 ± 0.020	0.327 ± 0.204
		DHS	0.914 ± 0.016	4.407 ± 2.658	0.539 ± 0.091	1.335 ± 0.602	1.208 ± 0.703	1.764 ± 0.022	2.747 ± 0.162
		SPME	0.019 ± 0.001	0.282 ± 0.024	0.027 ± 0.002	0.067 ± 0.003	0.069 ± 0.008	0.0611 ± 0.0002	0.0679 ± 0.0003
Methyl benzenemethanol (1-Phenylethanol) (CAS: 98–85–1; 1818)	1818 ^{b,2}	HSSE	0.074 ± 0.011	0.048 ± 0.009	0.026 ± 0.002	0.070 ± 0.006	0.074 ± 0.008	0.065 ± 0.009	0.150 ± 0.031
		DHS	3.027 ± 0.192	0.877 ± 0.690	1.273 ± 0.240	3.399 ± 1.588	3.241 ± 1.790	2.696 ± 0.025	9.984 ± 0.43
		SPME	0.024 ± 0.002	0.027 ± 0.002	0.026 ± 0.003	0.055 ± 0.002	0.058 ± 0.012	0.035 ± 0.001	0.069 ± 0.007
Benzyl alcohol (Phenylmethanol) (CAS: 100–51–6; LRI: 1896)	1896 ^a	HSSE	0.037 ± 0.006	0.093 ± 0.024	0.021 ± 0.003	0.0313 ± 0.0004	0.029 ± 0.003	0.057 ± 0.019	0.038 ± 0.007
		DHS	1.496 ± 0.091	1.871 ± 1.472	1.023 ± 0.195	1.383 ± 0.682	1.328 ± 0.749	1.730 ± 0.007	2.724 ± 0.183

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Table 2 (continued)

Compound	LRI	Technique	Mean relative peak areas						
			CC1_C	CCN_C	CR1_C	CR4_C	CR6_C	ICS_C	PMC_C
Phenylethyl alcohol (2-Phenylethanol) (CAS: 60–12–8; LRI: 1934)	1934 ^a	SPME	0.014 ± 0.002	0.054 ± 0.003	0.025 ± 0.001	0.025 ± 0.002	0.025 ± 0.006	0.025 ± 0.001	0.022 ± 0.004
		HSSE	2.897 ± 0.484	1.748 ± 0.396	2.020 ± 0.135	1.703 ± 0.148	1.420 ± 0.199	2.584 ± 0.38	2.738 ± 0.522
		DHS	57.20 ± 3.401	30.357 ± 25.677	44.729 ± 7.675	43.155 ± 18.128	41.837 ± 23.788	60.269 ± 2.165	96.642 ± 0.872
		SPME	1.133 ± 0.219	1.561 ± 0.119	2.233 ± 0.163	1.656 ± 0.005	1.411 ± 0.365	1.650 ± 0.112	1.525 ± 0.287
Aldehydes									
Benzaldehyde (CAS: 100–52–7; LRI: 1512)	1512 ^a	HSSE	0.074 ± 0.005	0.083 ± 0.015	0.127 ± 0.004	0.109 ± 0.001	0.124 ± 0.007	0.103 ± 0.001	0.155 ± 0.015
		DHS	0.596 ± 0.029	0.365 ± 0.187	0.683 ± 0.080	0.898 ± 0.270	0.707 ± 0.344	0.904 ± 0.017	1.594 ± 0.042
		SPME	0.148 ± 0.021	0.349 ± 0.055	0.485 ± 0.008	0.513 ± 0.079	0.444 ± 0.079	0.405 ± 0.012	0.493 ± 0.024
Benzeneacetaldehyde (Phenylacetaldehyde) (CAS: 122–78–1; LRI: 1637)	1637 ^a	HSSE	0.050 ± 0.003	0.054 ± 0.011	0.072 ± 0.003	0.063 ± 0.002	0.022 ± 0.001	0.059 ± 0.003	0.062 ± 0.008
		SPME	0.013 ± 0.001	0.027 ± 0.006	0.042 ± 0.004	0.028 ± 0.005	0.022 ± 0.007	0.027 ± 0.002	0.031 ± 0.006
		DHS	0.479 ± 0.02	0.968 ± 1.014	0.310 ± 0.089	0.098 ± 0.037	0.401 ± 0.289	0.442 ± 0.017	1.559 ± 0.155
Alkanes									
Dodecane (CAS: 112–40–3; LRI: 1195)	1195 ^{b,2}	HSSE	0.231 ± 0.043	0.164 ± 0.041	0.198 ± 0.004	0.160 ± 0.031	0.209 ± 0.027	0.210 ± 0.023	0.238 ± 0.031
		DHS	4.377 ± 0.238	1.790 ± 1.986	3.290 ± 0.634	1.579 ± 0.374	3.157 ± 1.597	2.735 ± 0.180	6.512 ± 0.692
		SPME	0.052 ± 0.001	0.092 ± 0.024	0.131 ± 0.045	0.0674 ± 0.0003	0.113 ± 0.039	0.081 ± 0.008	0.076 ± 0.007
Tetradecane (CAS: 629–59–4; LRI: 1390)	1390 ^a	HSSE	0.091 ± 0.019	0.088 ± 0.021	0.069 ± 0.004	0.069 ± 0.014	0.065 ± 0.007	0.115 ± 0.030	0.054 ± 0.003
		DHS	4.253 ± 0.246	2.699 ± 2.448	4.623 ± 0.903	3.578 ± 1.377	3.583 ± 1.976	5.308 ± 0.237	7.073 ± 0.270
		SPME	0.011 ± 0.002	0.034 ± 0.019	0.040 ± 0.021	0.022 ± 0.013	0.035 ± 0.014	0.028 ± 0.004	0.014 ± 0.003
Esters									
2-Heptanol acetate (1-Methylhexyl acetate) (CAS: 5921–82–4; LRI: 1247)	1247 ^a	HSSE	0.012 ± 0.002	0.105 ± 0.023	0.0034 ± 0.0001	0.0032 ± 0.0004	0.012 ± 0.001	0.014 ± 0.002	0.011 ± 0.002
		DHS	0.046 ± 0.002	0.184 ± 0.110	0.027 ± 0.004	0.026 ± 0.009	0.038 ± 0.018	0.0335 ± 0.0001	0.078 ± 0.003
		SPME	0.010 ± 0.002	0.120 ± 0.024	0.015 ± 0.002	0.016 ± 0.005	0.021 ± 0.002	0.0194 ± 0.0001	0.020 ± 0.001
Ethyl 2-hydroxypropanoate (Ethyl lactate) (CAS: 97–64–3; LRI: 1330)	1330 ^a	HSSE	0.239 ± 0.026	0.332 ± 0.064	0.099 ± 0.003	0.120 ± 0.005	0.212 ± 0.017	0.051 ± 0.001	0.056 ± 0.005
		DHS	0.485 ± 0.044	2.938 ± 0.941	0.275 ± 0.004	0.698 ± 0.182	0.809 ± 0.359	0.135 ± 0.002	0.127 ± 0.009
		SPME	0.097 ± 0.020	0.260 ± 0.001	0.065 ± 0.019	0.085 ± 0.018	0.136 ± 0.023	0.0215 ± 0.0004	0.029 ± 0.001
Ethyl octanoate (CAS: 106–32–1; LRI: 1420)	1420 ^a	HSSE	0.072 ± 0.013	0.135 ± 0.022	0.058 ± 0.002	0.060 ± 0.010	0.070 ± 0.010	0.088 ± 0.013	0.090 ± 0.012
		DHS	2.470 ± 0.150	1.917 ± 1.669	1.796 ± 0.323	1.571 ± 0.553	1.978 ± 1.077	2.555 ± 0.055	4.040 ± 0.210
		SPME	0.0156 ± 0.0004	0.050 ± 0.015	0.041 ± 0.015	0.030 ± 0.009	0.037 ± 0.013	0.032 ± 0.002	0.022 ± 0.002
2-Nonanol acetate (CAS: 14936–66–4; LRI:1446)	1446 ^a	HSSE	0.006 ± 0.001	0.026 ± 0.005	0.0012 ± 0.0004	0.0014 ± 0.0002	0.005 ± 0.001	0.008 ± 0.003	0.0044 ± 0.0004
		DHS	0.323 ± 0.020	0.392 ± 0.33	0.090 ± 0.019	0.090 ± 0.036	0.171 ± 0.091	0.281 ± 0.007	0.245 ± 0.006
		SPME	0.005 ± 0.001	0.021 ± 0.006	0.0045 ± 0.0004	0.003 ± 0.001	0.0075 ± 0.0001	0.0064 ± 0.0001	0.002 ± 0.001
2,3-Butanediol diacetate, isomer 1 (LRI: 1470)	1470 ^c	HSSE	0.004 ± 0.001	0.183 ± 0.03	0.0045 ± 0.0001	0.007 ± 0.001	0.012 ± 0.001	0.010 ± 0.005	0.007 ± 0.002
		DHS	0.082 ± 0.002	1.441 ± 0.974	0.101 ± 0.016	0.163 ± 0.055	0.196 ± 0.103	0.133 ± 0.004	0.182 ± 0.001
		SPME	0.0009 ±	0.137 ± 0.022	0.0037 ± 0.0001	0.0071 ± 0.0003	0.009 ± 0.001	0.0029 ± 0.0001	0.0028 ± 0.0001
2,3-Butanediol diacetate, isomer 2 (LRI: 1507)	1507 ^c	HSSE	0.009 ± 0.002	0.107 ± 0.019	0.0072 ± 0.0004	0.008 ± 0.001	0.021 ± 0.002	0.013 ± 0.001	0.009 ± 0.002
		DHS	0.0564 ± 0.0002	0.860 ± 0.615	0.071 ± 0.011	0.151 ± 0.06	0.336 ± 0.173	0.078 ± 0.002	0.120 ± 0.001
		SPME	0.0014 ± 0.0001	0.083 ± 0.012	0.0054 ± 0.0001	0.009 ± 0.001	0.016 ± 0.002	0.0035 ± 0.0001	0.0035 ± 0.0001
Benzyl acetate (CAS: 140–11–4; LRI: 1737)	1737 ^a	HSSE	0.003 ± 0.001	0.040 ± 0.008	0.0031 ± 0.0005	0.0050 ± 0.0002	0.0055 ± 0.0004	0.008 ± 0.002	0.004 ± 0.001
		SPME	0.0007 ± 0.0001	0.015 ± 0.003	0.0024 ± 0.0003	0.0024 ± 0.0003	0.0023 ± 0.0008	0.0015 ± 0.0001	0.0014 ± 0.0002
		DHS	0.069 ± 0.006	0.563 ± 0.473	0.054 ± 0.010	0.083 ± 0.033	0.107 ± 0.058	0.085 ± 0.002	0.1351 ± 0.0007
Ethyl phenylacetate (CAS: 101–97–3; LRI: 1784)	1784 ^a	HSSE	0.186 ± 0.015	0.199 ± 0.031	0.065 ± 0.004	0.065 ± 0.01	0.119 ± 0.016	0.111 ± 0.016	0.252 ± 0.042
		DHS	7.977 ± 0.635	3.958 ± 3.385	2.916 ± 0.584	2.623 ± 1.072	4.445 ± 2.376	4.577 ± 0.093	14.177 ± 0.262
		SPME	0.024 ± 0.002	0.053 ± 0.005	0.031 ± 0.006	0.022 ± 0.003	0.038 ± 0.012	0.027 ± 0.001	0.038 ± 0.005
2-Phenylethyl acetate (CAS: 103–45–7; LRI: 1813)	1813 ^a	HSSE	0.220 ± 0.029	1.368 ± 0.238	0.226 ± 0.014	0.299 ± 0.052	0.435 ± 0.057	0.244 ± 0.03	0.306 ± 0.044
		DHS	9.323 ± 0.714	21.84 ± 8.779	10.21 ± 1.961	11.24 ± 4.576	14.76 ± 7.978	10.43 ± 0.24	17.91 ± 0.281
		SPME	0.050 ± 0.001	0.314 ± 0.069	0.134 ± 0.03	0.116 ± 0.027	0.154 ± 0.048	0.083 ± 0.007	0.076 ± 0.015
Ketones									
3-Hydroxy–2-butanone (Acetoin) (CAS: 513–86–0; LRI: 1275)	1275 ^a	HSSE	0.171 ± 0.023	1.967 ± 0.311	0.233 ± 0.001	0.172 ± 0.059	0.4380 ± 0.0001	0.228 ± 0.011	0.207 ± 0.010
		DHS	0.867 ± 0.039	6.299 ± 2.965	1.161 ± 0.098	0.994 ± 0.211	1.219 ± 0.499	1.298 ± 0.070	1.254 ± 0.079
		SPME	0.340 ± 0.109	6.796 ± 0.423	0.539 ± 0.074	0.439 ± 0.084	0.638 ± 0.024	0.559 ± 0.011	0.408 ± 0.008
1-Hydroxy–2-propanone (Hydroxyacetone) (CAS: 116–09–6; LRI: 1288)	1288 ^a	HSSE	0.552 ± 0.136	0.593 ± 0.005	0.516 ± 0.164	0.251 ± 0.036	0.396 ± 0.054	0.313 ± 0.082	0.910 ± 0.301
		DHS	0.949 ± 0.004	3.019 ± 2.208	0.760 ± 0.129	1.207 ± 0.258	1.023 ± 0.549	1.284 ± 0.031	2.053 ± 0.099
		SPME	0.113 ± 0.038	0.307 ± 0.011	0.132 ± 0.006	0.2074 ± 0.0001	0.152 ± 0.014	0.200 ± 0.001	0.218 ± 0.009
1-Hydroxy–2-butanone (CAS: 5077–67–8; LRI: 1363)		HSSE	0.055 ± 0.001	0.058 ± 0.003	0.065 ± 0.020	0.025 ± 0.005	0.041 ± 0.007	0.034 ± 0.002	0.129 ± 0.064

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Table 2 (continued)

