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Fingerprint analysis of tea leaves by HS-SPME-GC×GC-QTOF.

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Introduction

Comprehensive two-dimensional gas chromatography (GC×GC) provides very high resolution power and unmatched peak capacity. In addition, the 2D chromatograms are highly structured and allow linking the compound position in the 2D space to properties such as volatility and polarity. As a consequence, 2D patterns are very informative and specific and can be exploited for comparison and classification of complex samples. Here we show the use HS-SPME-GC×GC-QTOF for the fingerprinting of Earl Grey tea leaves from different commercial brands.

Experimental details

- Head-space SPME sampling of Earl Grey tea leaves from 7 different commercial brands.
- Agilent 7890A GC with a cryogen-free Zoex ZX2 thermal modulator and an Agilent 7200B QTOF detector.
- Data display and processing with the GC Image software.

Results and discussion

All samples generate very complex chromatographic profiles characterized by a large amount of compounds (Fig. 1). Many components are present at a very low level and would not be efficiently separated in one dimension by standard GC, leading to a significant loss of information. On the other hand, the 2D counterplots are very detailed. The 2D blob patterns show several hundred compounds as well as clear similarities/differences between the samples.

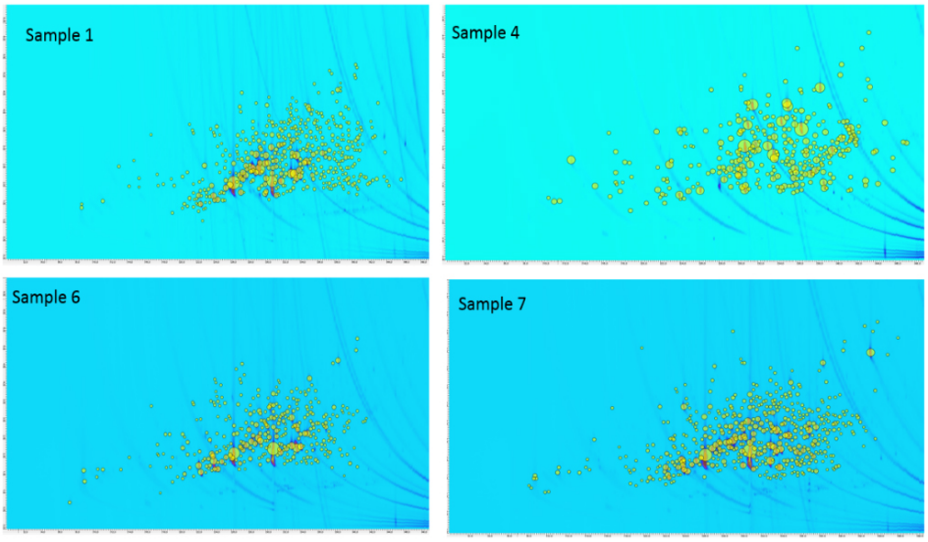

Fig. 1 – Examples of 2D chromatograms obtained for the different tea samples.

Degree of similarity

The QTOF allows two different acquisition modes:

- High Resolution (HR): maximum instrumental the resolution (≥ 13,000 FWHM), preferable at trace level.
- Extended Dynamic Range (EDR): lower resolution (6,500 FWHM) but less susceptible to saturation.

Hereby we used the HR mode. The integration settings aimed at producing very detailed patterns and keeping into account also minor components. We detected 245 to 473 blobs per sample.

Individual blob templates were created for each sample and applied to all the others for pair-wise comparison. Peak matching was based on retention times and MS spectrum. Some sample show high similarity while others are clearly very different (Table 1). Some compounds (e.g. 2-hexenal and benzyl alcohol) are found in all samples while others (e.g. benzyl benzoate or cinammaldehyde) are specific for a certain sample.

Table 1 – Degree of similarity calculated as template matching (%).

	Template Matching (%)						
	template 1	template 2	template 3	template 4	template 5	template 6	template 7
sample 1		52.7	66.3	62.5	74.6	68.0	59.8
sample 2	41.9		50.1	54.1	60.1	49.4	38.7
sample 3	53.5	58.0		58.0	75.6	67.7	54.1
sample 4	24.8	60.8	39.8		61.4	40.7	28.8
sample 5	54.6	59.6	67.4	62.2		64.3	50.1
sample 6	50.9	52.2	63.8	55.1	67.3		54.8
sample 7	65.7	55.9	75.8	66.1	74.6	79.2	

Comprehensive fingerprinting

In a different approach we built a global template by adding cumulatively the blobs of all samples. The integration threshold was set in order to make the blob amount manageable (about 200 blobs in total). The measurements were performed with the QTOF in HR and EDR acquisition mode, respectively.

