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Instrument: Pegasus® BTX 4D

Characterization and Comparison of Cocoa from Three Different Geographical Areas

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Key Words: ChromaTOF Sync 2D; Aroma Profiling; Cocoa Analysis; Chocolate; SPME; GCxGC; TOFMS

Introduction

Due to its unique, specific organoleptic properties, chocolate may be considered as the most appealing confectionery worldwide. The quality of chocolate and chocolate-containing products depends heavily on the type of cocoa used and its manufacturing process. Factors such as genotype and geographical origin, but also post-harvest treatments—including fermentation and roasting—have a major contribution to the cocoa aroma quality. In general, the cocoa market distinguishes between bulk cocoa (approximately 85 % of the world production) and fine or flavor cocoa (15 %)[1]. Bulk cocoa is typically characterized by its strong and intense basic cocoa flavor, whereas fine cocoa additionally displays ancillary aromas, such as floral and fruity notes. The volatile fraction of cocoa is very complex and consists of more than 500 non-polar and very polar compounds. These compounds cover a wide concentration range and belong to several chemical classes, such as hydrocarbons, alcohols, ketones, aldehydes, furanones, acids, esters, and pyrazines. However, it has been demonstrated that only some of the compounds are present at organoleptically perceptible levels and these produce the chocolate specific aroma[2]. Detecting these main contributors among the very complex mixture of hundreds of analytes without a cost- and labor-intensive sample preparation step poses a major challenge for analytics.

Characterizing the comprehensive volatile profile requires a technique that provides full separation and detection of these compounds in a single analysis. In this respect, comprehensive two-dimensional gas chromatography (GCxGC) coupled with time-of-flight mass spectrometry (TOFMS) presents an excellent option to elucidate cocoa profiles and detect aroma relevant compounds. As in any analytical workflow, the coupling of an appropriate sampling technique is also crucial for a meaningful outcome. Solvent-assisted flavor extraction (SAFE) is a state-of-the-art method for the artifact-avoiding removal of non-volatiles from solvent extracts obtained from food samples; however, the high level of manpower required to perform this technique represents a major drawback. Typical alternative sampling techniques for cocoa-based matrices include static head space (SHS) and head space solid phase microextraction (HS-SPME). The latter can be considered as an additional preconcentration step prior to sample injection.

In this application note, SHS and HS-SPME were considered and compared as sampling techniques for GCxGC-TOFMS analysis. HS-SPME and GCxGC-TOFMS were then employed for the extraction, separation, and detection of cocoa profiles from three different geographical origins. Data investigation was conducted using ChromaTOF® Sync 2D software.

Experimental

Cocoa liquor samples from three different geographical origins (Ecuador, Ivory Coast, and the Dominican Republic) were analyzed by GCxGC-TOFMS. Ecuador and Dominican Republic are both considered fine flavor cocoa producing countries, whereas Ivory Coast is a bulk cocoa producer[3].

Initially, for sample introduction and sample preparation, SHS and HS-SPME were considered and compared. The comparison of peak numbers detected as well as the compounds' peak areas (see Results section) emphasized the superiority of HS-SPME in comparison to SHS. All cocoa liquor samples were analyzed in triplicate with HS-SPME and GCxGC-TOFMS. Final conditions for the sampling and GCxGC-TOFMS analysis are reported in Table 1 and Table 2, respectively. In addition, an n-Alkane standard (C7–C30) was analyzed for calculation of linear retention indices (RI).

Table 1: HS-SPME parameters.

HS-SPME	
Sample	1.5 g cocoa liquor in a 20 ml HS-SPME vial
Fibre Type	Grey fibre (50/30 μm DVB/Carbon/PDMS 24Ga)
Incubation	3 min at 80 °C
Extraction	10 min at 80 °C
Desorption	2 min

Table 2: GCxGC-TOFMS analysis parameters.