Compound	LRI	Technique	Mean relative peak areas							
			CC1_C	CCN_C	CR1_C	CR4_C	CR6_C	ICS_C	PMC_C	
Acetoin acetate (2-Acetoxy-3-butanone) (CAS: 4906-24-5; LRI: 1364)	1363 ^{b,2}	DHS	0.059 ± 0.001	0.177 ± 0.070	0.042 ± 0.006	0.104 ± 0.017	0.084 ± 0.044	0.1064 ± 0.0002	0.145 ± 0.006	
		SPME	0.006 ± 0.001	0.008 ± 0.005	0.006 ± 0.001	0.012 ± 0.001	0.0091 ± 0.0001	0.015 ± 0.0002	0.013 ± 0.001	
		HSSE	0.021 ± 0.004	0.364 ± 0.080	0.013 ± 0.001	0.016 ± 0.001	0.019 ± 0.001	0.0158 ± 0.002	0.018 ± 0.003	
	1364 ^{b,2}	DHS	0.0517 ± 0.0003	1.414 ± 0.214	0.038 ± 0.006	0.032 ± 0.013	0.045 ± 0.021	0.077 ± 0.003	0.089 ± 0.001	
		SPME	0.003 ± 0.001	0.814 ± 0.024	0.010 ± 0.001	0.010 ± 0.001	0.024 ± 0.003	0.0077 ± 0.0002	0.0061 ± 0.00001	
		HSSE	0.080 ± 0.014	0.337 ± 0.051	0.0216 ± 0.0002	0.024 ± 0.003	0.046 ± 0.005	0.185 ± 0.027	0.050 ± 0.009	
2-Nonanone (CAS: 821-55-6; LRI: 1377)	1377 ^a	DHS	0.996 ± 0.028	2.400 ± 1.799	0.2014 ± 0.030	0.176 ± 0.061	0.441 ± 0.210	1.980 ± 0.001	0.716 ± 0.039	
		SPME	0.020 ± 0.003	0.165 ± 0.053	0.020 ± 0.004	0.020 ± 0.003	0.025 ± 0.005	0.069 ± 0.004	0.020 ± 0.008	
1-Phenylethanone (Acetophenone) (CAS: 98-86-2; LRI: 1651)	1651 ^a	HSSE	0.331 ± 0.132	0.538 ± 0.114	0.105 ± 0.040	0.387 ± 0.043	0.243 ± 0.099	1.757 ± 1.378	0.350 ± 0.015	
		DHS	2.201 ± 0.040	1.141 ± 0.856	1.028 ± 0.168	2.243 ± 0.823	2.304 ± 1.155	2.103 ± 0.012	4.444 ± 0.170	
1-(1H-pyrrol-2-yl) ethanone (2-Acetylpyrrole) (CAS: 1072-83-9; LRI: 1991)		SPME	0.028 ± 0.003	0.0388 ± 0.0002	0.032 ± 0.004	0.058 ± 0.001	0.066 ± 0.015	0.041 ± 0.001	0.064 ± 0.002	
		HSSE	0.07 ± 0.013	0.768 ± 0.169	0.049 ± 0.004	0.102 ± 0.009	0.114 ± 0.015	0.124 ± 0.017	0.142 ± 0.027	
	1991 ^{b,2}	SPME	0.026 ± 0.001	0.485 ± 0.059	0.071 ± 0	0.092 ± 0.012	0.112 ± 0.023	0.088 ± 0.003	0.085 ± 0.008	
		DHS	2.792 ± 0.163	16.04 ± 3.3	2.469 ± 0.474	5.041 ± 2.478	5.390 ± 3.133	5.626 ± 0.057	9.465 ± 0.494	
Monoterpenoids										
<i>trans</i> -linalyl oxide (<i>trans</i> -linalool oxide) (furanoid) (CAS: 34995-77-2; LRI: 1439)	1439 ^{b,3}	HSSE	0.012 ± 0.002	0.011 ± 0.002	0.0062 ± 0.0002	0.019 ± 0.002	0.045 ± 0.006	0.015 ± 0.002	0.017 ± 0.003	
		SPME	0.018 ± 0.003	0.026 ± 0.004	0.021 ± 0.001	0.0595 ± 0.0001	0.108 ± 0.010	0.027 ± 0.003	0.0291 ± 0.0003	
		DHS	0.446 ± 0.018	0.144 ± 0.108	0.166 ± 0.023	0.548 ± 0.208	1.330 ± 0.679	0.518 ± 0.012	0.774 ± 0.016	
		HSSE	0.080 ± 0.013	0.079 ± 0.013	0.025 ± 0.001	0.090 ± 0.012	0.227 ± 0.027	0.078 ± 0.010	0.059 ± 0.011	
<i>cis</i> -linalyl oxide (<i>cis</i> -linalool oxide) (furanoid) (CAS: 5989-33-3; LRI: 1467)		DHS	2.581 ± 0.123	0.812 ± 0.632	0.563 ± 0.086	1.893 ± 0.744	5.173 ± 2.687	2.268 ± 0.041	2.190 ± 0.025	
		SPME	0.043 ± 0.009	0.064 ± 0.010	0.031 ± 0.001	0.0991 ± 0.0003	0.199 ± 0.020	0.0532 ± 0.0002	0.035 ± 0.001	
Linalool (3,7-Dimethylocta-1,6-dien-3-ol) (CAS: 78-70-6; LRI: 1537)	1467 ^{b,3}	HSSE	0.030 ± 0.006	0.136 ± 0.024	0.014 ± 0.001	0.059 ± 0.006	0.125 ± 0.009	0.045 ± 0.006	0.034 ± 0.007	
		DHS	0.857 ± 0.060	1.351 ± 1.127	0.256 ± 0.049	1.002 ± 0.405	2.552 ± 1.331	0.996 ± 0.02	0.916 ± 0.013	
<i>cis</i> -Linalool 3,7-oxide (pyranoid) (CAS: 24048-52-0; LRI: 1773)	1537 ^a	SPME	0.011 ± 0.002	0.092 ± 0.021	0.012 ± 0.002	0.050 ± 0.007	0.098 ± 0.012	0.0218 ± 0.0001	0.0131 ± 0.0005	
		HSSE	0.016 ± 0.002	0.029 ± 0.006	0.007 ± 0.001	0.031 ± 0.005	0.075 ± 0.009	0.020 ± 0.002	0.015 ± 0.003	
		DHS	0.862 ± 0.065	0.697 ± 0.590	0.474 ± 0.102	1.746 ± 0.737	3.603 ± 1.84	1.129 ± 0.009	1.166 ± 0.022	
		SPME	0.012 ± 0.003	0.031 ± 0.003	0.0142 ± 0.0003	0.048 ± 0.002	0.101 ± 0.009	0.0204 ± 0.0001	0.013 ± 0.001	
3,7-Dimethyl-6-octen-1-ol (beta-Citronellol) (CAS: 106-22-9; LRI: 1774)	1773 ^c	HSSE	0.008 ± 0.004	0.017 ± 0.003	0.0029 ± 0.0003	0.004 ± 0.001	0.0086 ± 0.0001	0.016 ± 0.008	0.0072 ± 0.0003	
		DHS	0.056 ± 0.002	0.390 ± 0.280	0.050 ± 0.007	0.043 ± 0.016	0.095 ± 0.047	0.077 ± 0.003	0.157 ± 0.003	
		1774 ^{b,2}	SPME	0.001 ± 0.001	0.007 ± 0.003	0.0007 ± 0.0003	0.001 ± 0.001	0.0018 ± 0.0003	0.0013 ± 0.0001	0.0005 ± 0.0001
Pyrazines										
2-Methylpyrazine (CAS: 109-08-0; LRI: 1261)	1261 ^a	HSSE	0.008 ± 0.001	0.020 ± 0.003	0.011 ± 0.001	0.025 ± 0.001	0.013 ± 0.001	0.015 ± 0.001	0.036 ± 0.009	
		DHS	0.0120 ± 0.0002	0.023 ± 0.011	0.016 ± 0.002	0.035 ± 0.012	0.021 ± 0.011	0.0304 ± 0.0003	0.091 ± 0.002	
		SPME	0.0033 ± 0.0001	0.034 ± 0.004	0.0212 ± 0.0001	0.043 ± 0.007	0.026 ± 0.003	0.0231 ± 0.0002	0.068 ± 0.002	
2,5-Dimethyl pyrazine/ 2,6-Dimethyl pyrazine (CAS: 123-32-0/108-50-9; LRI: 1317/1335)	1317 ^a / 1335 ^a	HSSE	0.016 ± 0.003	0.056 ± 0.009	0.019 ± 0.001	0.051 ± 0.004	0.032 ± 0.003	0.036 ± 0.001	0.082 ± 0.012	
		DHS	0.021 ± 0.001	0.111 ± 0.037	0.022 ± 0.002	0.060 ± 0.201	0.045 ± 0.022	0.038 ± 0.001	0.145 ± 0.004	
		SPME	0.0041 ± 0.0001	0.070 ± 0.016	0.0156 ± 0.0002	0.036 ± 0.005	0.030 ± 0.005	0.0194 ± 0.0004	0.054 ± 0.002	
HSSE		0.004 ± 0.001	0.061 ± 0.009	0.017 ± 0.001	0.024 ± 0.002	0.022 ± 0.002	0.026 ± 0.001	0.029 ± 0.005		
2,3-Dimethylpyrazine (CAS: 5910-89-4; LRI: 1348)	1348 ^a	DHS	0.0065 ± 0.0003	0.161 ± 0.054	0.023 ± 0.002	0.033 ± 0.009	0.038 ± 0.018	0.033 ± 0.001	0.054 ± 0.001	
		SPME	0.0023 ± 0.0001	0.119 ± 0.035	0.021 ± 0.001	0.026 ± 0.005	0.030 ± 0.004	0.0208 ± 0.0001	0.025 ± 0.001	
		HSSE	0.010 ± 0.002	0.495 ± 0.124	0.025 ± 0.001	0.042 ± 0.005	0.062 ± 0.008	0.047 ± 0.006	0.068 ± 0.014	
Trimethylpyrazine (CAS: 14667-55-1; LRI: 1410)	1410 ^a	DHS	0.123 ± 0.005	4.509 ± 3.253	0.314 ± 0.057	0.624 ± 0.233	0.782 ± 0.400	0.490 ± 0.002	1.116 ± 0.084	
		SPME	0.0048 ± 0.0003	0.451 ± 0.090	0.035 ± 0.003	0.041 ± 0.009	0.070 ± 0.014	0.028 ± 0.001	0.042 ± 0.003	
5-Ethyl-2,3-dimethylpyrazine (CAS: 15707-34-3; LRI: 1463)	1463 ^a	HSSE	0.002 ± 0.001	0.048 ± 0.010	0.0044 ± 0.0003	0.006 ± 0.001	0.008 ± 0.001	0.010 ± 0.001	0.011 ± 0.002	
		DHS	0.065 ± 0.003	0.621 ± 0.537	0.095 ± 0.014	0.141 ± 0.061	0.203 ± 0.109	0.246 ± 0.002	0.402 ± 0.011	
		SPME	0.0015 ± 0.0004	0.032 ± 0.001	0.0050 ± 0.0004	0.0058 ± 0.0001	0.006 ± 0.001	0.0058 ± 0.0001	0.005 ± 0.001	
Tetramethylpyrazine (CAS: 1124-11-4; LRI: 1480)	1480 ^a	HSSE	0.025 ± 0.005	1.596 ± 0.363	0.021 ± 0.002	0.016 ± 0.002	0.058 ± 0.006	0.048 ± 0.008	0.054 ± 0.011	
		DHS	0.794 ± 0.039	18.91 ± 15.979	0.535 ± 0.087	0.407 ± 0.184	1.648 ± 0.870	1.432 ± 0.026	2.116 ± 0.083	
		SPME	0.010 ± 0.001	1.175 ± 0.019	0.044 ± 0.004	0.018 ± 0.002	0.051 ± 0.011	0.029 ± 0.001	0.026 ± 0.005	
2,3,5-Trimethyl-6-ethylpyrazine (CAS: 17398-16-2; LRI: 1514)	1514 ^c	HSSE	0.0018 ± 0.00003	0.089 ± 0.020	0.0024 ± 0.0002	0.005 ± 0.001	0.007 ± 0.001	0.008 ± 0.002	0.008 ± 0.002	
		DHS	0.0006 ± 0.0001	0.053 ± 0.008	0.0030 ± 0.0001	0.0037 ± 0.0001	0.005 ± 0.001	0.0039 ± 0.0001	0.0033 ± 0.0003	
		SPME	0.041 ± 0.003	1.031 ± 0.896	0.044 ± 0.008	0.089 ± 0.039	0.169 ± 0.089	0.202 ± 0.007	0.272 ± 0.005	
Others										

(continued on next page)

Table 2 (continued)

Compound	LRI	Technique	Mean relative peak areas						
			CCI_C	CCN_C	CRI_C	CR4_C	CR6_C	ICS_C	PMC_C
Gamma-Butyrolactone (CAS: 96-48-0; LRI: 1636)	1636 ^a	HSSE	0.500 ± 0.035	0.443 ± 0.083	0.477 ± 0.040	0.311 ± 0.002	0.400 ± 0.048	0.614 ± 0.011	0.644 ± 0.103
		SPME	0.256 ± 0.046	0.244 ± 0.002	0.417 ± 0.013	0.261 ± 0.040	0.273 ± 0.015	0.455 ± 0.010	0.379 ± 0.002
		DHS	4.347 ± 2.148	3.511 ± 3.309	2.597 ± 1.234	3.790 ± 2.138	3.477 ± 1.748	4.904 ± 1.788	5.357 ± 0.357
		HSSE	0.070 ± 0.011	0.169 ± 0.017	0.053 ± 0.024	0.029 ± 0.002	0.061 ± 0.010	0.053 ± 0.002	0.144 ± 0.088
2(5 H)-Furanone (CAS: 497-23-4; LRI: 1769)	1769 ^{b,2}	DHS	0.063 ± 0.001	0.380 ± 0.271	0.072 ± 0.010	0.121 ± 0.047	0.152 ± 0.083	0.155 ± 0.002	0.271 ± 0.022
		SPME	0.002 ± 0.001	0.022 ± 0.002	0.004 ± 0.001	0.0065 ± 0.0003	0.007 ± 0.001	0.0078 ± 0.0001	0.0067 ± 0.0003
		HSSE	0.037 ± 0.007	0.145 ± 0.028	0.010 ± 0.001	0.041 ± 0.009	0.050 ± 0.007	0.046 ± 0.005	0.029 ± 0.003
		SPME	0.0075 ± 0.0001	0.043 ± 0.011	0.012 ± 0.003	0.017 ± 0.003	0.028 ± 0.007	0.016 ± 0.001	0.009 ± 0.001
Unknown 108 (LRI: 1840)	1840	DHS	1.833 ± 0.139	4.192 ± 3.716	0.589 ± 0.117	2.384 ± 0.947	2.772 ± 1.487	2.728 ± 0.077	2.413 ± 0.080
		HSSE	0.047 ± 0.010	0.117 ± 0.013	0.030 ± 0.014	0.040 ± 0.002	0.039 ± 0.002	0.087 ± 0.025	0.072 ± 0.042
		SPME	0.003 ± 0.001	0.016 ± 0.002	0.0086 ± 0.0004	0.008 ± 0.002	0.0098 ± 0.0003	0.0058 ± 0.0001	0.0083 ± 0.0004
		DHS	0.104 ± 0.006	0.193 ± 0.149	0.116 ± 0.023	0.135 ± 0.066	0.169 ± 0.092	0.121 ± 0.001	0.281 ± 0.019

¹n = 2; LRI: mean experimental linear retention index. reliability of identification: a) mass spectrum and LRI agreed with standards, b) mass spectrum agreed with mass spectral database and LRI agreed with the literature data, c) mass spectrum agreed with mass spectral data base; literature references agreed with LRI data: ² Kim, et al. (2025), ³ Mancini, et al. (2009).

detection of 5 alcohols that when using HS-SPME and HSSE were not detected or were not properly integrated due to notably low signal levels (Table S3): 1-hexanol, 3-phenylpropanol (benzenepropanol), 4,5-dimethyl-2-hepten-3-ol, 4-methyl-5-hexen-2-ol and nicotinyl alcohol (3-pyridinemethanol). This observation supports the importance of the selection of the sampling technique in VOCs analysis.

Regarding peak areas of the extracted alcohols, DHS extraction yielded higher relative peak areas for this chemical group compared to HS-SPME or HSSE, with some exceptions (Tables S1 and S3). This can be attributed to factors such as the nature of the DHS adsorbent, Tenax TA™, which has affinity to high-boiling compounds (Buchem n.d.; Dettmer and Engewald, 2002; Mastrogiamco et al., 1995), the continuous headspace flushing and the markedly higher amount of extraction phase compared to HS-SPME and HSSE. This behavior has also been reported for other food matrices as in milk chocolate, red wine, yogurt, and wine vinegar (Cucu et al., 2021; Ríos-Reina et al., 2018). Some examples of alcohols which area values were notably higher after sampling with DHS were: 2,3-butanediol isomers, phenylethyl alcohol (2-phenylethanol), 2-heptanol, 2-nonanol, 2-furanmethanol (furfuryl alcohol), benzyl alcohol (phenylmethanol), methyl benzenemethanol (1-phenylethanol), 4-methyl-5-hexen-2-ol, among others.

In general, regarding the kind of alcohols extracted, they were mainly higher alcohols (C₄-C₉) (Tables S1, S2 and S3) which have been previously reported for cacao and derivatives (Escobar et al., 2021; Frauendorfer and Schieberle, 2019; Hegmann et al., 2020; Herrera-Rocha et al., 2023; Rodríguez-González et al., 2025; Rottiers et al., 2019). Alcohols can be found in the fresh cacao seeds or can be developed during processing, playing and active role in quality (Deuscher et al., 2020; Herrera-Rocha et al., 2023) and expressing origin factors linked to genetics, climate, local microbiota, among others. Certain alcohols, such as 1-pentanol, 2-pentanol, 1-hexanol, 2-heptanol, 2-octanol, 2-nonanol and 3-methyl-1-butanol—all of them detected in this study—are initially present in the cacao pulp, imparting fruity, flowery, herbal and spicy fine notes, and can vary among cacao varieties and season effects (Hegmann et al., 2020). These can diffuse into the bean, as has been studied for phenylethyl alcohol (Besançon et al., 2024; Chetschik et al., 2018). Other alcohols, as for example 2-methyl-1-propanol (isobutyl alcohol), might cause undesirable off-flavors (Rottiers et al., 2019). This is why their determination can be relevant when assessing cocoa quality.

Post-harvest steps, especially fermentation and drying, and industrial processing, with a major influence of roasting, also shape the profile of alcohols. For instance, 3-methyl-1-butanol (isoamyl alcohol) and phenylethyl alcohol—which were detected with the three sampling techniques—can be obtained by means of microbial metabolism during cocoa fermentation (De Vuyst and Leroy, 2020; Dias et al., 2025; Frauendorfer and Schieberle, 2019; Hegmann et al., 2020). Moreover, alcohols can persist after industrial processing, albeit volatilization and chemical reactions that arise during roasting, refining and conching steps. Examples include 1-pentanol, phenylethyl alcohol and 2-heptanol (Escobar et al., 2021; Guckenbiehl et al., 2025; Quelal et al., 2023).

3.1.2. Esters

Esters constituted one of the main chemical groups extracted in this study, accounting for 21 in total (Tables S1, S2 and S3). DHS and HS-SPME enabled the detection of 16 esters, while HSSE led to 11 VOCs (Fig. 2), most of which were esterification products of acetic acid. Moreover, 9 of these esters were detected across all three techniques (Tables 2 and 3), all of which have previously been reported in cocoa masses and chocolates (Cemin et al., 2024; Escobar et al., 2021).

As occurred for alcohols, higher relative peak areas were also obtained after DHS compared to the other techniques, with more pronounced effects on ethyl octanoate, ethyl phenylacetate and 2-phenylethyl acetate. Furthermore, 5 compounds were detected only after sampling with DHS (ethyl 3-phenylpropenoate, ethyl 3-phenylpropionate, ethyl tetradecanoate, geranyl acetate and phenethyl

Table 3

Mean relative¹ peak areas and correspondent standard deviations of VOCs detected in common after sampling with three headspace techniques in different cocoa masses made from estate 'O' cocoa.