The matching percent of the comprehensive template for each sample provides an assessment of the samples complexity. Fig. 2 summarized the results obtained. As can be seen, the two acquisition modes are consistent. The matching ranges from 20% to 60%, indicating clearly the very significantly different nature of the tea leaves under exam. These very significant differences can be expressed in a more relevant way in terms of detection/absence (Table 2) or relative abundance (Fig. 3) of significant aroma-key compounds.

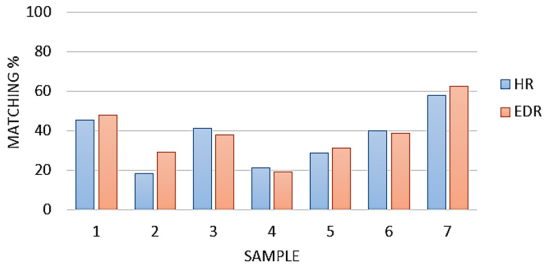

Fig. 2 – HR and EDR matching results of the comprehensive template.

Table 2 – Examples of matched/unmatched (+/-) compounds.

Compound	Sample						
	1	2	3	4	5	6	7
Longifolene	+	-	+	-	-	-	+
Citronellal	+	+	+	-	+	-	-
Linalool	+	-	-	-	-	-	+
Estragole	-	+	-	+	+	-	-
2-Hexenal	-	+	-	-	-	-	-
Caryophyllene	-	+	+	-	-	+	+
Geranyl isovalerate	+	+	+	-	+	+	+
Decanal	+	-	-	+	-	-	+

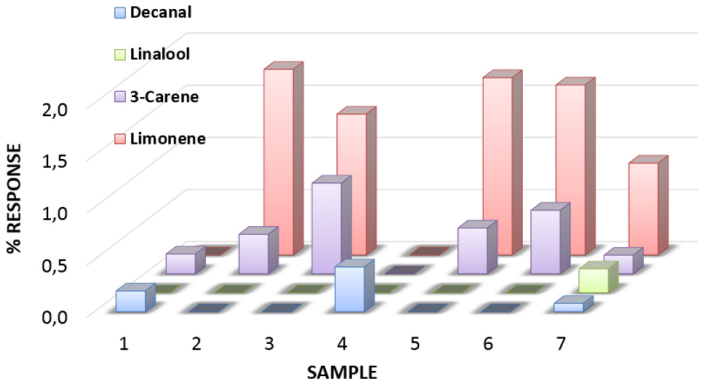

Fig. 3 – Examples of compounds response in the different samples.

Accurate mass

The accurate masses can be used to calculate the most likely formula for any MS fragment. The formula suggested can be compared to that expected for structure of the library match. This process can be applied to several fragments. If the errors obtained are very small for all fragments, identification confidence is greatly improved (Table 3).

Table 3 – Identity confirmation by multi-fragment mass accuracy evaluation.

Compound	Fragment (m/z)	Library match formula	Exact mass	Measured mass	Suggested formula	Error (ppm)
Citral	152	C10H16O+ (M+)	152.1196	152,1195	C10H16O+	0.35
	123	C9H15+	123.1168	123.1171	C9H15+	2.46
	119	C9H15+	119.0855	119.0855	C9H15+	0.02
3-Carene	136	C10H16+ (M+)	136.1246	136.1244	C10H16+	1.93
	121	C9H13+	121.1012	121.1010	C9H13+	1.47
	105	C8H9+	105.0699	105.0696	C8H9+	2.52
Longifolene	204	C15H24+ (M+)	204.1873	204.1862	C15H24+	5.33
	189	C14H21+	189.1638	189.1638	C14H21+	0.04
	161	C12H7+	161.1325	161.1324	C12H7+	0.46

Conclusions

- HS-SPME-GC×GC-QTOF allows detailed characterization of volatiles profiles of complex food matrices such as tea.
- Many minor potentially significant compounds are successfully separated from the highly complex matrix.
- The detailed and specific two-dimensional separation patterns are ideal for fingerprint analysis.
- The presence or absence of specific markers or aroma-key compounds can be used to differentiate or associate samples, e.g. in terms of geographical origin, treatment or sophistication.
- The high mass accuracy provided by the QTOF is very useful for identity confirmation and identification of unknowns.

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