GC Parameters	**Agilent 8890 w/LECO *QuadJet* Thermal Modulator**
Inlet	Split (10:1), 250 °C
Carrier Gas	Helium, 1.40 mL/min, constant flow
Columns	1D: Stabilwax 30 m x 0.25 mm x 0.25 μm (Restek) 2D: Rxi-17Silms 1.1 m x 0.15 mm x 0.15 μm (Restek)
Oven Program	40 °C (2 min), 4 °C/min to 200 °C, 10 °C/min to 230 °C (3 min)
Secondary Oven Temp	+5 °C (relative to the GC oven temperature)
Modulator Temp	+15 °C (relative to the secondary oven temperature)
Modulation Period	3.5 s
Transfer Line	280 °C
TOFMS	**LECO *Pegasus* BTX**
Ion Source Temp	250 °C
Mass Range	40–450 m/z
Acquisition Rate	100 spectra/s

Results and Discussion

One of the initial goals was to compare SHS and HS-SPME as sampling techniques for these sample types. Both sampling procedures were optimized to maximize the sum of all peak areas for volatile compounds in cocoa liquor. SHS sampling is simple, clean, and convenient as a gas extraction technique. HS-SPME is a more complex technique where the adsorption and concentration steps are performed simultaneously, then desorption is performed by heating in the GC inlet. Comparing SHS and HS-SPME in terms of peak numbers detected and their corresponding areas revealed that HS-SPME is a more efficient technique regarding the determination of cocoa volatiles, considering its increased sensitivity and its wider range of chemical analytes detected. Figure 1 displays GCxGC contour plots obtained with SHS and HS-SPME.

The contour plot on the left of Figure 1 was obtained by SHS and resulted in about 130 tentatively identified analytes, whereas the contour plot on the right was attained with HS-SPME and is substantially richer, revealing more than 700 compounds. For this peak number comparison, a simple filter tool available within *ChromaTOF* was applied to show features with a similarity score ≥800 and RI matching of ±20. The zoomed-in area at RT1 ~1120 s highlights the enhancement of a low-level compound: methional (CAS no: 3268-49-3), which was only detected using HS-SPME but not with SHS. Methional is an important cocoa aroma compound with cooked roasted potato odor[4]; moreover, it is likely to contribute to the savory note of cocoa[2].

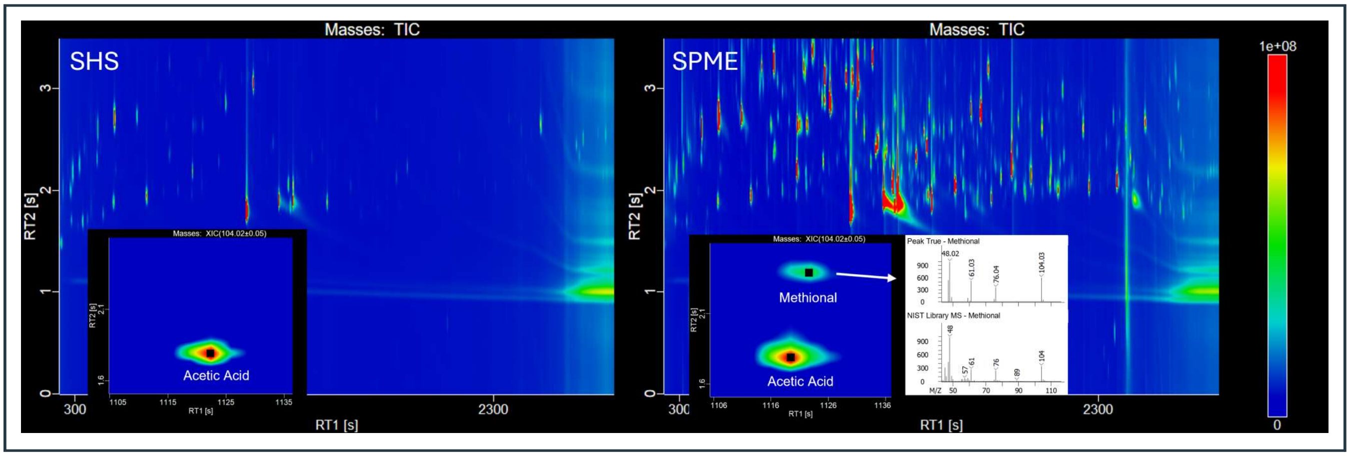


Figure 1: GCxGC-contour plots obtained from SHS (left) and HS-SPME (right) on the cocoa liquor sample from Ecuador. The same color scale was applied for both chromatograms. The zoomed-in area displays the XIC 104.02 visualizing the detection of methional (CAS: 3268-49-3) only with HS-SPME. The spectral plot shows the deconvoluted mass spectrum on the top and the NIST library match on the bottom.

To compare the HS-SPME data for all the samples, processing was conducted in *ChromaTOF* Sync 2D allowing for a streamlined data processing workflow including peak deconvolution, retention index (RI) calculation, matched identification, and alignment of the sample set data. Figure 2 shows the workspace within *ChromaTOF* Sync 2D. After processing, visualization options such as side-by-side profiles or bar charts provide easy access to data exploration.