Compound	LRI	Technique	Mean relative peak areas			
			CCN_O	CR1_O	ICS_O	TSH_O
Acids						
Acetic acid (CAS: 64–19–7; LRI: 1425)	1425 ^a	HSSE	0.309 ± 0.166	1.028 ± 0.919	0.557 ± 0.435	0.412 ± 0.181
		DHS	7.473 ± 3.338	9.428 ± 0.139	8.208 ± 2.920	16.439 ± 14.734
		SPME	4.026 ± 1.601	8.423 ± 0.838	4.468 ± 0.154	5.154 ± 0.030
Propanoic acid (Propionic acid) (CAS: 79–09–4; LRI: 1532)	1532 ^a	HSSE	0.018 ± 0.003	0.033 ± 0.016	0.053 ± 0.032	0.032 ± 0.001
		DHS	0.386 ± 0.176	0.113 ± 0.008	0.230 ± 0.104	0.447 ± 0.314
		SPME	0.094 ± 0.039	0.048 ± 0.003	0.074 ± 0.002	0.088 ± 0.001
Butanoic acid (Butyric acid) (CAS: 107–92–6; LRI: 1629)	1629 ^a	HSSE	0.0214 ± 0.0002	0.032 ± 0.007	0.065 ± 0.025	0.045 ± 0.002
		SPME	0.090 ± 0.026	0.048 ± 0.003	0.115 ± 0.002	0.077 ± 0.003
		DHS	0.345 ± 0.157	0.090 ± 0.112	0.400 ± 0.191	0.413 ± 0.201
3-Methylbutanoic acid (Isovaleric acid) (CAS: 503–74–2; LRI: 1672)	1672 ^a	HSSE	0.711 ± 0.008	0.163 ± 0.050	0.545 ± 0.372	0.607 ± 0.105
		DHS	7.758 ± 3.626	3.288 ± 0.560	5.322 ± 3.383	22.802 ± 22.341
		SPME	2.012 ± 0.819	0.240 ± 0.017	0.798 ± 0.008	1.427 ± 0.018
2-Methylbutanoic acid (CAS: 116–53–0; LRI: 1675)	1675 ^a	HSSE	0.265 ± 0.001	0.043 ± 0.014	0.219 ± 0.135	0.252 ± 0.045
		SPME	0.518 ± 0.211	0.036 ± 0.002	0.230 ± 0.002	0.431 ± 0.003
		DHS	3.475 ± 4.356	0.516 ± 0.237	3.117 ± 2.013	16.267 ± 5.782
Alcohols						
2-Pentanol (CAS: 6032–29–7; LRI: 1114)	1114 ^a	HSSE	0.0039 ± 0.0003	0.021 ± 0.002	0.009 ± 0.002	0.0098 ± 0.0004
		DHS	0.006 ± 0.002	0.024 ± 0.003	0.014 ± 0.005	0.018 ± 0.004
		SPME	0.026 ± 0.011	0.137 ± 0.012	0.050 ± 0.002	0.079 ± 0.002
3-Methyl–1-butanol (Isoamyl alcohol) (CAS: 123–51–3; LRI: 1201)	1201 ^a	HSSE	0.081 ± 0.010	0.130 ± 0.012	0.116 ± 0.045	0.078 ± 0.002
		DHS	0.131 ± 0.026	0.227 ± 0.003	0.237 ± 0.091	0.344 ± 0.155
		SPME	0.121 ± 0.046	0.162 ± 0.020	0.171 ± 0.005	0.189 ± 0.008
1-Pentanol (CAS: 71–41–0; LRI: 1244)	1244 ^b	HSSE	0.018 ± 0.001	0.035 ± 0.002	0.025 ± 0.006	0.023 ± 0.001
		DHS	0.038 ± 0.001	0.117 ± 0.011	0.063 ± 0.019	0.120 ± 0.012
		SPME	0.026 ± 0.010	0.054 ± 0.005	0.040 ± 0.001	0.060 ± 0.003
2-Heptanol (CAS: 543–49–7; LRI: 1316)	1316 ^a	HSSE	1.528 ± 0.129	0.566 ± 0.070	0.949 ± 0.450	2.088 ± 0.186
		DHS	6.483 ± 2.711	1.902 ± 0.323	3.369 ± 2.315	37.07 ± 9.394
		SPME	1.310 ± 0.345	0.483 ± 0.018	0.721 ± 0.013	2.040 ± 0.061
3-Ethoxy–1-propanol (CAS: 111–35–3; LRI: 1372)	1372 ^{b,2}	HSSE	0.013 ± 0.001	0.044 ± 0.011	0.040 ± 0.019	0.023 ± 0.002
		DHS	0.057 ± 0.013	0.159 ± 0.022	0.123 ± 0.059	0.169 ± 0.069
		SPME	0.019 ± 0.008	0.0507 ± 0.0003	0.044 ± 0.001	0.034 ± 0.005
2-Octanol (CAS: 123–96–6; LRI: 1414)	1414 ^a	HSSE	0.038 ± 0.003	0.012 ± 0.002	0.031 ± 0.016	0.053 ± 0.007
		DHS	0.311 ± 0.137	0.045 ± 0.009	0.110 ± 0.069	2.027 ± 2.424
		SPME	0.032 ± 0.004	0.0145 ± 0.0002	0.0212 ± 0.0001	0.043 ± 0.004
2-Nonanol (CAS: 628–99–9; LRI: 1515)	1515 ^a	HSSE	0.310 ± 0.013	0.053 ± 0.008	0.527 ± 0.338	0.283 ± 0.030
		DHS	0.859 ± 0.438	0.127 ± 0.025	0.649 ± 0.398	4.647 ± 5.677
		SPME	0.224 ± 0.007	0.056 ± 0.008	0.2896 ± 0.0003	0.166 ± 0.017
2,3-Butanediol, isomer 1 (LRI: 1554)	1554 ^c	HSSE	3.971 ± 0.163	12.38 ± 1.883	12.29 ± 5.016	8.792 ± 1.782
		DHS	33.113 ± 15.658	41.338 ± 7.116	41.510 ± 24.616	179.548 ± 192.760
		SPME	5.304 ± 1.751	9.698 ± 0.844	7.232 ± 0.165	7.702 ± 0.332
2,3-Butanediol, isomer 2 (LRI: 1590)	1590 ^c	HSSE	2.664 ± 0.157	7.659 ± 1.007	6.857 ± 3.081	5.283 ± 0.674
		DHS	28.261 ± 12.939	36.621 ± 5.895	32.654 ± 18.929	129.213 ± 131.062
		SPME	4.345 ± 1.364	7.333 ± 0.803	5.399 ± 0.128	5.953 ± 0.254
2-Furanmethanol (Furfuryl alcohol) (CAS: 98–00–0; LRI: 1661)	1661 ^a	HSSE	0.068 ± 0.002	0.116 ± 0.049	0.166 ± 0.139	0.124 ± 0.046
		DHS	0.128 ± 0.059	0.284 ± 0.032	0.064 ± 0.037	0.876 ± 0.918
		SPME	0.029 ± 0.005	0.051 ± 0.004	0.039 ± 0.001	0.033 ± 0.003
Methyl benzenemethanol (1-Phenylethanol) (CAS: 98–85–1; 1818)	1818 ^{b,2}	HSSE	0.0243 ± 0.0001	0.038 ± 0.006	0.060 ± 0.026	0.041 ± 0.004
		DHS	0.306 ± 0.135	0.354 ± 0.059	0.472 ± 0.325	2.694 ± 2.942
		SPME	0.020 ± 0.004	0.037 ± 0.004	0.0401 ± 0.0002	0.038 ± 0.006
Benzyl alcohol (Phenylmethanol) (CAS: 100–51–6; LRI: 1896)	1896 ^a	HSSE	0.052 ± 0.003	0.056 ± 0.010	0.070 ± 0.020	0.058 ± 0.008
		DHS	0.749 ± 0.327	0.566 ± 0.086	0.515 ± 0.349	2.658 ± 2.670
		SPME	0.060 ± 0.010	0.065 ± 0.009	0.0579 ± 0.0004	0.057 ± 0.004
Phenylethyl alcohol (2-Phenylethanol) (CAS: 60–12–8; LRI: 1934)	1934 ^a	HSSE	4.228 ± 0.192	3.467 ± 0.587	6.975 ± 4.220	4.790 ± 0.494
		DHS	19.541 ± 9.557	14.149 ± 2.356	20.100 ± 13.154	116.082 ± 134.065
		SPME	3.606 ± 0.692	3.484 ± 0.339	4.516 ± 0.007	3.998 ± 0.395
Aldehydes						
Benzaldehyde (CAS: 100–52–7; LRI: 1512)	1512 ^a	HSSE	0.209 ± 0.013	0.221 ± 0.020	0.184 ± 0.105	0.143 ± 0.034
		DHS	0.538 ± 0.213	0.484 ± 0.045	0.633 ± 0.306	2.955 ± 3.147
		SPME	0.666 ± 0.107	0.945 ± 0.135	0.958 ± 0.016	1.162 ± 0.114
Benzeneacetaldehyde (Phenylacetaldehyde) (CAS: 122–78–1; LRI: 1637)	1637 ^a	HSSE	0.0691 ± 0.0001	0.069 ± 0.001	0.064 ± 0.035	0.045 ± 0.004
		SPME	0.0321 ± 0.0004	0.033 ± 0.004	0.0469 ± 0.0002	0.027 ± 0.004
		DHS	0.028 ± 0.028	0.035 ± 0.001	0.010 ± 0.008	0.863 ± 1.194
Alkanes						
Dodecane (CAS: 112–40–3; LRI: 1195)	1195 ^{b,2}	HSSE	0.200 ± 0.006	0.217 ± 0.030	0.321 ± 0.218	0.125 ± 0.025
		DHS	1.237 ± 0.474	0.543 ± 0.069	0.883 ± 0.470	5.652 ± 6.855
		SPME	0.087 ± 0.002	0.135 ± 0.004	0.125 ± 0.003	0.067 ± 0.012
Tetradecane (CAS: 629–59–4; LRI: 1390)	1390 ^a	HSSE	0.063 ± 0.002	0.071 ± 0.001	0.136 ± 0.044	0.052 ± 0.007
		DHS	1.281 ± 0.626	0.935 ± 0.184	1.063 ± 0.678	5.690 ± 6.896
		SPME	0.023 ± 0.003	0.041 ± 0.001	0.033 ± 0.001	0.011 ± 0.002
Esters						

(continued on next page)

Table 3 (continued)

Compound	LRI	Technique	Mean relative peak areas			
			CCN_O	CR1_O	ICS_O	TSH_O
2-Heptanol acetate (1-Methylhexyl acetate) (CAS: 5921–82–4; LRI: 1247)	1247 ^a	HSSE	0.086 ± 0.005	0.037 ± 0.006	0.039 ± 0.021	0.154 ± 0.019
		DHS	0.079 ± 0.006	0.0231 ± 0.0001	0.022 ± 0.007	0.302 ± 0.273
		SPME	0.085 ± 0.012	0.058 ± 0.001	0.036 ± 0.001	0.146 ± 0.012
Ethyl 2-hydroxypropanoate (Ethyl lactate) (CAS: 97–64–3; LRI: 1330)	1330 ^a	HSSE	0.0147 ± 0.0002	0.195 ± 0.032	0.022 ± 0.009	0.020 ± 0.003
		DHS	0.030 ± 0.007	0.545 ± 0.054	0.049 ± 0.019	0.079 ± 0.031
		SPME	0.008 ± 0.003	0.0864 ± 0.0002	0.0110 ± 0.0002	0.0190 ± 0.0001
Ethyl octanoate (CAS: 106–32–1; LRI: 1420)	1420 ^a	HSSE	0.042 ± 0.002	0.081 ± 0.009	0.098 ± 0.061	0.070 ± 0.011
		DHS	0.443 ± 0.203	0.492 ± 0.092	0.469 ± 0.296	4.119 ± 5.027
		SPME	0.025 ± 0.002	0.059 ± 0.002	0.039 ± 0.002	0.031 ± 0.005
2-Nonanol acetate (CAS: 14936–66–4; LRI:1446)	1446 ^a	HSSE	0.040 ± 0.002	0.009 ± 0.001	0.022 ± 0.009	0.065 ± 0.010
		DHS	0.424 ± 0.199	0.063 ± 0.012	0.108 ± 0.069	3.953 ± 4.88
		SPME	0.0471 ± 0.0001	0.016 ± 0.001	0.021 ± 0.001	0.065 ± 0.008
2,3-Butanediol diacetate, isomer 1 (LRI: 1470)		HSSE	0.0067 ± 0.0001	0.031 ± 0.005	0.013 ± 0.008	0.0068 ± 0.0001
	1470 ^c	DHS	0.053 ± 0.020	0.133 ± 0.027	0.091 ± 0.058	0.191 ± 0.196
		SPME	0.004 ± 0.002	0.030 ± 0.001	0.0079 ± 0.0002	0.0053 ± 0.0001
2,3-Butanediol diacetate, isomer 2 (LRI: 1507)		HSSE	0.0039 ± 0.0004	0.027 ± 0.003	0.009 ± 0.003	0.0065 ± 0.0001
	1507 ^c	DHS	0.021 ± 0.009	0.095 ± 0.018	0.023 ± 0.013	0.127 ± 0.142
		SPME	0.003 ± 0.001	0.0272 ± 0.0004	0.0041 ± 0.0001	0.0044 ± 0.0001
Benzyl acetate (CAS: 140–11–4; LRI: 1737)		HSSE	0.0053 ± 0.0001	0.011 ± 0.001	0.009 ± 0.001	0.006 ± 0.001
	1737 ^a	SPME	0.002 ± 0.001	0.0062 ± 0.0001	0.0029 ± 0.0002	0.0026 ± 0.0001
		DHS	0.038 ± 0.018	0.051 ± 0.010	0.026 ± 0.017	0.236 ± 0.280
Ethyl phenylacetate (CAS: 101–97–3; LRI: 1784)		HSSE	0.082 ± 0.001	0.124 ± 0.019	0.101 ± 0.064	0.098 ± 0.012
	1784 ^a	DHS	1.047 ± 0.498	0.996 ± 0.185	0.683 ± 0.437	7.345 ± 8.662
		SPME	0.029 ± 0.005	0.0526 ± 0.0002	0.031 ± 0.002	0.034 ± 0.007
2-Phenylethyl acetate (CAS: 103–45–7; LRI: 1813)		HSSE	0.552 ± 0.005	0.870 ± 0.130	0.916 ± 0.622	0.581 ± 0.072
	1813 ^a	DHS	5.948 ± 2.881	5.791 ± 1.003	5.567 ± 3.549	36.27 ± 2.641
		SPME	0.189 ± 0.013	0.348 ± 0.012	0.258 ± 0.006	0.175 ± 0.024
Ketones						
3-Hydroxy–2-butanone (Acetoin) (CAS: 513–86–0; LRI: 1275)	1275 ^a	HSSE	0.544 ± 0.039	0.724 ± 0.101	0.794 ± 0.327	0.244 ± 0.068
		DHS	2.668 ± 0.984	1.563 ± 0.148	4.052 ± 1.200	2.180 ± 0.693
		SPME	1.284 ± 0.554	1.574 ± 0.086	1.745 ± 0.046	0.921 ± 0.020
1-Hydroxy–2-propanone (Hydroxyacetone) (CAS: 116–09–6; LRI: 1288)	1288 ^a	HSSE	0.347 ± 0.023	0.494 ± 0.122	0.535 ± 0.351	0.406 ± 0.037
		DHS	0.481 ± 0.176	0.390 ± 0.033	0.287 ± 0.075	1.046 ± 0.833
		SPME	0.118 ± 0.051	0.1726 ± 0.0001	0.062 ± 0.002	0.109 ± 0.003
1-Hydroxy–2-butanone (CAS: 5077–67–8; LRI: 1363)		HSSE	0.041 ± 0.003	0.063 ± 0.028	0.061 ± 0.054	0.031 ± 0.003
	1363 ^{b,2}	DHS	0.029 ± 0.008	0.076 ± 0.001	0.016 ± 0.004	0.065 ± 0.048
		SPME	0.008 ± 0.003	0.015 ± 0.004	0.0050 ± 0.0001	0.0084 ± 0.0004
Acetoin acetate (2-Acetoxy–3-butanone) (CAS: 4906–24–5; LRI: 1364)	1364 ^{b,2}	HSSE	0.019 ± 0.001	0.038 ± 0.006	0.028 ± 0.008	0.016 ± 0.002
		DHS	0.055 ± 0.031	0.031 ± 0.003	0.034 ± 0.020	0.286 ± 0.335
		SPME	0.019 ± 0.008	0.046 ± 0.001	0.024 ± 0.001	0.0134 ± 0.0001
2-Nonanone (CAS: 821–55–6; LRI: 1377)		HSSE	0.455 ± 0.024	0.069 ± 0.008	0.449 ± 0.251	0.315 ± 0.038
	1377 ^a	DHS	2.150 ± 0.844	0.210 ± 0.023	1.065 ± 0.593	7.116 ± 7.881
		SPME	0.213 ± 0.003	0.047 ± 0.002	0.187 ± 0.006	0.130 ± 0.021
1-Phenylethanone (Acetophenone) (CAS: 98–86–2; LRI: 1651)	1651 ^a	HSSE	0.179 ± 0.097	0.463 ± 0.01	1.280 ± 1.507	0.806 ± 0.543
		DHS	0.120 ± 0.190	0.362 ± 0.06	0.465 ± 0.274	2.487 ± 2.600
		SPME	0.035 ± 0.005	0.049 ± 0.005	0.0513 ± 0.0002	0.056 ± 0.005
1-(1H-pyrrol–2-yl) ethanone (2-Acetylpyrrole) (CAS: 1072–83–9; LRI: 1991)	1991 ^{b,2}	HSSE	0.023 ± 0.001	0.095 ± 0.015	0.022 ± 0.011	0.030 ± 0.002
		SPME	0.027 ± 0.009	0.114 ± 0.023	0.018 ± 0.002	0.041 ± 0.003
		DHS	0.272 ± 0.117	0.918 ± 0.125	0.135 ± 0.092	1.307 ± 1.265
Monoterpenoids						
trans-linalyl oxide (trans-linalool oxide) (furanoid) (CAS: 34995–77–2; LRI: 1439)	1439 ^{b,3}	HSSE	0.0108 ± 0.0003	0.0100 ± 0.0005	0.014 ± 0.005	0.022 ± 0.003
		SPME	0.0297 ± 0.0073	0.029 ± 0.001	0.0252 ± 0.0002	0.0621 ± 0.0001
		DHS	0.128 ± 0.056	0.053 ± 0.013	0.071 ± 0.044	1.269 ± 1.521
cis-linalyl oxide (cis-linalool oxide) (furanoid) (CAS: 5989–33–3; LRI: 1467)	1467 ^{b,3}	HSSE	0.081 ± 0.001	0.063 ± 0.009	0.115 ± 0.066	0.125 ± 0.016
		DHS	0.760 ± 0.341	0.274 ± 0.062	0.498 ± 0.316	5.854 ± 7.121
		SPME	0.083 ± 0.022	0.069 ± 0.003	0.079 ± 0.001	0.131 ± 0.001
Linalool (3,7-Dimethylocta–1,6-dien–3-ol) (CAS: 78–70–6; LRI: 1537)	1537 ^a	HSSE	0.175 ± 0.011	0.072 ± 0.005	0.092 ± 0.050	0.293 ± 0.034
		DHS	1.457 ± 0.791	0.272 ± 0.055	0.274 ± 0.158	12.13 ± 4.779
		SPME	0.142 ± 0.023	0.073 ± 0.003	0.051 ± 0.001	0.231 ± 0.009
cis-Linalool 3,7-oxide (pyranoid) (CAS: 24048–52–0; LRI: 1773)	1773 ^c	HSSE	0.023 ± 0.001	0.016 ± 0.002	0.033 ± 0.021	0.026 ± 0.003
		DHS	0.448 ± 0.206	0.203 ± 0.036	0.357 ± 0.217	2.837 ± 3.265
		SPME	0.037 ± 0.011	0.0260 ± 0.0002	0.034 ± 0.001	0.044 ± 0.001
3,7-Dimethyl–6-octen–1-ol (beta-Citronellol) (CAS: 106–22–9; LRI: 1774)	1774 ^{b,2}	HSSE	0.007 ± 0.001	0.060 ± 0.008	0.051 ± 0.029	0.011 ± 0.002
		DHS	0.063 ± 0.037	0.777 ± 0.124	0.486 ± 0.309	0.781 ± 0.910
		SPME	0.003 ± 0.001	0.067 ± 0.001	0.031 ± 0.003	0.0048 ± 0.0004
Pyrazines						
2-Methylpyrazine (CAS: 109–08–0; LRI: 1261)	1261 ^a	HSSE	0.010 ± 0.001	0.013 ± 0.002	0.012 ± 0.001	0.016 ± 0.001
		DHS	0.007 ± 0.003	0.007 ± 0.001	0.004 ± 0.002	0.029 ± 0.030
		SPME	0.012 ± 0.003	0.032 ± 0.005	0.0093 ± 0.0002	0.022 ± 0.003
2,5-Dimethylpyrazine/2,6-Dimethylpyrazine (CAS: 123–32–0/108–50–9; LRI: 1317/1335)	1317 ^a / 1335 ^a	HSSE	0.0128 ± 0.0003	0.025 ± 0.003	0.010 ± 0.002	0.015 ± 0.001
		DHS	0.010 ± 0.002	0.014 ± 0.001	0.003 ± 0.002	0.033 ± 0.030
		SPME	0.008 ± 0.002	0.021 ± 0.003	0.0044 ± 0.0002	0.012 ± 0.002

(continued on next page)

Table 3 (continued)

Compound	LRI	Technique	Mean relative peak areas			
			CCN_O	CR1_O	ICS_O	TSH_O
2,3-Dimethylpyrazine (CAS: 5910–89–4; LRI: 1348)	1348 ^a	HSSE	0.032 ± 0.001	0.078 ± 0.01	0.107 ± 0.032	0.057 ± 0.004
		DHS	0.035 ± 0.003	0.081 ± 0.001	0.066 ± 0.029	0.140 ± 0.109
		SPME	0.034 ± 0.007	0.125 ± 0.024	0.109 ± 0.002	0.070 ± 0.008
Trimethylpyrazine (CAS: 14667–55–1; LRI: 1410)	1410 ^a	HSSE	0.0350 ± 0.0003	0.127 ± 0.017	0.072 ± 0.040	0.040 ± 0.004
		DHS	0.184 ± 0.056	0.448 ± 0.102	0.220 ± 0.171	0.898 ± 0.997
		SPME	0.029 ± 0.007	0.134 ± 0.028	0.054 ± 0.001	0.046 ± 0.005
5-Ethyl–2,3-dimethylpyrazine (CAS: 15707–34–3; LRI: 1463)	1463 ^b	HSSE	0.0168 ± 0.0001	0.100 ± 0.014	0.064 ± 0.039	0.024 ± 0.003
		DHS	0.127 ± 0.052	0.435 ± 0.100	0.262 ± 0.182	1.018 ± 1.225
		SPME	0.013 ± 0.001	0.096 ± 0.007	0.0415 ± 0.0003	0.019 ± 0.002
Tetramethylpyrazine (CAS: 1124–11–4; LRI: 1480)	1480 ^a	HSSE	0.0799 ± 0.0004	0.300 ± 0.043	0.394 ± 0.241	0.069 ± 0.008
		DHS	0.625 ± 0.211	1.452 ± 0.336	1.749 ± 1.217	3.425 ± 4.165
		SPME	0.068 ± 0.010	0.320 ± 0.028	0.264 ± 0.002	0.061 ± 0.005
2,3,5-Trimethyl–6-ethylpyrazine (CAS: 17398–16–2; LRI: 1514)	1514 ^{b,2}	HSSE	0.0070 ± 0.0001	0.026 ± 0.004	0.013 ± 0.007	0.007 ± 0.001
		DHS	0.004 ± 0.001	0.025 ± 0.001	0.0065 ± 0.0002	0.0046 ± 0.0003
		SPME	0.047 ± 0.020	0.098 ± 0.019	0.042 ± 0.028	0.287 ± 0.357
Others						
Gamma-Butyrolactone (CAS: 96–48–0; LRI: 1636)	1636 ^a	HSSE	0.178 ± 0.007	0.311 ± 0.045	0.244 ± 0.106	0.214 ± 0.006
		SPME	0.149 ± 0.048	0.232 ± 0.015	0.144 ± 0.003	0.181 ± 0.002
		DHS	1.193 ± 0.006	1.158 ± 0.335	0.596 ± 0.218	1.352 ± 0.803
2(5 H)-Furanone (CAS: 497–23–4; LRI: 1769)	1769 ^{b,2}	HSSE	0.032 ± 0.002	0.054 ± 0.019	0.102 ± 0.070	0.070 ± 0.022
		DHS	0.014 ± 0.006	0.030 ± 0.005	0.001 ± 0.002	0.076 ± 0.073
		SPME	0.0032 ± 0.0002	0.008 ± 0.001	0.0047 ± 0.0001	0.0039 ± 0.0001
Unknown 108 (LRI: 1840)	1840	HSSE	0.0266 ± 0.0001	0.024 ± 0.002	0.053 ± 0.033	0.029 ± 0.004
		SPME	0.013 ± 0.001	0.023 ± 0.002	0.019 ± 0.001	0.013 ± 0.001
		DHS	0.558 ± 0.273	0.358 ± 0.058	0.552 ± 0.352	3.483 ± 4.123
Unknown 126 (LRI: 2009)	2009	HSSE	0.023 ± 0.004	0.043 ± 0.012	0.081 ± 0.013	0.068 ± 0.008
		SPME	0.007 ± 0.002	0.010 ± 0.002	0.0065 ± 0.0003	0.0101 ± 0.0002
		DHS	0.030 ± 0.014	0.026 ± 0.004	0.021 ± 0.015	0.157 ± 0.170

¹n = 2; LRI: mean experimental linear retention index. reliability of identification: **a)** mass spectrum and LRI agreed with standards, **b)** mass spectrum agreed with mass spectral database and LRI agreed with the literature data, **c)** mass spectrum agreed with mass spectral data base; literature references agreed with LRI data: ²Kim, et al. (2025), ³Mancini, et al. (2009).

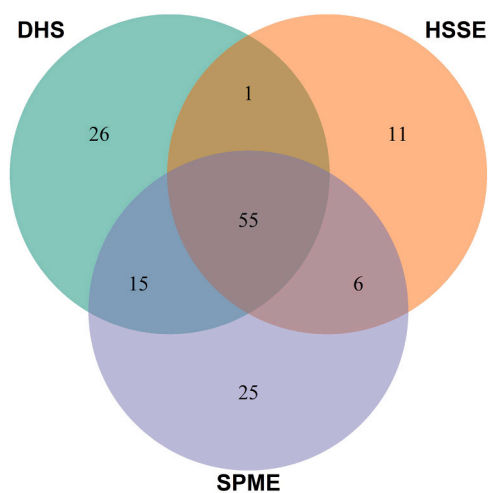


Fig. 1. Comparison of the number of VOCs identified after headspace sampling with three techniques and the number of shared compounds between techniques.

isobutyrate), highlighting the influence of the analytical technique on the results.

Regarding their presence in cocoa samples, esters are known to have a relevant and positive contributions in the aroma profile of cocoa derivatives, mostly related to fruity and floral notes (Bagnulo et al., 2023; Colonges et al., 2021; Herrera-Rocha et al., 2023; Marseglia et al., 2020; Rodríguez-González et al., 2025; Rottiers et al., 2019; Ullrich et al., 2022). Their presence in cocoa products derives from multiple sources. For example, 2-heptanol acetate (1-methylhexyl acetate) —detected when using the three headspace techniques— can be found in the cocoa pulp (Hegmann et al., 2020). Other esters are produced by the yeasts during fermentation, involving reactions of alcohols with organic acids

as acetic acid or other medium-chain fatty acids (De Vuyst and Leroy, 2020; Erazo Solorzano et al., 2023). Some esters can even persist after roasting, as shown in the study of Marseglia et al. (2020) and in our results, such as ethyl octanoate, ethyl acetate, 2-heptanol acetate, and ethyl dodecanoate.

3.1.3. Pyrazines

A total of 9 pyrazines were detected in the samples (Fig. 2, Tables S1, S2 and S3), all of them observed with the three techniques, except for 2-ethyl–5(6)-methylpyrazine. When sampled with HSSE, the relative peak areas of this compound were too small to allow reliable integration, therefore, it was excluded from the HSSE analysis. All of these pyrazines have been identified in cocoa mass in previous studies (Bagnulo et al., 2023; Braga et al., 2018; Magi et al., 2012; Rodríguez-González et al., 2025).

As was observed for the other chemical groups, relative peak areas were influenced by the sampling technique. For example, compared to the HS-SPME and HSSE, tetramethylpyrazine showed relative peak areas that were 10- to 100-fold higher after DHS extraction (Table 2, Table 3 and Tables S1, S2, and S3).

Regarding their presence in cocoa samples, pyrazines are mainly derived from Maillard reactions which occur primarily during cocoa drying and roasting of cocoa beans (De Vuyst and Leroy, 2020; Hinnneh et al., 2018; Llano et al., 2024; Santander et al., 2025; Shakoore et al., 2022; Toker et al., 2019; Yu et al., 2021). These compounds play a fundamental role in shaping the sensory profile by contributing to the overall aroma with earthy, roasty, cocoa, chocolate, and nutty notes (Crafack et al., 2014; Herrera-Rocha et al., 2023; Owusu et al., 2012; Peña-Correa et al., 2022; Rojas et al., 2022; Ullrich et al., 2022); thereby influencing consumer acceptance (Cemin et al., 2022). Moreover, pyrazines have been linked to potential quality indices, for instance the ratio of tetramethyl pyrazine (TMP) and trimethyl pyrazine (TrMP) provide insights on the fermentation and roasting performance thereby in the resulting aroma potential (Van Durme et al., 2016; Ziegler, 2025).

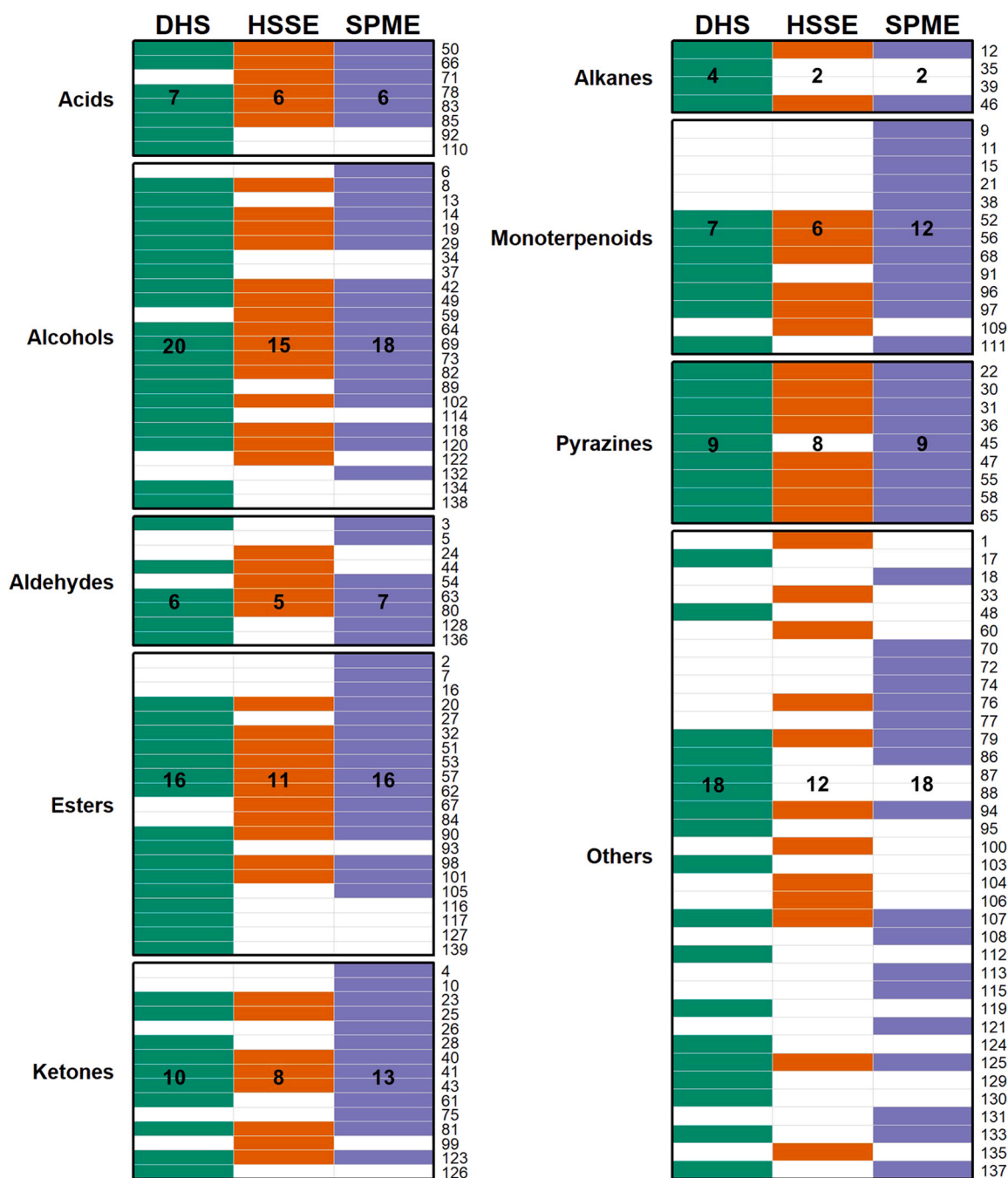


Fig. 2. Heatmap of the VOCs annotated after sampling with three headspace techniques (numbers outside the boxes refer to the VOCs codes which can be found in [Supplementary Material, Tables S1, S2 and S3](#)).

2017).

3.1.4. Ketones

Fifteen ketones were detected in the samples ([Tables S1, S2 and S3](#)): 7 of them were detected in common for the three techniques ([Tables 2 and 3](#)); 13 of them were detected after sampling with HS-SPME; 10 when using DHS extraction; and 8 after HSSE ([Fig. 2](#)). In addition, sampling with HS-SPME differentiated from the other two techniques because it enabled the detection of 4 ketones (2,3-butanedione (diacetyl), 2-heptanone, 3-hepten-2-one and 3-methyl-2-cyclohexen-1-one) that were not found or could not be integrated because of markedly low peak areas, when sampling with DHS or HSSE. Moreover, the effect of the sampling technique in terms of relative peak areas was variable, but

overall, DHS extraction yielded again higher relative peak areas, particularly for 3-hydroxy-2-butanone (acetoin), 1-phenylethanone (acetophenone), 2-nonanone and 1-(1H-pyrrol-2-yl)ethanone (2-acetylpyrrole) ([Tables 2 and 3](#)).