In Figure, 3 the curated aligned results table (ART) exemplifies some of the representative compounds that were detected. On the right side of the ART, a heat map visualizes the relative abundance of the compounds in the samples. Columns 1-3 correspond to the samples from Ecuador, columns 4-6 display the samples from Ivory Coast, and columns 7-9 show the samples from Dominican Republic. This part of the ART can be used to easily filter and find compounds which are either elevated or present at low levels in one of the tested samples. Red indicates high levels and blue indicates low levels. Aroma related information, such as threshold levels for human perception and odor descriptions, were researched via the Leibniz-LSB@TUM Odorant Database[4] and added to the table.

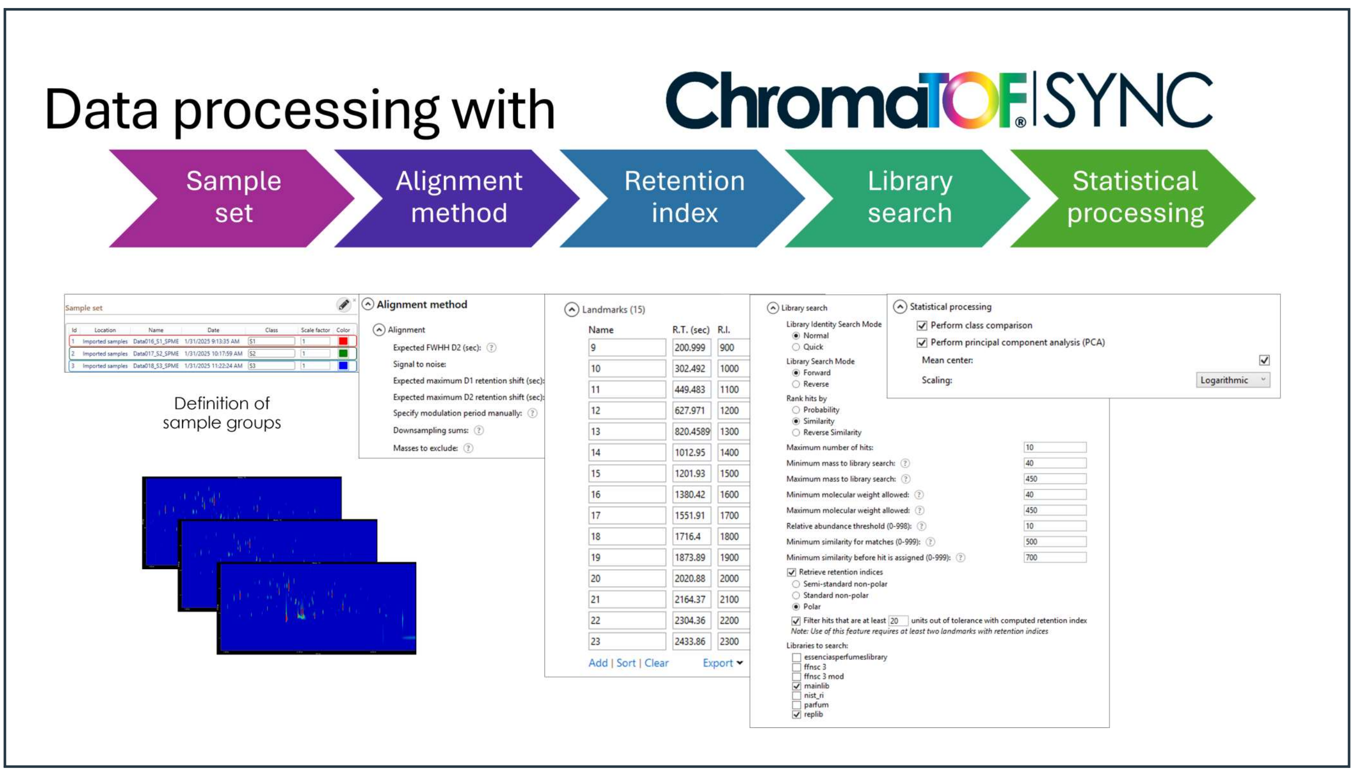
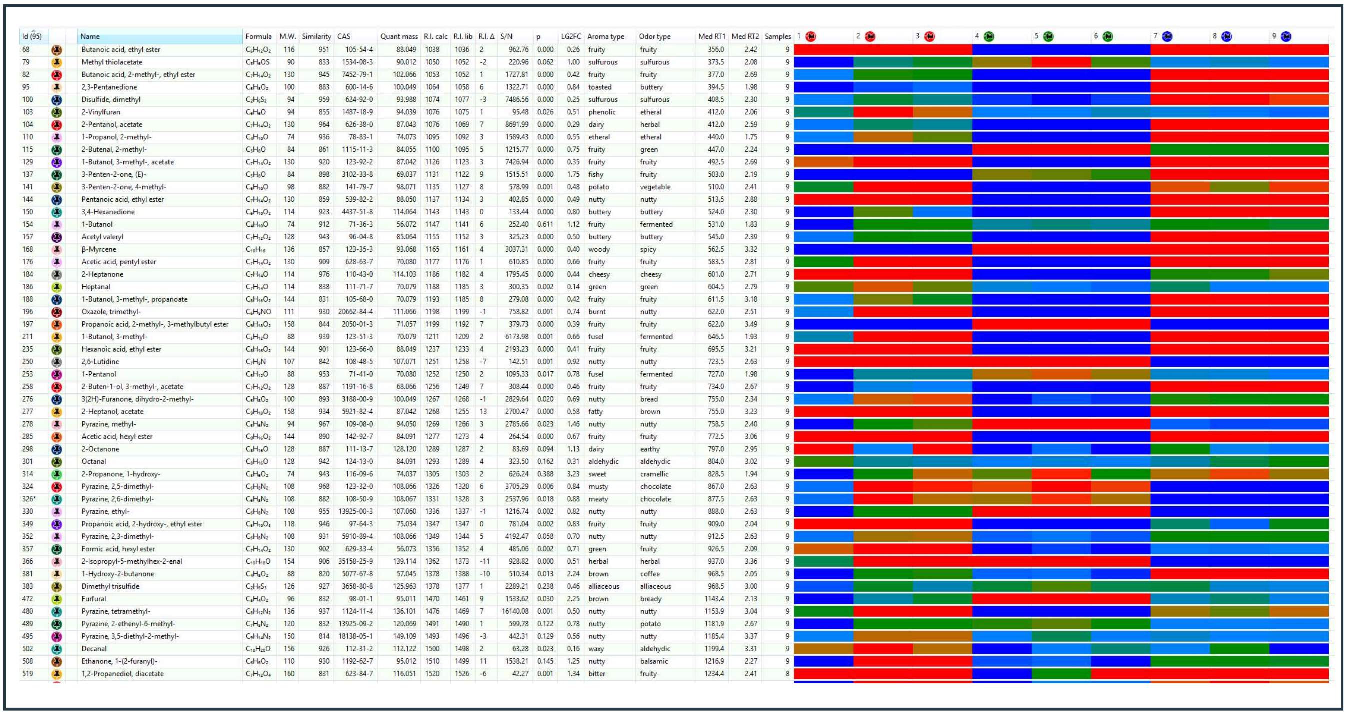


Figure 2: Workflow for data processing in ChromaTOF Sync 2D.



Id (95)	Name	Formula	M.W.	Similarity	CAS	Quant mass	R.I. calc	R.I. lib	R.I. Δ	S/N	p	LG2FC	Aroma type	Odor type	Med RT1	Med RT2	Samples
68	Butanoic acid, ethyl ester	$C_6H_{12}O_2$	116	951	105-54-4	88.049	1038	1036	2	962.76	0.000	0.26	fruity	fruity	356.0	2.42	9
79	Methyl thiolacetate	C_3H_6OS	90	833	1534-08-3	90.012	1050	1052	-2	220.96	0.062	1.00	sulfurous	sulfurous	373.5	2.08	9
82	Butanoic acid, 2-methyl-, ethyl ester	$C_7H_{14}O_2$	130	945	7452-79-1	102.066	1053	1052	1	1727.81	0.000	0.42	fruity	fruity	377.0	2.69	9
95	2,3-Pentanedione	$C_5H_8O_2$	100	883	600-14-6	100.049	1064	1058	6	1322.71	0.000	0.84	toasted	buttery	394.5	1.98	9
100	Disulfide, dimethyl	$C_2H_6S_2$	94	959	624-92-0	93.988	1074	1077	-3	7486.56	0.000	0.25	sulfurous	sulfurous	408.5	2.30	9
103	2-Vinylfuran	C_6H_6O	94	855	1487-18-9	94.039	1076	1075	1	95.48	0.026	0.51	phenolic	etheral	412.0	2.06	9
104	2-Pentanol, acetate	$C_7H_{14}O_2$	130	964	626-38-0	87.043	1076	1069	7	8691.99	0.000	0.29	dairy	herbal	412.0	2.59	9
110	1-Propanol, 2-methyl-	$C_4H_{10}O$	74	936	78-83-1	74.073	1095	1092	3	1589.43	0.000	0.55	etheral	etheral	440.0	1.75	9
115	2-Butenal, 2-methyl-	C_5H_8O	84	861	1115-11-3	84.055	1100	1095	5	1215.77	0.000	0.75	fruity	green	447.0	2.24	9
129	1-Butanol, 3-methyl-, acetate	$C_7H_{14}O_2$	130	920	123-92-2	87.042	1126	1123	3	7426.94	0.000	0.35	fruity	fruity	492.5	2.69	9
137	3-Penten-2-one, (E)-	C_5H_8O	84	898	3102-33-8	69.037	1131	1122	9	1515.51	0.000	1.75	fishy	fruity	503.0	2.19	9
141	3-Penten-2-one, 4-methyl-	$C_6H_{10}O$	98	882	141-79-7	98.071	1135	1127	8	578.99	0.001	0.48	potato	vegetable	510.0	2.41	9
144	Pentanoic acid, ethyl ester	$C_7H_{14}O_2$	130	859	539-82-2	88.050	1137	1134	3	402.85	0.000	0.49	nutty	nutty	513.5	2.88	9
150	3,4-Hexanedione	$C_6H_{10}O_2$	114	923	4437-51-8	114.064	1143	1143	0	133.44	0.000	0.80	buttery	buttery	524.0	2.30	9
154	1-Butanol	$C_4H_{10}O$	74	912	71-36-3	56.072	1147	1141	6	252.40	0.611	1.12	fruity	fermented	531.0	1.83	9
157	Acetyl valeryl	$C_7H_{12}O_2$	128	943	96-04-8	85.064	1155	1152	3	325.23	0.000	0.50	buttery	buttery	545.0	2.39	9
168	β-Myrcene	$C_{10}H_{16}$	136	857	123-35-3	93.068	1165	1161	4	3037.31	0.000	0.40	woody	spicy	562.5	3.32	9
176	Acetic acid, pentyl ester	$C_7H_{14}O_2$	130	909	628-63-7	70.080	1177	1176	1	610.85	0.000	0.66	fruity	fruity	583.5	2.81	9
184	2-Heptanone	$C_7H_{14}O$	114	976	110-43-0	114.103	1186	1182	4	1795.45	0.000	0.44	cheesy	cheesy	601.0	2.71	9
186	Heptanal	$C_7H_{14}O$	114	838	111-71-7	70.079	1188	1185	3	300.35	0.002	0.14	green	green	604.5	2.79	9
188	1-Butanol, 3-methyl-, propanoate	$C_8H_{16}O_2$	144	831	105-68-0	70.079	1193	1185	8	279.08	0.000	0.42	fruity	fruity	611.5	3.18	9
196	Oxazole, trimethyl-	C_6H_9NO	111	930	20662-84-4	111.066	1198	1199	-1	758.82	0.001	0.74	burnt	nutty	622.0	2.51	9
197	Propanoic acid, 2-methyl-, 3-methylbutyl ester	$C_9H_{18}O_2$	158	844	2050-01-3	71.057	1199	1192	7	379.73	0.000	0.39	fruity	fruity	622.0	3.49	9
211	1-Butanol, 3-methyl-	$C_5H_{12}O$	88	939	123-51-3	70.079	1211	1209	2	6173.98	0.001	0.66	fusel	fermented	646.5	1.93	9
235	Hexanoic acid, ethyl ester	$C_8H_{16}O_2$	144	901	123-66-0	88.049	1237	1233	4	2193.23	0.000	0.41	fruity	fruity	695.5	3.21	9