With regards to their presence in the samples, 2-heptanone, 2-nonanone, 1-hydroxy-2-propanone (hydroxyacetone), 1-phenylethanone and 3-hydroxy-2-butanone have been previously found in cacao beans and derivatives as nibs, cocoa masses and dark chocolates, contributing to the aroma with fruity, creamy, buttery, among other notes ([Akoa et al., 2023](#); [Bagnulo et al., 2023](#); [Braga et al., 2018](#); [Llano et al., 2024](#); [Magi et al., 2012](#); [Marseglia et al., 2020](#); [Otero Rojas et al., 2022](#); [Rodríguez-González et al., 2025](#); [Rottiers et al., 2019](#)). Moreover, 2-heptanone and 2-nonanone have also been detected in cacao pulp,

suggesting a possible migration from the pulp to the beans during post-harvest processing, which might be influenced by cocoa variety and weather variations (Bickel Haase et al., 2021; Hegmann et al., 2020).

3.1.5. Monoterpenoids

Thirteen monoterpenoids were detected in the samples under study (Tables S1, S2 and S3) and 5 of them were observed in common after sampling with each technique. Moreover, HS-SPME enabled the highest number of detected monoterpenoids (Fig. 2) being 5 of them not detected when using DHS and HSSE: 1-methyl-4-prop-1-en-2-ylcyclohexene (*D*-limonene), 1-methyl-4-propan-2-ylbenzene (*p*-cymene), 7-methyl-3-methylene-1,6-octadiene (*beta*-myrcene), neo-alloocimene (*(E,Z)*-2,6-dimethyl-2,4,6-octatriene), and trans- β -ocimene (*(3E)*-3,7-dimethylocta-1,3,6-triene). In contrast, regarding the relative peak areas extracted, once again, sampling with DHS yielded the highest relative peak areas for this VOCs group (Tables 2 and 3).

Monoterpenoids are widely known for their correlation with floral, herbal and fruity fine aroma notes, among others, thereby their presence in these samples contributes to their sensory complexity. They can be present in cacao pulp, as linalool and linalool oxides—which in this study were detected in common after sampling with the three techniques—and may migrate into cocoa beans during processing, remaining even in cocoa derivatives such as cocoa mass (Besançon et al., 2024; Collin et al., 2023; Colonges et al., 2021; Deucher et al., 2020; Hegmann et al., 2020; Quelal et al., 2023; Rottiers et al., 2019; Ullrich et al., 2022).

3.1.6. Aldehydes

Nine aldehydes were detected in the cocoa masses (Tables S1, S2 and S3), among which benzaldehyde and benzeneacetaldehyde (phenylacetaldehyde) were the only ones observed in common after sampling with the three techniques (Tables 2 and 3). The higher number of aldehydes was obtained after sampling with HS-SPME (7), followed by DHS (6) and HSSE (5) (Fig. 2).

In general, aldehydes are recognized to be key contributors in the aromatic profile of cocoa and cocoa derivatives (Herrera-Rocha et al., 2023). They can be present in the fresh cocoa pulp as hexanal, nonanal, octanal (Bickel Haase et al., 2021; Hegmann et al., 2020) or be formed during fermentation as product of the metabolism of lactic acid bacteria, as 3-methylbutanal (isovaleraldehyde) and benzaldehyde (De Vuyst and Leroy, 2020). Moreover, roasting cocoa beans also yield Strecker aldehydes as for example, 3-methylbutanal, benzaldehyde, and benzeneacetaldehyde (Escobar et al., 2021; Frauendorfer and Schieberle, 2019; Ullrich et al., 2022).

3.1.7. Acids

Eight acids with carbon chains ranging from C2 to C8 were detected in the samples (Tables S1, S2 and S3), 5 of which were observed in common after sampling with the three techniques (Tables 2 and 3). DHS yielded a higher number (7) of detected acids compared to HS-SPME and HSSE, that accounted for 6 each one (Fig. 2). Noteworthy, hexanoic and pentanoic acids (valeric acid) were observed only after sampling with DHS.

As occurs with other chemical groups, again DHS enabled substantially higher values in relative peak areas of acids when compared to HS-SPME and HSSE. Acetic acid, 3-methylbutanoic acid (isovaleric acid), and 2-methylbutanoic acid clearly evidenced this. For example, relative peak areas of acetic acid in some samples were over 5-fold higher when using DHS compared to HS-SPME, and more than 100-fold compared to HSSE (Tables 2 and 3).

With regards to their presence in the studied samples, some of them could arise from microbial metabolism, for instance production of acetic acid is one of the most notable changes during cacao fermentation (De Vuyst and Leroy, 2020; Dias et al., 2025). This VOC imparts sour taste and vinegar aroma notes that although its levels decrease through volatilization when drying, roasting, and conching, it may persist in final

products such as cocoa mass and chocolates (De Vuyst and Leroy, 2020; Guckenbiehl et al., 2022; Rottiers et al., 2019; Toker et al., 2019; Wexler et al., 2025). Other acids can act as precursors of aroma-active VOCs such as esters, for instance ethyl dodecanoate and 3-methyl-1-butanol acetate (isoamyl acetate), contributing with fruity notes. In contrast, others exhibit undesirable off-flavors, as propanoic (propionic), butyric, 2-methylpropanoic (isobutyric), and 3-methylbutanoic acids (Colonges et al., 2022; De Vuyst and Leroy, 2020; Gutiérrez-Ríos et al., 2022; Mota-Gutiérrez et al., 2019; Rottiers et al., 2019).

3.1.8. Others

The volatile complexity of the samples was further characterized by 16 annotated VOCs belonging to various chemical families, including alkanes, alkenes, furans, furanones, and lactones (Tables S1, S2 and S3), while 22 additional VOCs grouped in this category remained with unknown nature.

As observed with most of the chemical groups discussed above, the choice of sampling technique significantly influenced the detection of VOCs. In this case, gamma-butyrolactone and 2(5H)-furanone were the only two annotated compounds in this group observed in common after sampling with DHS, HS-SPME, and HSSE. The latter is a furanone, a class of compounds that, together with furans, act as key intermediate products of the Maillard reaction, with particular interest due to their positive contribution to the roasted and sweet notes of cocoa (Cui et al., 2021; Rojas et al., 2022; Santander et al., 2025). Another detected compound, grouped in this chemical family, was 3-phenylfuran, which was in this case exclusively captured via DHS, highlighting again the influence of the analytical technique used on the results obtained.

3.1.9. Overall findings

HS-SPME, DHS, and HSSE sampling techniques enabled the detection of a broad range of volatile compounds in cocoa masses. To further compare the three sampling techniques, a Principal Component Analysis (PCA) was performed considering only the VOCs detected in common (Figure S1. Supplementary Material). As was observed, the integrated PCA considering VOCs detected by all three techniques further highlighted the distinct extraction behavior of DHS compared to HS-SPME and HSSE. Thus, each technique exhibited distinct performance characteristics in terms of number of observed VOCs, relative peak areas and variability between replicates. These results are consistent with several studies that compared different sampling techniques for VOCs analysis in other food matrices (Dias et al., 2023; Diez-Simon et al., 2020; Li et al., 2020; Liu et al., 2022; Petretto et al., 2017; Ríos-Reina et al., 2018). It should be noted that these differences arise from a combination of factors, including physicochemical properties and geometry of the sorptive materials (coating versus bulk adsorbent), the amount of extracting phase and adsorption capacity, and the underlying extraction mechanisms (static versus dynamic sampling). While a fully controlled comparison isolating individual variables would be valuable from a mechanistic perspective, the present study intentionally adopts a configuration that reflects the practical implementation of these techniques in routine VOCs analysis. Therefore, the individual contribution of each factor cannot be fully disentangled, which represents an inherent limitation of this type of comparative approach. Accordingly, the sorptive material was considered an intrinsic component of each extraction methodology rather than an experimental variable to be independently optimized. Therefore, the observed differences among techniques should be interpreted as the combined effect of both the extraction approach and the physicochemical properties of the corresponding sorptive material.

As discussed previously, HS-SPME enabled the detection of the highest number of VOCs compared to DHS and HSSE (Figs. 1 and 2). This could be partially attributed to the fiber composition, which combines three different adsorbing phases—divinylbenzene (DVB), carboxen (CAR), and polydimethylsiloxane (PDMS)—thereby enhancing its capacity to adsorb compounds with diverse polarities and molecular

weights. This broader sorption spectrum likely contributed to the wider VOC coverage observed for HS-SPME compared to the other evaluated techniques. The effect of the adsorbent nature in HS-SPME was investigated in the study of [Otero Rojas et al. \(2022\)](#) who reported superior performance when using a DVB/CAR/PDMS fiber compared to CAR/PDMS and DVB/PDMS fibers for the analysis of cocoa masses. Moreover, DHS yielded in general the highest values of relative peak areas, which eased integration and identification, compared to the results of the other two techniques, nonetheless, in general, a higher variability between replicates was observed in terms of standard deviations ([Tables S1, S2 and S3](#)). This higher variability observed for DHS

may be attributed to several factors inherent to the technique. Firstly, the dynamic nature of the extraction involves continuous purging with nitrogen, where slight fluctuations in flow rate may influence the transfer efficiency of VOCs from the headspace to the adsorbent. Secondly, the trapping efficiency of Tenax TA™ can vary depending on compound volatility. Thirdly, the additional steps involved in thermal desorption may introduce further variability compared to more straightforward techniques such as HS-SPME. Furthermore, DHS sampler in general implies higher operational costs.

In contrast, HSSE yielded the lower number of detected compounds, likely due to the PDMS adsorbent, which has major affinity for non-polar

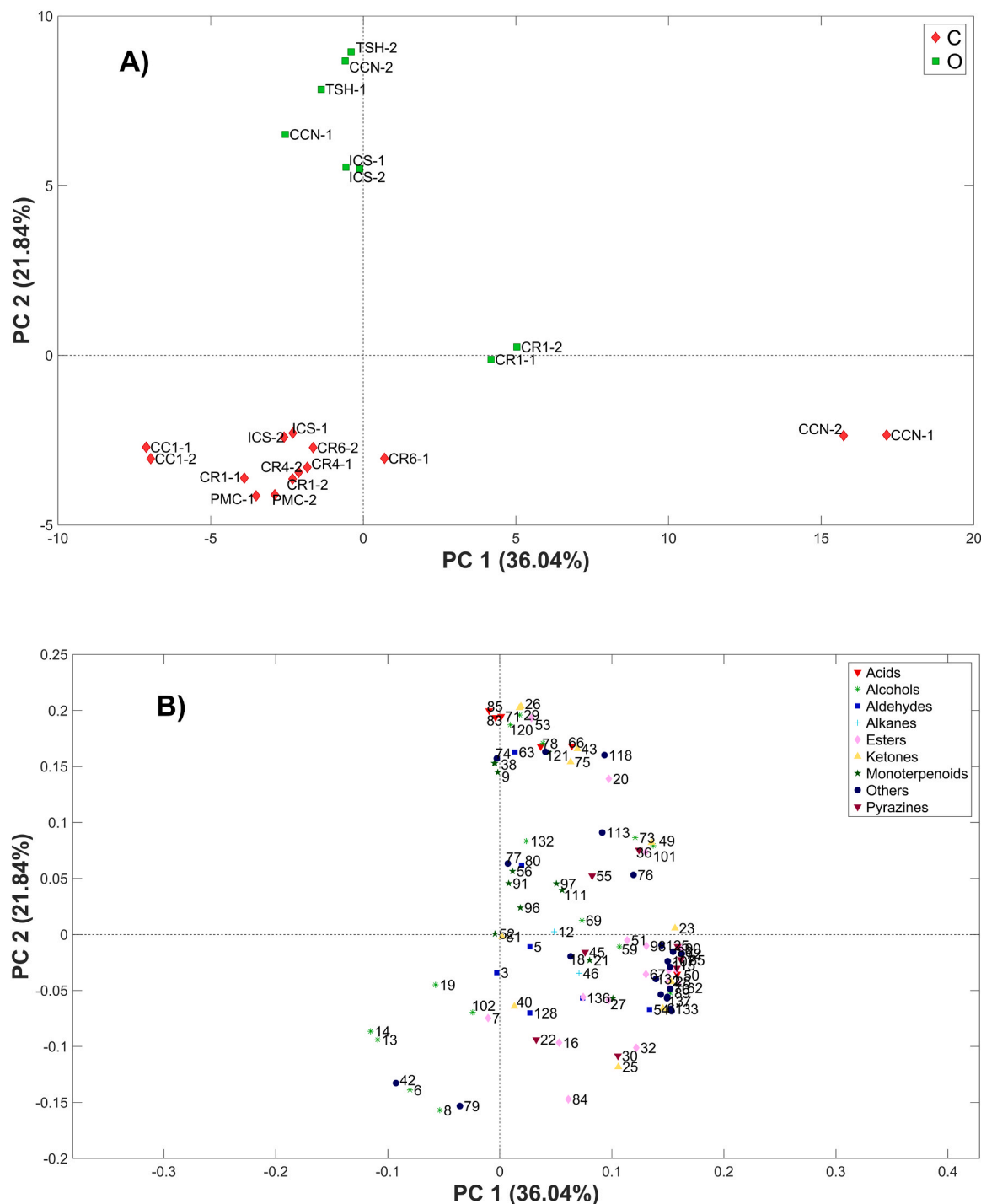


Fig. 3. Principal Component Analysis based on HS-SPME data: A) score plot of the samples labeled according to their origin ('C' and 'O'); B) loading plot of VOCs labeled by chemical groups (numbers refer to the VOCs codes which can be found in [Supplementary Material, Tables S1, S2 and S3](#)).

or medium-polar compounds (Gerstel, 2015), and was more laborious as the extraction step was non-automatized. This selectivity may have limited the extraction of certain VOC families present in cocoa masses, thereby contributing to the lower number of detected compounds observed for HSSE.

In addition, the extraction techniques differ fundamentally in their retention mechanisms based on the phase state; solid sorbents like Tenax^{TA} or SPME coatings (DVD/CAR/PDMS) exhibit adsorption behavior, while polymeric liquids like PDMS rely on absorption. Consequently, this governs their susceptibility to competitive displacement effects during volatile extraction, being this phenomenon higher in the case of the adsorption type coatings (Jeleń and Wiczorek, 2025).

To facilitate method selection depending on analytical priorities, a comparative decision matrix of the three extraction techniques is presented as [Supplementary Material \(Table S4\)](#). It should be noted that the comparative assessment presented in this table is based on harmonized experimental conditions. Therefore, the relative performance of each technique may vary if technique-specific optimization is applied. The present comparison is intended to provide a consistent and controlled framework rather than to reflect the maximum achievable performance of each method.

Although HS-SPME demonstrated a favorable balance between VOCs coverage, variability, and operational simplicity, the results also highlight that HSSE and DHS may provide complementary analytical

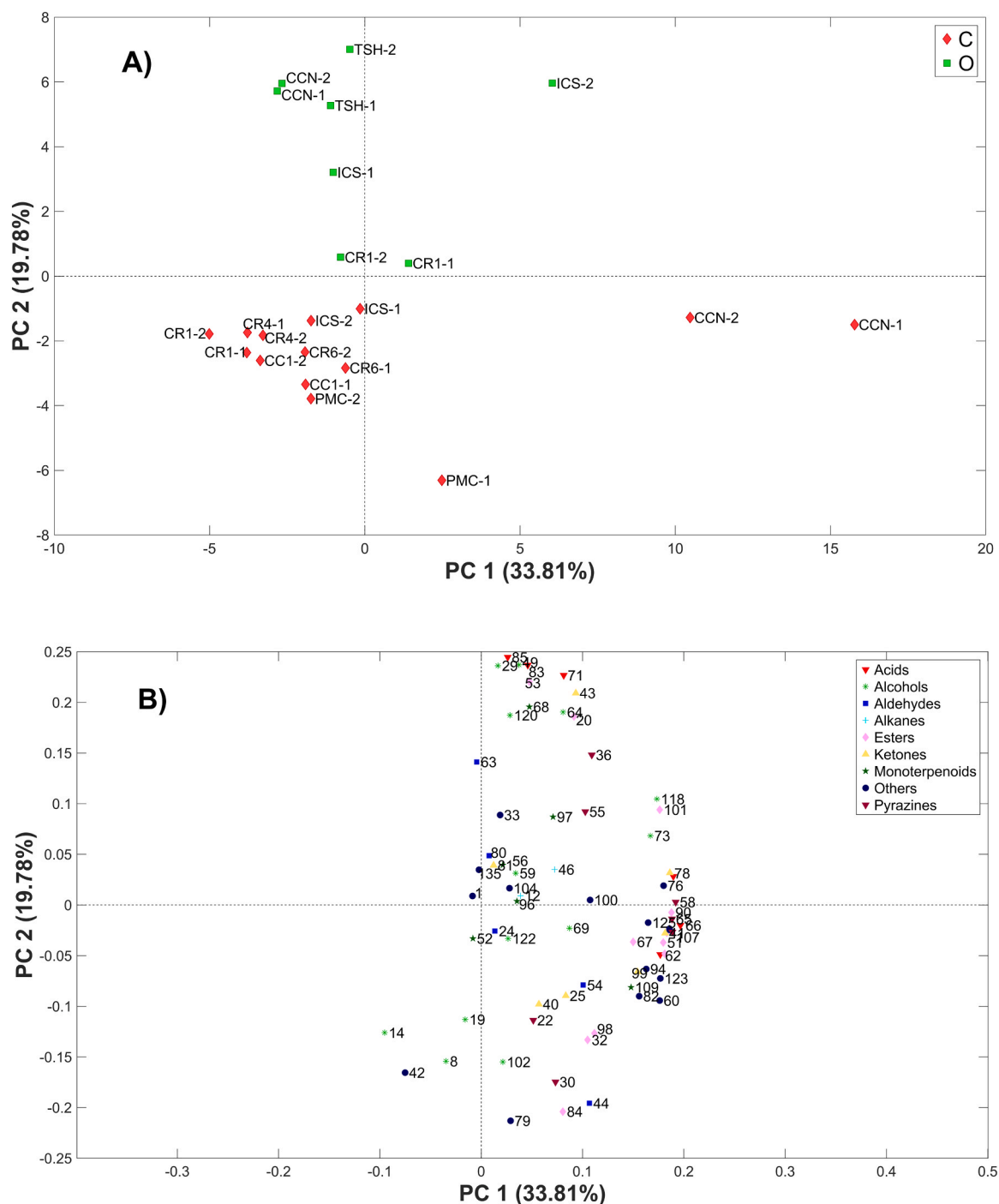


Fig. 4. Principal Component Analysis based on HSSE data: A) score plot of the samples labeled according to their origin ('C' and 'O'); B) loading plot of VOCs labeled by chemical groups (numbers refer to the VOCs codes which can be found in [Supplementary Material, Tables S1, S2 and S3](#)).

information. In particular, DHS showed enhanced peak areas, facilitating the detection and integration of certain compounds, while HSSE and DHS enabled the detection of specific VOCs not captured by HS-SPME. Therefore, the selection of the sampling technique should not rely solely on general performance, but rather on the specific analytical objective, such as sensitivity requirements or target compound families.