250	2,6-Lutidine	C_7H_9N	107	842	108-48-5	107.071	1251	1258	-7	142.51	0.001	0.92	nutty	nutty	723.5	2.63	9
253	1-Pentanol	$C_5H_{12}O$	88	953	71-41-0	70.080	1252	1250	2	1095.33	0.017	0.78	fusel	fermented	727.0	1.98	9
258	2-Buten-1-ol, 3-methyl-, acetate	$C_7H_{12}O_2$	128	887	1191-16-8	68.066	1256	1249	7	308.44	0.000	0.46	fruity	fruity	734.0	2.67	9
276	3(2H)-Furanone, dihydro-2-methyl-	$C_5H_8O_2$	100	893	3188-00-9	100.049	1267	1268	-1	2829.64	0.020	0.69	nutty	bread	755.0	2.34	9
277	2-Heptanol, acetate	$C_9H_{18}O_2$	158	934	5921-82-4	87.042	1268	1255	13	2700.47	0.000	0.58	fatty	brown	755.0	3.23	9
278	Pyrazine, methyl-	$C_5H_6N_2$	94	967	109-08-0	94.050	1269	1266	3	2785.66	0.023	1.46	nutty	nutty	758.5	2.40	9
285	Acetic acid, hexyl ester	$C_8H_{16}O_2$	144	890	142-92-7	84.091	1277	1273	4	264.54	0.000	0.67	fruity	fruity	772.5	3.06	9
298	2-Octanone	$C_8H_{16}O$	128	887	111-13-7	128.120	1289	1287	2	83.69	0.094	1.13	dairy	earthy	797.0	2.95	9
301	Octanal	$C_8H_{16}O$	128	942	124-13-0	84.091	1293	1289	4	323.50	0.162	0.31	aldehydic	aldehydic	804.0	3.02	9
314	2-Propanone, 1-hydroxy-	$C_3H_6O_2$	74	943	116-09-6	74.037	1305	1303	2	626.24	0.388	3.23	sweet	cramellic	828.5	1.94	9
324	Pyrazine, 2,5-dimethyl-	$C_6H_8N_2$	108	968	123-32-0	108.066	1326	1320	6	3705.29	0.006	0.84	musty	chocolate	867.0	2.63	9
326*	Pyrazine, 2,6-dimethyl-	$C_6H_8N_2$	108	882	108-50-9	108.067	1331	1328	3	2537.96	0.018	0.88	meaty	chocolate	877.5	2.63	9
330	Pyrazine, ethyl-	$C_6H_8N_2$	108	955	13925-00-3	107.060	1336	1337	-1	1216.74	0.002	0.82	nutty	nutty	888.0	2.63	9
349	Propanoic acid, 2-hydroxy-, ethyl ester	$C_5H_{10}O_3$	118	946	97-64-3	75.034	1347	1347	0	781.04	0.002	0.83	fruity	fruity	909.0	2.04	9
352	Pyrazine, 2,3-dimethyl-	$C_6H_8N_2$	108	931	5910-89-4	108.066	1349	1344	5	4192.47	0.058	0.70	nutty	nutty	912.5	2.63	9
357	Formic acid, hexyl ester	$C_7H_{14}O_2$	130	902	629-33-4	56.073	1356	1352	4	485.06	0.002	0.71	green	fruity	926.5	2.09	9
366	2-Isopropyl-5-methylhex-2-enal	$C_{10}H_{18}O$	154	906	35158-25-9	139.114	1362	1373	-11	928.82	0.000	0.51	herbal	herbal	937.0	3.36	9
381	1-Hydroxy-2-butanone	$C_4H_8O_2$	88	820	5077-67-8	57.045	1378	1388	-10	510.34	0.013	2.24	brown	coffee	968.5	2.05	9
383	Dimethyl trisulfide	$C_2H_6S_3$	126	927	3658-80-8	125.963	1378	1377	1	2289.21	0.238	0.46	alliaceous	alliaceous	968.5	3.00	9
472	Furfural	$C_5H_4O_2$	96	832	98-01-1	95.011	1470	1461	9	1533.62	0.030	2.25	brown	bready	1143.4	2.13	9
480	Pyrazine, tetramethyl-	$C_8H_{12}N_2$	136	937	1124-11-4	136.101	1476	1469	7	16140.08	0.001	0.50	nutty	nutty	1153.9	3.04	9
489	Pyrazine, 2-ethenyl-6-methyl-	$C_7H_8N_2$	120	832	13925-09-2	120.069	1491	1490	1	599.78	0.122	0.78	nutty	potato	1181.9	2.67	9
495	Pyrazine, 3,5-diethyl-2-methyl-	$C_9H_{14}N_2$	150	814	18138-05-1	149.109	1493	1496	-3	442.31	0.129	0.56	nutty	nutty	1185.4	3.37	9
502	Decanal	$C_{10}H_{20}O$	156	926	112-31-2	112.122	1500	1498	2	63.28	0.023	0.16	waxy	aldehydic	1199.4	3.31	9
508	Ethanone, 1-(2-furanyl)-	$C_6H_6O_2$	110	930	1192-62-7	95.012	1510	1499	11	1538.21	0.145	1.25	nutty	balsamic	1216.9	2.27	9
519	1,2-Propanediol, diacetate	$C_7H_{12}O_4$	160	831	623-84-7	116.051	1520	1526	-6	42.27	0.001	1.34	bitter	fruity	1234.4	2.41	8