3.2. VOCs sampling techniques as tools for distinguishing provenance

One of the aims of this study was to determine whether the VOCs profiles obtained from each sampling technique could be used to differentiate the provenance of the cocoa masses, which were elaborated

from eight varieties of cacao from two different estates (Table 1). Notably, three of these varieties were cultivated in both origins, allowing a partial assessment of the relative influence of varietal and provenance-related factors. To further explore this, a principal component analysis (PCA) was performed with data acquired from each technique (Figs. 3, 4 and 5). In all cases, successful distinction was achieved, with samples clustering primarily according to their origin rather than their varietal background, as varieties shared between origins did not group together.

3.2.1. HS-SPME

HS-SPME data analysis led to a PCA model in which the first two

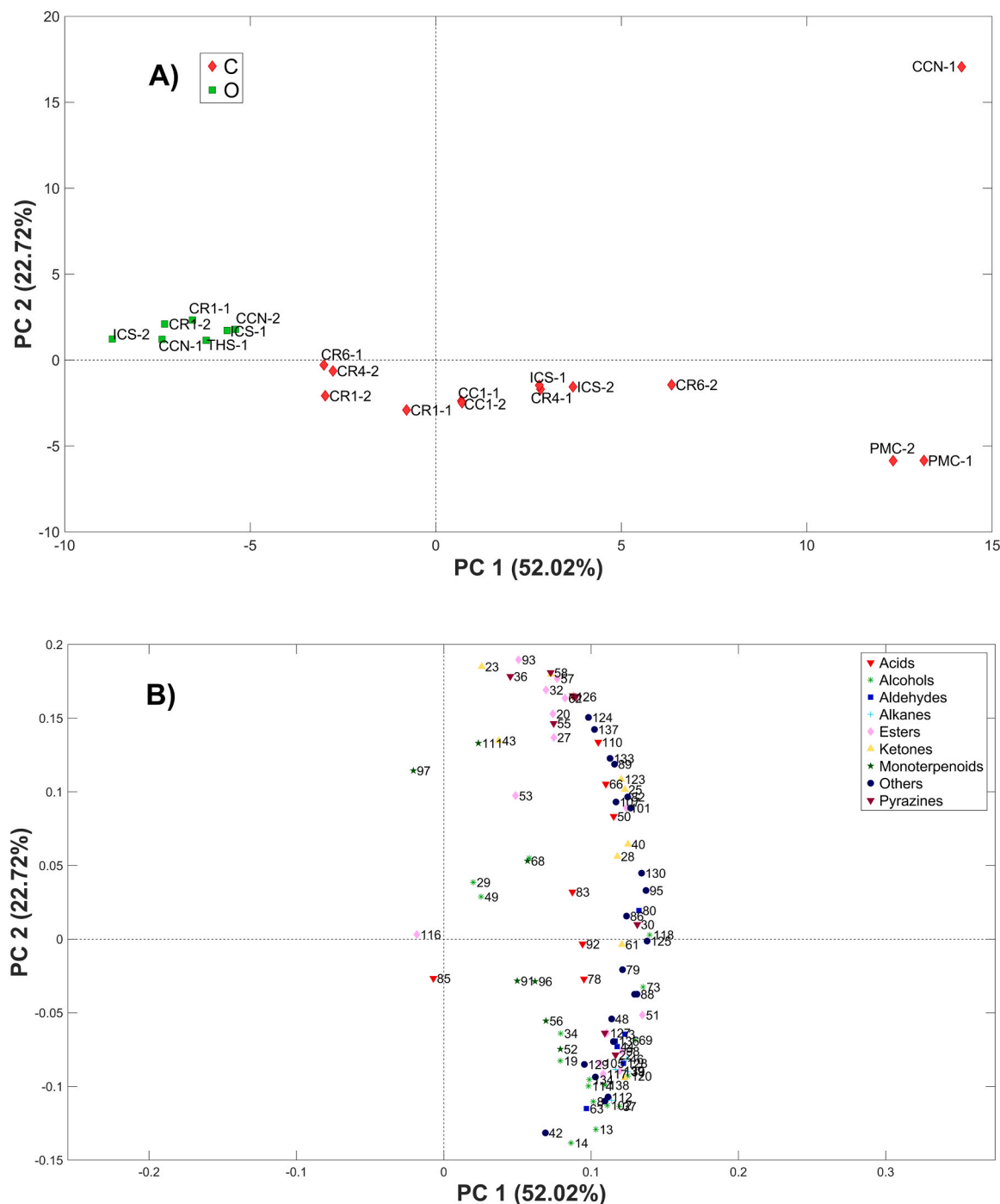


Fig. 5. Principal Component Analysis based on DHS data: A) score plot of the samples labeled according to their origin ('C' and 'O'); B) loading plot of VOCs labeled by chemical groups (numbers refer to the VOCs codes which can be found in [Supplementary Material, Tables S1, S2 and S3](#)).

principal components explained more than half of the variability of the dataset (Fig. 3), being the second principal component (PC2), the one that mainly explained the variations regarding the provenance of the samples. In contrast, the first principal component (PC1) mainly highlighted the differentiated profile of sample CCN_C compared to the others. This could be explained by the fact that this sample had notably higher values of relative peak areas of some pyrazines such as for example trimethylpyrazine, tetramethylpyrazine and 2,3,5-trimethyl-6-ethylpyrazine, esters as for example 2,3-butanediol diacetate isomers, as well as ketone 3-hydroxy-2-butanone and acetic acid (Tables S1, S2 and S3). These findings might be related to the fermentation step, where the intrinsic characteristics of cacao together with microbial metabolism produce a variety of compounds as 3-hydroxy-2-butanone, acetic acid and derived acetate esters, and other compounds (Aprotosoie et al., 2016; De Vuyst and Leroy, 2020; Dias et al., 2025; Erazo Solorzano et al., 2023). Differences in diversity, abundance and activity of the fermentation microbiota can be linked to origin characteristics (González-Orozco et al., 2024; Velásquez-Reyes et al., 2025).

Regarding the chemical markers responsible for geographical differentiation according to the PCA results, in general, samples from estate 'O' were characterized by higher relative peak areas of some VOCs, such as specific ketones (2-heptanone and 2-nonanone), acids (3-methylbutanoic acid, 2-methylbutanoic acid, and 2-methylpropanoic acid), and alcohols such as 2-heptanol and phenylethyl alcohol. In contrast, samples from estate 'C' generally exhibited higher levels of 2-pentanol and 3-ethoxy-1-propanol, along with other compounds such as gamma-butyrolactone and 1-hydroxy-2-propanone (Tables S1, S2 and S3).

These findings align with recent literature suggesting that the cocoa volatile profile is highly dependent on provenance, encompassing genetics, climate, and postharvest processing (Liberto et al., 2025). For instance, a variety of key markers have been previously identified for distinguishing cocoa origins across America and Africa, according to Liberto et al. (2025) who studied the VOCs composition of cocoa beans and cocoa masses from different origins (Colombia, Cameroon and West Africa countries) and varieties (Forastero, Arriba and CCN-51), and Marseglia et al. (2020), who analyzed the volatilome of cacao beans from countries across different continents (America, Africa and Asia). In fact, this last study unveiled that samples from American countries were differentially characterized by diverse VOCs including 2-heptanone and 2-heptanol, also relevant in the differentiation by origin of the samples under study. Similarly, the higher abundance of 2-pentanol observed in estate 'C' is consistent with studies correlating specific alcohol profiles with environmental factors like altitude and precipitation (Velásquez-Reyes et al., 2025), in which a higher abundance of 2-pentanol was obtained in samples of cocoa beans from localities of a region of Mexico with higher altitude and annual precipitations.

3.2.2. HSSE

HSSE results were consistent with the findings of the HS-SPME technique despite the differences in the chemistry of the adsorbent, as was previously discussed. Thus, PC1, accounting close to a third of the explained variability of the experimental data, highlighted the differentiated chemical profile of CCN_C sample compared to the others, meanwhile PC2 explained variations related to the origin of the samples (Fig. 4), as was observed in the PCA model obtained from HS-SPME data. By looking at the loadings and relative peak areas, tetramethyl and trimethylpyrazines, 3-hydroxy-2-butanone, 2,3-butanediol diacetate isomers, and acetic acid exhibited much higher values in CCN_C sample, as seen after sampling with HS-SPME, as expected. Propanoic acid was also a characteristic of CCN_C sample, showing higher values compared to other samples (Table S3).

In contrast, the differentiation of samples regarding the origin of the cacao was influenced by several acids such as 2-methylbutanoic acid, 3-methylbutanoic acid and 2-methylpropanoic acid, as well as 2-heptanol. All of them, in general, showed markedly elevated values of relative peak areas in samples from estate 'O'. Despite the differences in the

chemical profiles obtained when using different sampling techniques, these VOCs were also relevant contributors of origin differentiation in the HS-SPME model previously described.

As aforementioned, this is in agreement with the findings of Marseglia et al. (2020), as 2-heptanol played a role in the differentiation of cacao beans from different countries, moreover Calva-Estrada (2020) identified 2-methylpropanoic acid as a potential dark-chocolate marker made from South-American cocoa beans. In another study, Tuentler et al. (2020) reported that 3-methyl butanoic acid was detected in a commercial cocoa mass made from a blend of cocoa beans originating from West Africa, but not in one produced from cocoa beans of an Ecuadorian province. All these VOCs are relevant in terms of flavor as they contribute to a diversity of aromas as fruity notes, which is the case of 2-heptanol, or rancid, sweaty or buttery notes associated with 3-methylbutanoic acid and 2-methylpropanoic acid (Herrera-Rocha et al., 2023).

3.2.3. DHS

Differentiation between samples according to cacao origin was also observed in the PCA obtained from DHS data, mostly explained by PC1 and PC2 (Fig. 5). CCN_C sample was again notably different from the others, as was observed in the PCA model obtained from the other techniques. By looking at the loadings and relative peak areas, tetramethyl and trimethylpyrazines, 3-hydroxy-2-butanone, and acetic acid exhibited much higher values in CCN_C sample, as seen after sampling with the other two techniques (Table S3).

According to the loadings plot (Fig. 5), regarding sample provenance, samples from estate 'O' were generally characterized by higher relative peak areas of monoterpenoids such as 3,7-dimethyl-6-octen-1-ol (beta-citronellol), together with phenethyl isobutyrate and 2-methylbutanoic acid. In contrast, samples of estate 'C' were associated with higher values for alcohols such as 1-hexanol, 2,3-butanediol isomers, and benzyl alcohol, together with ketones such as 1-phenylethanol and 1-(1H-pyrrol-2-yl)-ethanone. Notably, sample PMC_C (estate 'C'), stood out from the others, due to its high relative peak areas of 1-hexanol and 1-phenylethanol, among others (Table S3).

The PCA results showed that DHS also enabled the detection of VOCs that contributed to differentiating samples by their origin. Nonetheless, the compounds that were more influential in this differentiation were not the same as the ones obtained after HS-SPME and HSSE sampling, likely due to differences in adsorbent amount and nature, and in the dynamic sampling principle, as discussed previously. Despite these differences, all the three techniques proved to find VOCs that were characteristic of the provenance of the samples studied.

In the study of Marseglia et al. (2020), in which roasted cocoa beans of 22 different origins from America, Africa and Southeast Asia were analyzed, the findings point out that 1-(1H-pyrrol-2-yl)-ethanone had a high presence in these samples, which might be related to fermentation degree or cacao variety. In addition, authors indicated that 1-phenylethanol was characteristic of samples from Africa, although high variation was observed. Similarly, Bagnulo et al. (2023) highlighted that 1-phenylethanol had higher abundances in the samples of West African-origin blends. Calva-Estrada et al., (2020) observed that certain compounds contributed more significantly to the VOCs profile of five chocolates of different origins. For example, 3-hydroxy-2-butanone was detected only in the chocolate or cocoa mass produced from Criollo cacao of the Amazons, and not in the chocolates made from cacao originating in Mexico, Venezuela and Ecuador.

As observed across all the PCA models, despite the differences in the VOCs profiles resulting from the sampling technique applied (HS-SPME, HSSE and DHS), each method successfully extracted VOCs that contributed to the differentiation of cocoa masses according to their origin in Costa Rica: estate 'C' in South Caribbean region and estate 'O' in Northern region (Table 1). For instance, 3-methylbutanoic acid, 2-methylpropanoic acid, and 2-pentanol were influential when sampling with HS-SPME and HSSE, and ketones such as 3-hydroxy-2-butanone, 1-(1H-pyrrol-2-yl)-ethanone and 1-phenylethanol when sampling

with DHS. Although these results suggest that VOC profiling may capture differences associated with cacao origin, the present findings should be considered preliminary due to the limited number of cacao varieties and geographical regions included in this study. Therefore, further investigations involving a larger and more representative sample set will be necessary to confirm the robustness and general applicability of the observed geographical patterns.

4. Conclusions

This study demonstrates that the choice of headspace sampling technique (HS-SPME, HSSE, or DHS) significantly influences the volatile profile characterization of cocoa masses. Although the three methods differed in number of detected VOCs, relative peak area values, and variation between replicates, each provided valuable perspectives. These findings highlight the importance of selecting the most appropriate technique according to the specific research question, as it may provide different insights.

Among the assessed techniques, HS-SPME was considered to have more advantages as it enabled the detection of a higher number of VOCs of all the three techniques, has lower costs and less operational demand than DHS and HSSE. However, the results also highlight the complementary nature of the evaluated techniques. In particular, DHS provided enhanced higher detection capacity, while HSSE and DHS enabled the detection of specific VOCs not captured by HS-SPME. Rather than identifying a universally superior technique, these findings show that each method exhibits a distinct balance between the different performance parameters. Therefore, the selection of the extraction technique should depend on the specific analytical objective, reinforcing the importance of a fit-for-purpose approach when applying headspace sampling techniques to complex matrices.

The VOC profiles obtained after sampling with the three techniques were proved effective for distinguishing samples regarding their origin (estate 'C' in South Caribbean region and estate 'O' in Northern region, both located in Costa Rica). Despite these differences observed by each technique, key markers such as 2-heptanol, 2-heptanone, 3-methylbutanoic acid, 2-methylpropanoic acid, 1-(1H-pyrrol-2-yl)-ethanone, and 1-phenylethanone, among others, were consistently identified as influential for origin-based differentiation, aligning with international literature on cocoa traceability. Moreover, the complexity of the chemical profile in cocoa beans is product of an interplay among the agronomical varieties, geographical factors and postharvest practices linked to the origin, and this study suggests that these factors can also be expressed in cocoa derivatives as cocoa masses. However, the observations related to geographical differentiation should be regarded as preliminary and require confirmation through studies including a larger number of samples, varieties, and production regions.

To conclude, this study provides new insights regarding the use of three headspace techniques for the analysis of cacao masses. Results demonstrate the relevance and usefulness of the assessed sampling techniques in the analysis of VOCs, encouraging new research focused on traceability strategies for cocoa derivatives as cocoa masses.

CRedit authorship contribution statement

Raquel M. Callejón: Writing – review & editing, Supervision, Methodology, Conceptualization. **M. Pilar Segura-Borrego:** Investigation. **Rocío Ríos-Reina:** Writing – review & editing, Visualization, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **M. Lourdes Morales:** Software, Methodology, Formal analysis, Data curation. **Adriana M. Arciniegas-Leal:** Writing – review & editing, Resources. **Ana Lucía Mayorga-Gross:** Writing – original draft, Visualization, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation.