Figure 3: Peak identification metrics including similarity score and RI information are tabulated in the ART, and relative trends are displayed via a heat map where the color scale indicates the relative concentration for representative analytes from selected cocoa volatiles.

Individual analytes can be explored further, as shown in Figure 4. For example, an important cocoa-aroma compound is 2,3,5-trimethyl-6-ethylpyrazine (CAS: 17398-16-2), which was detected in all three cocoa samples. Figure 4 illustrates some of the identification parameters such as the similarity score against the NIST libraries (here 944/1000) and RI matching, here with a RI_{Δ} (retention index delta) of 8. The relative peak areas of 2,3,5-trimethyl-6-ethylpyrazine are shown on the right side with elevated levels for the samples from Ecuador and the Dominican Republic—both considered to be fine cocoa producers, and lower levels in the samples from Ivory Coast (bulk cocoa). With its intense earthy smell, as well as vegetal and roasted notes, 2,3,5-trimethyl-6-ethylpyrazine is considered a main odorant of cocoa[2].

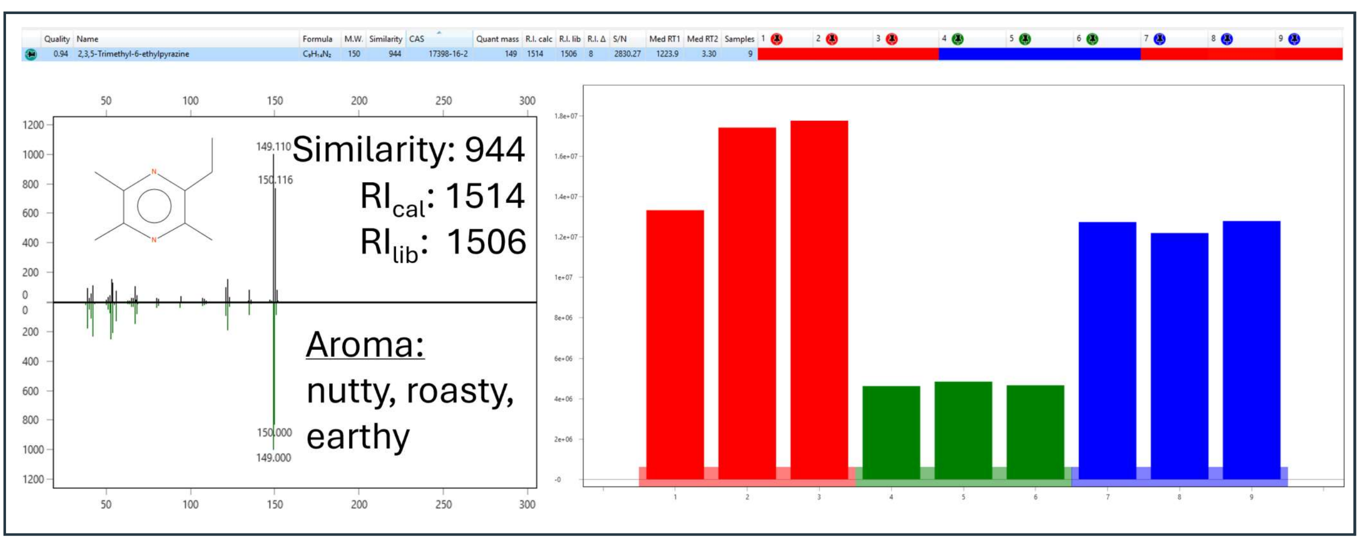


Figure 4: Relative trend of 2,3,5-trimethyl-6-ethylpyrazine (CAS: 17398-16-2) identified through mass spectral comparison and supported by RI matching.

Another pyrazine, trimethylpyrazine (CAS: 14667-55-1), was also detected and is displayed in Figure 5. It was identified with a similarity score of 926/1000 and a RI_{Δ} = 4 showing highest values for the Ecuadorian cocoa. Trimethlypyrazine has earthy and roasty aroma notes[4], therefore it is often added in chocolate flavorings.