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Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this manuscript, the authors used ChatGPT (OpenAI) to assist with English language editing and improve the clarity of the text. In addition, AI-assisted image generation tools were used to create some illustrative elements included in the graphical abstract. These images were used exclusively for schematic purposes and do not represent experimental data or scientific results. The graphical abstract was designed and assembled by the authors. The authors carefully reviewed and edited all AI-generated content and take full responsibility for the final version of the manuscript.

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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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jfca.2026.109416.

Data availability

Data will be made available on request.

References

- Akoa, S.P., Boulanger, R., Effa Onomo, P., Lebrun, M., Ondobo, M.L., Lahon, M.-C., Ntyam Mendo, S.A., Niemenak, N., Djocgoue, P.F., 2023. Sugar profile and volatile aroma composition in fermented dried beans and roasted nibs from six controlled pollinated Cameroonian fine-flavor cocoa (*Theobroma cacao* L.) hybrids (Article). Food Biosci. 53, 102603. <https://doi.org/10.1016/j.fbio.2023.102603>.
- Aprotosoaie, A.C., Luca, S.V., Miron, A., 2016. Flavor chemistry of cocoa and cocoa products—An overview. Compr. Rev. Food Sci. Food Saf. 15 (1), 73–91. <https://doi.org/10.1111/1541-4337.12180>.
- Bagnulo, E., Scavarda, C., Bortolini, C., Cordero, C., Bicchi, C., Liberto, E., 2023. Cocoa quality: Chemical relationship of cocoa beans and liquors in origin identification (Article). Food Res. Int. 172, 113199. <https://doi.org/10.1016/j.foodres.2023.113199>.
- Becerra, L.D., Ruiz, R.Y., Rodríguez Cortina, J., Quintanilla-Carvajal, M.X., Coy-Barrera, E., Escobar Parra, S., 2023. Chemical characterization of quality-related compounds in cocoa matrices: An overview of analytical methods applied for their analysis. Crit. Rev. Anal. Chem. 53 (3), 689–717. <https://doi.org/10.1080/10408347.2021.1971511>.
- Besançon, L., Poirot, P., Lebrun, M., Ortiz-Julien, A., Boulanger, R., 2024. Investigating key volatile compound diffusion in cocoa beans during yeast fermentation-like incubation. J. Agric. Food Chem. 72, 15788–15800. <https://doi.org/10.1021/acs.jafc.4c03086>.

- Bickel Haase, T., Schweiggert-Weisz, U., Ortner, E., Zorn, H., Naumann, S., 2021. Aroma properties of cocoa fruit pulp from different origins (Article). *Molecules* 26 (24), 7618. <https://doi.org/10.3390/molecules26247618>.
- Braga, S.C.G.N., Oliveira, L.F., Hashimoto, J.C., Gama, M.R., Efraim, P., Poppi, R.J., Augusto, F., 2018. Study of volatile profile in cocoa nibs, cocoa liquor and chocolate on production process using GC × GC-QMS. *Microchem. J.* 141, 353–361. <https://doi.org/10.1016/j.microc.2018.05.042>.
- Britto de Andrade, A., Lins da Cruz, M., De Souza Oliveira, F.A., Soares, S.E., Druzian, J. I., Radomille de Santana, L.R., Oliveira de Souza, C., da Silva Bispo, E., 2021. Influence of under-fermented cocoa mass in chocolate production: Sensory acceptance and volatile profile characterization during the processing (Article). *LWT Food Sci. Technol.* 149, 112048. <https://doi.org/10.1016/j.lwt.2021.112048>.
- Buchem B.V. (n.d.). *TENAX*. <https://buchem.com/product/tenax/tenax-ta/>.
- Cacao of Excellence, 2023. Guide for the assessment of cacao quality and flavour (compiled by the Cacao of Excellence programme of the Alliance of Bioversity International and CIAT, in collaboration with the members of the Working Group on the development of the International Standards for the Assessment of Cocoa Quality and Flavour (ISCQF)). Bioversity International. ISBN 978-92-9255-296-1.
- Calva-Estrada, S.J., Utrilla-Vázquez, M., Vallejo-Cardona, A., Roblero-Pérez, D.B., Lugo-Cervantes, E., 2020. Thermal properties and volatile compounds profile of commercial dark-chocolates from different genotypes of cocoa beans (*Theobroma cacao* L.) from Latin America (Article). *Food Res. Int.* 136, 109594. <https://doi.org/10.1016/j.foodres.2020.109594>.
- Cemin, P., Ribeiro, S.R., Oliveira, F.C., Leal Leães, F., Dos Santos Nunes, M.R., Wagner, R., Sant'Anna, V., 2022. Chocolates with Brazilian cocoa: tracking volatile compounds according to consumers' preference (Article). *Food Res. Int.* 159, 111618. <https://doi.org/10.1016/j.foodres.2022.111618>.
- Cemin, P., Ribeiro, S.R., Oliveira, F.C., Wagner, R., Sant'Anna, V., 2024. Study of volatile compounds and sensory profile of Brazilians' cocoa liquors (Article). *Int. J. Gastron. Food Sci.* 37, 100967. <https://doi.org/10.1016/j.ijgfs.2024.100967>.
- Chaudhary, V., Mongia, G., Drake, M., 2025. Approaches to determine the flavor and flavor chemistry of cocoa beans and cocoa liquor. *J. Food Sci.* 90 (9), e70481. <https://doi.org/10.1111/1750-3841.70481>.
- Chetschik, I., Kneubühl, M., Chatelain, K., Schlüter, A., Bernath, K., Hühn, T., 2018. Investigations on the aroma of cocoa pulp (*Theobroma cacao* L.) and its influence on the odor of fermented cocoa beans. *J. Agric. Food Chem.* 66 (10), 2467–2472. <https://doi.org/10.1021/acs.jafc.6b05008>.
- Collin, S., Fissette, T., Pinto, A., Souza, J., Rogez, H., 2023. Discriminating aroma compounds in five cocoa bean genotypes from two Brazilian states: White Kerosene-like Catongo, Red Whisky-like FL89 (Bahia), Forastero IMC67, PA121 and P7 (Pará) (Article). *Molecules* 28 (4), 1548. <https://doi.org/10.3390/molecules28041548>.
- Colonges, K., Jimenez, J.-C., Saltos, A., Seguine, E., Looor Solorzano, R.G., Fouet, O., Argout, X., Assemat, S., Davrieux, F., Cros, E., Boulanger, R., Lanaud, C., 2021. Two main biosynthesis pathways involved in the synthesis of the floral aroma of the Nacional cocoa variety (Article). *Front. Plant. Sci.* 12, 681979. <https://doi.org/10.3389/fpls.2021.681979>.
- Colonges, K., Jimenez, J.-C., Saltos, A., Seguine, E., Looor Solorzano, R.G., Fouet, O., Argout, X., Assemat, S., Davrieux, F., Cros, E., Lanaud, C., Boulanger, R., 2022. Integration of GWAS, metabolomics, and sensorial analyses to reveal novel metabolic pathways involved in cocoa fruit aroma GWAS of fruit aroma in *Theobroma cacao*. *Plant. Physiol. Biochem.* 171, 213–225. <https://doi.org/10.1016/j.plaphy.2021.11.006>.
- Cordero, C., Sgorbini, B., Liberto, E., Bicchì, C., Rubiolo, P., 2009. Stir bar sorptive extraction (SBSE) and headspace sorptive extraction (HSSE): an overview. *LCGC North. Am.* 27, 376–390.
- Crafack, M., Keul, H., Eskildsen, C.E., Petersen, M.A., Saerens, S., Blennow, A., Skovmand-Larsen, M., Swiegers, J.H., Petersen, G.B., Heimdal, H., Nielsen, D.S., 2014. Impact of starter cultures and fermentation techniques on the volatile aroma and sensory profile of chocolate. *Food Res. Int.* 63, 306–316. <https://doi.org/10.1016/j.foodres.2014.04.032>.
- Cucu, T., Devos, C., David, F., Sandra, P., 2021. High analytical performance for untargeted aroma profiling by combining dynamic headspace sampling, capillary gas chromatography and time-of-flight mass spectrometry. *LCGC Eur.* 34 (4), 130–138.
- Cui, H., Yu, J., Zhai, Y., Feng, L., Chen, P., Hayat, K., Xu, Y., Zhang, X., Ho, C.-T., 2021. Formation and fate of Amadori rearrangement products in Maillard reaction. *Trends Food Sci. & Technol.* 115, 391–408. <https://doi.org/10.1016/j.tifs.2021.06.055>.
- De Vuyst, L., Leroy, F., 2020. Functional role of yeasts, lactic acid bacteria and acetic acid bacteria in cocoa fermentation processes. *FEMS Microbiol. Rev.* 44 (4), 432–453. <https://doi.org/10.1093/femsre/fuaa014>.
- Dettmer, K., Engewald, W., 2002. Adsorbent materials commonly used in air analysis for adsorptive enrichment and thermal desorption of volatile organic compounds. *Anal. Bioanal. Chem.* 373 (6), 490–500. <https://doi.org/10.1007/s00216-002-1352-5>.
- Deuscher, Z., Gourrat, K., Repoux, M., Boulanger, R., Labouré, H., Le Qué, J.-L., 2020. Key aroma compounds of dark chocolates differing in organoleptic properties: A GC-O comparative study (Article). *Molecules* 25 (8), 1809. <https://doi.org/10.3390/molecules25081809>.
- Dias, R.P., Johnson, T.A., Ferrão, L.F.V., Munoz, P.R., de la Mata, A.P., Harynuk, J.J., 2023. Improved sample storage, preparation and extraction of blueberry aroma volatile organic compounds for gas chromatography (Article). *J. Chromatogr. Open.* 3, 100075. <https://doi.org/10.1016/j.jcoa.2022.100075>.
- Dias, S.R., Adami, L., Batista, N.N., Martinez, S.J., Bressani, A.P.P., Dias, D.R., Schwan, R. F., 2025. Metatransomic, chemical, and sensory aspects of cocoa fermentation using single hybrids and combinations of hybrids and their effects on chocolate quality (Article). *Food Res. Int.* 210, 116410. <https://doi.org/10.1016/j.foodres.2025.116410>.
- Diez-Simon, C., Ammerlaan, B., van den Berg, M., van Duynhoven, J., Jacobs, D., Mumm, R., Hall, R.D., 2020. Comparison of volatile trapping techniques for the comprehensive analysis of food flavourings by gas chromatography-mass spectrometry (Article). *J. Chromatogr. A* 1624, 461191. <https://doi.org/10.1016/j.chroma.2020.461191>.
- Djombou Feunang, Y., Eisner, R., Knox, C., Chepelev, L., Hastings, J., Owen, G., Fahy, E., Steinbeck, C., Subramanian, S., Bolton, E., Greiner, R., Wishart, D.S., 2016. ClassyFire: Automated chemical classification with a comprehensive, computable taxonomy. *J. Cheminform.* 8, 61. <https://doi.org/10.1186/s13321-016-0174-y>.
- End, M.J., Dand, R., 2023. CAOBISCO/ECA Cocoa Beans: Chocolate & Cocoa Industry Quality Requirements, 2nd ed.. CAOBISCO/ECA <https://www.eurococoa.com/wp-content/uploads/Cocoa-Beans-Guide-2023-v240124.pdf>, 2nd ed.
- Erazo Solorzano, C.Y., Tuárez García, D.A., Edison Zambrano, C., Moreno-Rojas, J.M., Rodríguez Solana, R., 2023. Monitoring changes in the volatile profile of Ecuadorian cocoa during different steps in traditional on-farm processing (Article). *Plants* 12 (22), 3904. <https://doi.org/10.3390/plants12223904>.
- Escobar, S., Santander, M., Zuluaga, M., Chacón, I., Rodríguez, J., Vaillant, F., 2021. Fine cocoa beans production: Tracking aroma precursors through a comprehensive analysis of flavor attributes formation (Article). *Food Chem.* 365, 130627. <https://doi.org/10.1016/j.foodchem.2021.130627>.
- Febrianto, N.A., Zhu, F., 2020. Changes in the composition of methylxanthines, polyphenols, and volatiles and sensory profiles of cocoa beans from the Sul 1 Genotype affected by fermentation. *J. Agric. Food Chem.* 68 (32), 8658–8675. <https://doi.org/10.1021/acs.jafc.0c02909>.
- Fraundorfer, F., Schieberle, P., 2019. Key aroma compounds in fermented Forastero cocoa beans and changes induced by roasting. *Eur. Food Res. Technol.* 245 (9), 1907–1915. <https://doi.org/10.1007/s00217-019-03292-2>.
- Food and Agriculture Organization of the United Nations, World Health Organization & Codex Alimentarius. (2022a). CXS 86-1981. Standard for cocoa butter. <https://www.fao.org/fao-who-codexalimentarius/codex-texts/list-standards/es/>.
- Food and Agriculture Organization of the United Nations, World Health Organization & Codex Alimentarius. (2022b). CXS 87-1981. Standard for chocolate and chocolate products. <https://www.fao.org/fao-who-codexalimentarius/codex-texts/list-standards/es/>.
- Food and Agriculture Organization of the United Nations, World Health Organization & Codex Alimentarius. (2022c). CXS 105—1981. Standard for cocoa powders (cocoas) and dry mixtures of cocoa and sugars. <https://www.fao.org/fao-who-codexalimentarius/codex-texts/list-standards/es/>.
- Food and Agriculture Organization of the United Nations, World Health Organization & Codex Alimentarius. (2022d). CXS 141-1983. Standard for cocoa (cacao) mass (cocoa/chocolate liquor) and cocoa cake. <https://www.fao.org/fao-who-codexalimentarius/codex-texts/list-standards/es/>.
- Gerstel, 2015. Gerstel certified Supplies. Twister®. Twister®. GERSTEL GmbH & Co. KG. Retrieved from: https://ric-technologies.com/wp-content/uploads/2021/01/Twister-Flyer_eng.pdf.
- González-Orozco, C.E., Porcel, M., Yockteng, R., Caro-Quintero, A., Rodríguez-Medina, C., Santander, M., Zuluaga, M., Soto, M., Rodríguez Cortina, J., Vaillant, F. E., Escobar Parra, S., 2024. Integrating new variables into a framework to support cacao denomination of origin: A case study in Southwest Colombia. *J. Sci. Food Agric.* 104 (3), 1367–1381. <https://doi.org/10.1002/jsfa.13016>.
- Guckenbiehl, Y., Martin, A., Ortner, E., Rothkopf, I., Schweiggert-Weisz, U., Buettner, A., Naumann-Gola, S., 2022. Aroma-active volatiles and rheological characteristics of the plastic mass during conching of dark chocolate (Article). *Food Res. Int.* 162, 112063. <https://doi.org/10.1016/j.foodres.2022.112063>.
- Guckenbiehl, Y., Ortner, E., Rothkopf, I., Buettner, A., 2025. Refining and conching alter the volatile composition of dark chocolate—Revealing profile changes in aroma-active volatiles and volatile organic compounds (Article). *J. Agric. Food Res.* 101664. <https://doi.org/10.1016/j.jafr.2025.101664>.
- Gutiérrez-Ríos, H.G., Suárez-Quiroz, M.L., Hernández-Estrada, Z.J., Castellanos-Onorio, O.P., Alonso-Villegas, R., Rayas-Duarte, P., Cano-Sarmiento, C., Figueroa-Hernández, C.Y., González-Ríos, O., 2022. Yeasts as producers of flavor precursors during cocoa bean fermentation and their relevance as starter cultures: A review (Article). *Fermentation* 8 (7), 331. <https://doi.org/10.3390/fermentation8070331>.
- Hamdouche, Y., Meile, J.C., Lebrun, M., Guehi, T., Boulanger, R., Teyssier, C., Montet, D., 2019. Impact of turning, pod storage and fermentation time on microbial ecology and volatile composition of cocoa beans. *Food Res. Int.* 119, 477–491. <https://doi.org/10.1016/j.foodres.2019.01.001>.
- Hegmann, E., Niether, W., Rohsius, C., Phillips, W., Lieberei, R., 2020. Besides variety, also season and ripening stage have a major influence on fruit pulp aroma of cacao (*Theobroma cacao* L.). *J. Appl. Bot. Food Qual.* 93, 266–275. <https://doi.org/10.5073/JABFQ.2020.093.033>.
- Herrera-Rocha, F., Cala, M.P., Aguirre Mejía, J.L., Rodríguez-López, C.M., Chica, M.J., Olarte, H.H., Fernández-Niño, M., Gonzalez Barrios, A.F., 2021. Dissecting fine-flavor cocoa bean fermentation through metabolomics analysis to break down the current metabolic paradigm (Article). *Sci. Rep.* 11, 21904. <https://doi.org/10.1038/s41598-021-01427-8>.
- Herrera-Rocha, F., Fernández-Niño, M., Cala, M.P., Duitama, J., Barrios, A.F.G., 2023. Omics approaches to understand cocoa processing and chocolate flavor development: A review (Article). *Food Res. Int.* 165, 112555. <https://doi.org/10.1016/j.foodres.2023.112555>.
- Herrera-Rocha, F., León-Inga, A.M., Aguirre Mejía, J.L., Rodríguez-López, C.M., Chica, M.J., Wessjohann, L.A., González Barrios, A.F., Cala, M.P., Fernández-Niño, M., 2024. Bioactive and flavor compounds in cocoa liquor and their traceability over the major steps of cocoa post-harvesting processes. *Article* 137529 *Food Chem.* 435. <https://doi.org/10.1016/j.foodchem.2023.137529>.

- Hinne, M., Semanhyia, E., Van de Walle, D., De Winne, A., Tzompa-Sosa, D.A., Scalone, G.L.L., De Meulenaer, B., Messens, K., Van Durme, J., Afoakwa, E.O., De Cooman, L., Dewettinck, K., 2018. Assessing the influence of pod storage on sugar and free amino acid profiles and the implications on some Maillard reaction related flavor volatiles in Forastero cocoa beans. *Food Res. Int.* 111, 607–620. <https://doi.org/10.1016/j.foodres.2018.05.064>.
- Jaimez, R.E., Barragan, L., Fernández-Niño, M., Wessjohann, L.A., Cedeño-García, G., Cantos, I.S., Arteaga, F., 2022. *Theobroma cacao* L. cultivar CCN 51: A comprehensive review on origin, genetics, sensory properties, production dynamics, and physiological aspects (Article). *PeerJ* 10, e12676. <https://doi.org/10.7717/peerj.12676>.
- Jelen, H.H., Majcher, M., Dziadas, M., 2012. Microextraction techniques in the analysis of food flavor compounds: A review. *Anal. Chim. Acta* 738, 13–26.
- Jelen, H.H., Wiczorek, M.N., 2025. Commentary: Selected factor influencing quantitative HS-SPME analysis of volatile compounds in alcoholic beverages. *J. Compos. Anal.* 145 (2025), 107852.
- Johnsen, L.G., Skou, P.B., Khakimov, B., Bro, R., 2017. Gas chromatography – mass spectrometry data processing made easy. *J. Chromatogr. A* 1503, 57–64. <https://doi.org/10.1016/j.chroma.2017.04.052>.
- Kadow, D., 2020. The biochemistry of cocoa flavor—A holistic analysis of its development along the processing chain. *J. Appl. Bot. Food Qual.* 93, 300–312. <https://doi.org/10.5073/JABFQ.2020.093.037>.
- Kim, S., Chen, J., Cheng, T., Gindulyte, A., He, J., He, S., Li, Q., Shoemaker, B.A., Thiessen, P.A., Yu, B., Zaslavsky, L., Zhang, J., Bolton, E.E., 2025. PubChem 2025 update. *Nucleic Acids Res.* 53, D1516–D1525. <https://doi.org/10.1093/nar/gkae1059>.
- Lemarcq, V., Van de Walle, D., Monterde, V., Sioriki, E., Dewettinck, K., 2022. Assessing the flavor of cocoa liquor and chocolate through instrumental and sensory analysis: A critical review. *Crit. Rev. Food Sci. Nutr.* 62 (20), 5523–5539. <https://doi.org/10.1080/10408398.2021.1887076>.
- Li, J., Fu, Y., Bao, X., Li, H., Zuo, J., Zhang, M., Wang, J., 2020. Comparison and analysis of tomato flavor compounds using different extraction methods. *J. Food Meas. Charact.* 14 (1), 465–475. <https://doi.org/10.1007/s11694-019-00102-x>.
- Liberto, E., Bagnulo, E., Giorgio, F., Caratti, C., Bortolini, C., Cordero, C., Bicchi, C., 2025. Machine learning models for terroir classification and blend similarity prediction: A proof-of-concept to enhance cocoa quality evaluation (Article). *Food Chem.* 486, 144620. <https://doi.org/10.2139/ssrn.5115360>.
- Liberto, E., Cagliero, C., Cordero, C., Rubiolo, P., Bicchi, C., Sgorbini, B., 2017. Fractionated dynamic headspace sampling in the analysis of matrices of vegetable origin in the food field. *J. Chromatogr. A* 1489, 18–28. <https://doi.org/10.1016/j.chroma.2017.02.009>.
- Liu, C., Yang, P., Wang, H., Song, H., 2022. Identification of odor compounds and odor-active compounds of yogurt using DHS, SPME, SAFE, and SBSE/GC-O-MS (Article). *LWT Food Sci. Technol.* 154, 112689. <https://doi.org/10.1016/j.lwt.2021.112689>.
- Llano, S., Vaillant, F., Santander, M., Zorro-González, A., González-Orozco, C.E., Maraval, I., Boulanger, R., Escobar, S., 2024. Exploring the impact of fermentation time and climate on quality of cocoa bean-derived chocolate: sensorial profile and volatolome analysis (Article). *Foods* 13 (16), 2614. <https://doi.org/10.3390/foods13162614>.
- Magi, E., Bono, L., Di Carro, M., 2012. Characterization of cocoa liquors by GC–MS and LC–MS/MS: Focus on alkylpyrazines and flavanols. *J. Mass. Spectrom.* 47 (9), 1191–1197. <https://doi.org/10.1002/jms.3034>.
- Mancini, E., Arnold, N.A., De Martino, L., De Feo, V., Formisano, C., Rigano, D., Senatore, F., 2009. Chemical Composition and Phytotoxic Effects of Essential Oils of *Salvia hirsutissima* Boiss and *Salvia multicaulis* Vahl. *Var. Simplicifolia* Boiss. Growing Wild in Lebanon. *Molecules* 14 (11), 4725–4736. <https://doi.org/10.3390/molecules14114725>.
- Marseglia, A., Musci, M., Rinaldi, M., Palla, G., Caligiani, A., 2020. Volatile fingerprint of unroasted and roasted cocoa beans (*Theobroma cacao* L.) from different geographical origins (Article). *Food Res. Int.* 132, 109101. <https://doi.org/10.1016/j.foodres.2020.109101>.
- Mastrogiovanni, A.R., Pierini, E., Sampaolo, L., 1995. A comparison of the critical parameters of some adsorbents employed in trapping and thermal desorption of organic pollutants. *Cromatographia* 41, 599–604. <https://doi.org/10.1007/BF02269726>.
- Mayorga-Gross, A.L., Quirós-Guerrero, L.M., Fourny, G., Vaillant, F., 2016. An untargeted metabolomic assessment of cocoa beans during fermentation. *Food Res. Int.* 89, 901–909. <https://doi.org/10.1016/j.foodres.2016.04.017>.
- Mota-Gutiérrez, J., Barbosa-Pereira, L., Ferrocino, I., Coccolin, L., 2019. Traceability of functional volatile compounds generated on inoculated cocoa fermentation and its potential health benefits (Article). *Nutrients* 11 (4), 884. <https://doi.org/10.3390/nu11040884>.
- Otero Rojas, E.J., Herrera Ruales, F.C., Arturo Perdomo, D., Jimenez Mora, J.P., 2022. Evaluación del método de extracción SPME-GC-MS para el análisis de compuestos orgánicos volátiles en licor de cacao de Nariño-Colombia. *Rev. ION* 35 (1), 103–116. <https://doi.org/10.18273/revion.v35n1-2022007>.
- Owusu, M., Petersen, M.A., Heimdal, H., 2012. Effect of fermentation method, roasting and conching conditions on the aroma volatiles of dark chocolate. *J. Food Process. Preserv.* 36 (5), 446–456. <https://doi.org/10.1111/j.1745-4549.2011.00602.x>.
- Paiva, A.C., Crucello, J., de Aguiar Porto, N., Hantao, L.W., 2021. Fundamentals of and recent advances in sorbent-based headspace extractions (Article). *TrAC Trends Anal. Chem.* 139, 116252. <https://doi.org/10.1016/j.trac.2021.116252>.
- Papalexandratou, Z., Kaasik, K., Kauffmann, L.V., Skorstengaard, A., Bouillon, G., Espensen, J.L., Hansen, L.H., Jakobsen, R.R., Blennow, A., Krych, L., Castro-Mejía, J. L., Nielsen, D.S., 2019. Linking cocoa varieties and microbial diversity of Nicaraguan fine cocoa bean fermentations and their impact on final cocoa quality appreciation. *Int. J. Food Microbiol.* 304, 106–118. <https://doi.org/10.1016/j.jfoodmicro.2019.05.012>.
- PARADISE. (2023). PARADISE. Retrieved from <https://ucphchemometrics.com/paradise/>. Accessed January, 28, 2026.
- Peña-Correa, R.F., Ataç Mogol, B., Fogliano, V., 2022. The impact of roasting on cocoa quality parameters. *Crit. Rev. Food Sci. Nutr.* 64 (13), 4348–4361. <https://doi.org/10.1080/10408398.2022.2141191>.
- Perez, M., Lopez-Yarena, A., Vallverdú-Queral, A., 2020. Traceability, authenticity and sustainability of cocoa and chocolate products: A challenge for the chocolate industry. *Crit. Rev. Food Sci. Nutr.* 62 (2), 475–489. <https://doi.org/10.1080/10408398.2020.1819769>.
- Petretto, G.L., Urghege, P.P., Mascia, I., Fadda, C., Rourke, J.P., Pintore, G., 2017. Stir bar sorptive extraction coupled with GC/MS applied to honey: Optimization of method and comparative study with headspace extraction techniques. *Eur. Food Res. Technol.* 243 (5), 735–741. <https://doi.org/10.1007/s00217-016-2787-9>.
- Phillips-Mora, W., Arciniegas-Leal, A., Mata-Quirós, A., Motamayor-Arias, J.C., 2012. *Catálogo de clones de cacao seleccionados por el CATIE para siembras comerciales* (1ed.). CATIE. <https://repositorio.catie.ac.cr/handle/11554/7280>.
- Putri, D.N., De Steur, H., Juvinal, J.G., Gellynck, X., Schouteten, J.J., 2024. Sensory attributes of fine flavor cocoa beans and chocolate: A systematic literature review. *J. Food Sci.* 89 (4), 1917–1943. <https://doi.org/10.1111/1750-3841.17006>.
- Quelal, O.M., Hurtado, D.P., Benavides, A.A., Alanes, P.V., Alanes, N.V., 2023. Key aromatic volatile compounds from roasted cocoa beans, cocoa liquor, and chocolate (Article). *Fermentation* 9 (2), 166. <https://doi.org/10.3390/fermentation9020166>.
- Quelal-Vásquez, M.A., Macchioni, R., Livi, G., Pérez-Esteve, E., Lerma-García, M.J., Talens, P., Barat, J.M., Petersen, M.A., Bro, R., 2022. Automatic and non-targeted analysis of the volatile profile of natural and alkalized cocoa powders using SBSE-GC-MS and chemometrics (Article). *Food Chem.* 389, 133074. <https://doi.org/10.1016/j.foodchem.2022.133074>.
- Quintanilla-Casas, B., Bro, R., Løve Hinrich, J., 2023. Tutor. Paradise. PARAFAC2-Based deconvolution Identif. Syst. Process. GCMS data. V. 1. <https://doi.org/10.21203/rs.3.pex-2143/v1>.
- Ríos-Reina, R., Morales, M.L., García-González, D.L., Amigo, J.M., Callejón, R.M., 2018. Sampling methods for the study of volatile profile of PDO wine vinegars. A comparison using multivariate data analysis. *Food Res. Int.* 105, 880–896. <https://doi.org/10.1016/j.foodres.2017.12.001>.
- Rodríguez-González, B.E., Amador-Espejo, G.G., Penagos-Velez, L., Ramírez-López, C., Mazo-Rivas, J.C., Ruiz-Espinosa, H., 2025. Chemical composition and volatile profile of cocoa liquors from Colombian Criollo cacao (Article). *J. Food Sci.* 90 (4), e70143. <https://doi.org/10.1111/1750-3841.70143>.
- Rojas, M., Hommes, A., Heeres, H.J., Chejne, F., 2022. Kinetic Studies on cocoa roasting including volatile characterization. *ACS Food Sci. & Technol.* 14, 509–533. <https://doi.org/10.1021/acsfodsctech.1c00481>.
- Rottiers, H., Tzompa Sosa, D.A., De Winne, A., Ruales, J., De Clippeleer, J., De Leersnyder, I., De Wever, J., Everaert, H., Messens, K., Dewettinck, K., 2019. Dynamics of volatile compounds and flavor precursors during spontaneous fermentation of fine flavor Trinitario cocoa beans. *Eur. Food Res. Technol.* 245 (9), 1917–1937. <https://doi.org/10.1007/s00217-019-03307-y>.
- Ruvalcaba, J.E., Durán-Guerrero, E., Barroso, C.G., Castro, R., 2020. Development of head space sorptive extraction method for the determination of volatile compounds in beer and comparison with stir bar sorptive extraction (Article). *Foods* 9 (3), 255. <https://doi.org/10.3390/foods9030255>.
- Santander, M., Chica, V., Correa, H.A.M., Rodríguez, J., Villagran, E., Vaillant, F., Escobar, S., 2025. Unravelling cocoa drying technology: A comprehensive review of the influence on flavor formation and quality (Article). *Foods* 14 (5), 721. <https://doi.org/10.3390/foods14050721>.
- Seguine, E., Mills, D., Marelli, J.-P., Motamayor-Arias, J.C., Coelho, I.D.S., 2014. Micro-Ferment. cocoa (Pat. No. US20140199437A1). U. S. <https://patents.google.com/patent/US20140199437A1/en>.
- Segura-Borrego, M.P., Ríos-Reina, R., Ubeda, C., Callejón, R.M., Morales, M.L., 2020. Comparison of the novel thin film-solid phase microextraction and sorptive extraction methods for Picual and Hojiblanca olive oil volatile fraction analysis in headspace (Article). *Foods* 9 (6), 748. <https://doi.org/10.3390/foods9060748>.
- Shakoor, A., Zhang, C., Xie, J., Yang, X., 2022. Maillard reaction chemistry in formation of critical intermediates and flavour compounds and their antioxidant properties (Article). *Food Chem.* 393, 133416. <https://doi.org/10.1016/j.foodchem.2022.133416>.
- Toker, O.S., Palabiyik, I., Konar, N., 2019. Chocolate quality and conching. *Trends Food Sci. & Technol.* 91, 446–453. <https://doi.org/10.1016/j.tifs.2019.07.047>.
- Tuenter, E., Delbaere, C., De Winne, A., Bijttebier, S., Custers, D., Foubert, K., Van Durme, J., Messens, K., Dewettinck, K., Pieters, L., 2020. Non-volatile and volatile composition of West African bulk and Ecuadorian fine-flavor cocoa liquor and chocolate (Article). *Food Res. Int.* 130, 108943. <https://doi.org/10.1016/j.foodres.2019.108943>.
- University of Reading, Cocoa Research Association (CRA) & Intercontinental Exchange (ICE Futures Europe). (n.d.). *International Cocoa Germplasm Database (ICGD)* [On line]. <https://icgd.reading.ac.uk/>.
- Ullrich, L., Casty, B., André, A., Hühn, T., Steinhaus, M., Chetschik, I., 2022. Decoding the fine flavor properties of dark chocolates. *J. Agric. Food Chem.* 70 (42), 13730–13740. <https://doi.org/10.1021/acs.jafc.2c04166>.
- Van Durme, J., Ingels, I., De Winne, A., 2016. Inline roasting hyphenated with gas chromatography–mass spectrometry as an innovative approach for assessment of cocoa fermentation quality and aroma formation potential. *Food Chem.* 205, 66–72. <https://doi.org/10.1016/j.foodchem.2016.03.004>.
- Velásquez-Reyes, D., García-Alamilla, P., Kirchmayr, M.R., Lugo-Cervantes, E., Gschaedler, A., 2025. Contribution of post-harvest processing in cocoa bean:

- Chemometric and metagenomic analysis in fermentation step (Article). *Food Chem.* 477, 143458. <https://doi.org/10.1016/j.foodchem.2025.143458>.
- Wahrens, S.S., Zhang, S., Hedelund, P.L., Petersen, M.A., Byrne, D.V., 2016. Application of the fast sensory method 'Rate-All-That-Apply' in chocolate quality control compared with DHS- GC-MS. *Int. J. Food Sci. & Technol.* 51 (8), 1877–1887. <https://doi.org/10.1111/ijfs.13161>.
- Wexler, L., Cubero-Castillo, E., Vega-Aguilar, C.A., Alvarado-Marenco, P., Hernández-Gómez, L., 2025. Fermentation time effect on the quality of ancestral cocoa cultivars from Upala, Costa Rica, using physicochemical and sensory characteristics (Article). *CyTA - J. Food* 23 (1), 2486226. <https://doi.org/10.1080/19476337.2025.2486226>.
- Yu, H., Zhang, R., Yang, F., Xie, Y., Guo, Y., Yao, W., Zhou, W., 2021. Control strategies of pyrazines generation from Maillard reaction. *Trends Food Sci. & Technol.* 112, 795–807. <https://doi.org/10.1016/j.tifs.2021.04.028>.
- Ziegler, G., 2017. Flavour development in cocoa and chocolate. In: Beckett, S.T., Fowler, M.S., Ziegler, G.R. (Eds.), *Beckett's industrial chocolate manufacture and use*. Wiley Blackwell, pp. 185–215.
- Wishart, D.S., Guo, A.C., Gautam, V., 2021. HMDB 5.0: the Human Metabolome Database for 2022. *Nucleic Acids Res.* 50 (D1), D622–D631. <https://doi.org/10.1093/nar/gkab1062>.