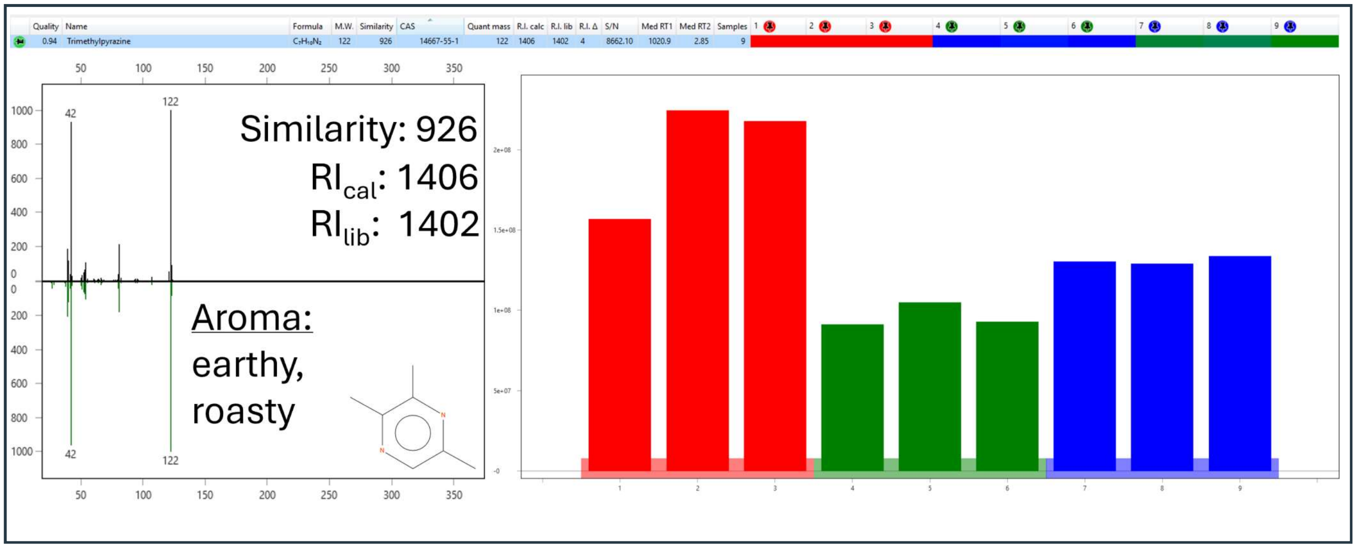


Figure 5: Relative trend of trimethylpyrazine (CAS: 14667-55-1) identified through mass spectral comparison and supported by RI matching.

Another interesting analyte is ethyl-phenylacetate (CAS: 101-97-3), shown in Figure 6. A good similarity score of 952/1000 and a RI matching with a RI_{Δ} of 4 provide high confidence in the identification. The bar chart on the right indicates that samples from the Dominican Republic show highest abundance, followed by the cocoa from Ecuador. On the contrary, Ivory Coast presents only low abundance of ethyl-phenylacetate, which is a key odorant with notes of dried fruit and flowers and is considered a potential marker for fine cocoa[5].

Figure 6: Relative trend of ethyl-phenylacetate (CAS: 101-97-3) identified through mass spectral comparison and supported by RI matching.

Dimethyl trisulfide (CAS: 3658-80-8), commonly found in a wide variety of food products, has been identified with a similarity score of 940/1000 and supported with a RI_{Δ} of 1 (see Figure 7). It is perceived with a sulfury aroma note like cooked cabbage[4], and it is known to be an important contributor to the cocoa aroma, especially for dark chocolate. The bar plot on the right side in Figure 7 shows a relatively equal distribution for all tested samples.

Figure 7: Relative trend of dimethyl trisulfide (CAS: 3658-80-8) identified through mass spectral comparison and supported by RI matching.

Conclusion

The determination of volatile compounds is essential for the chemical characterization of cocoa liquor and to investigate the correlation to organoleptic profiles and geographical origin. The volatile profile obtained by HS-SPME and GCxGC-TOFMS allowed for the determination of a large number of chemical compounds, present at both high and low concentrations. Key aroma compounds and major differences, as well as varying ratios depending on the origin of the raw material, could be easily determined by this approach. It was possible to identify differences between the samples of different origin using *ChromaTOF* Sync 2D.

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