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Unraveling the cannabis plant metabolome: A novel analytical framework for the comprehensive chemical profiling of cannabis utilizing GC-HRMS analysis

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A novel analytical framework including GC-HRMS was used for chemical profiling of 35 CBD-type cannabis varieties. This allowed the differentiation of cannabis varieties and growing conditions. These findings highlight the possibilities of not only looking at the main cannabinoids but also analyzing the whole cannabis metabolome.

Introduction

The analysis of cannabis plant samples usually focuses on major cannabinoids such as Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD). With the increasing complexity of the cannabis market and the availability of products with various legal classifications, more sophisticated analytical approaches are needed. By examining the complete metabolome beyond just major cannabinoids, the aim was to differentiate cannabis varieties and growing conditions. This approach may offer new possibilities for pharmaceutical quality control and forensic applications.

Methods

Sample preparation and analysis

Three flower clusters from 35 CBD-type cannabis strains each, grown under greenhouse (GH), outdoor (OD) or indoor (ID) conditions, were blended into a uniform mixture.

Samples
- 3 flowers per variety blended
- 3 x 100 mg sample per variety

Extraction
- 5 ml ethanol
- Ultrasonication
- Dilution 1:50 with methanol

GC
- Thermo TRACE 1300 GC system
- TraceGOLD TG-5SilMS capillary column
- Inj. vol 1 µl, temp. 50°C to 320°C (12 °C/min)

MS
- Thermo Q Exactive GC Orbitrap MS
- EI+ mode, m/z 40-500

Fig.1: Sample preparation and analysis

Negative controls, pool samples and pool dilution samples were prepared. The samples were analyzed in randomized order. Every 6th sample, a pool sample was analyzed.

Data processing

For data preprocessing steps, statistical analysis, and compound identification see Fig. 2.

Peak picking and alignment
- MS-DIAL v. 4.9.221218
- Parameters in Table 1
- Gap filling
- Export of peak areas

Filtering
- RSD within pool samples <25%
- Pearson correlation in pool dilution samples should be >0.7
- RSD within real samples >20% than within pool samples.

Normalization
- MetaboAnalyst 6.0
- Weight correction
- Probabilistic quotient normalization (PQN) using pool samples as reference

Scaling
- MetaboAnalyst 6.0
- Log transformation
- Pareto scaling

Statistical analysis
- MetaboAnalyst 6.0
- Principle component analysis (PCA)
- PLS-DA

Identification
- Reference standards
- NIST library
- MS-DIAL library with Kovats RI

Fig.2: Data processing

Table 1 MS-DIAL processing parameters

mass range	40-500 Da
minimum peak height	2000 cps
mass slice width	0.01 Da
mass accuracy for centroiding	0.025 Da
smoothing (lin. weighted moving av.)	3
sigma window value	0.5
average peak width	10 scans
retention time tolerance	0.05 min
EI similarity tolerance	70%

Results

A total of 113 features (representing mainly terpenes and cannabinoids, Fig. 3) remained for statistical analysis after filtering. PCA and PLS-DA allowed distinct grouping of the varieties (Fig. 4) and the growing conditions (Fig. 5). The variable importance in the projection (VIP) approach revealed that mainly terpene molecules, rather than cannabinoids, had the greatest discriminating power between varieties or growing conditions. Level 1 identification according to the Metabolomics Standard Initiative (MSI) was achieved for example, for the terpenes β-ocimene, γ-terpinene, caryophyllene oxide, α-bisabolol, and the alkanes pentacosane and heptacosane (Table 2).

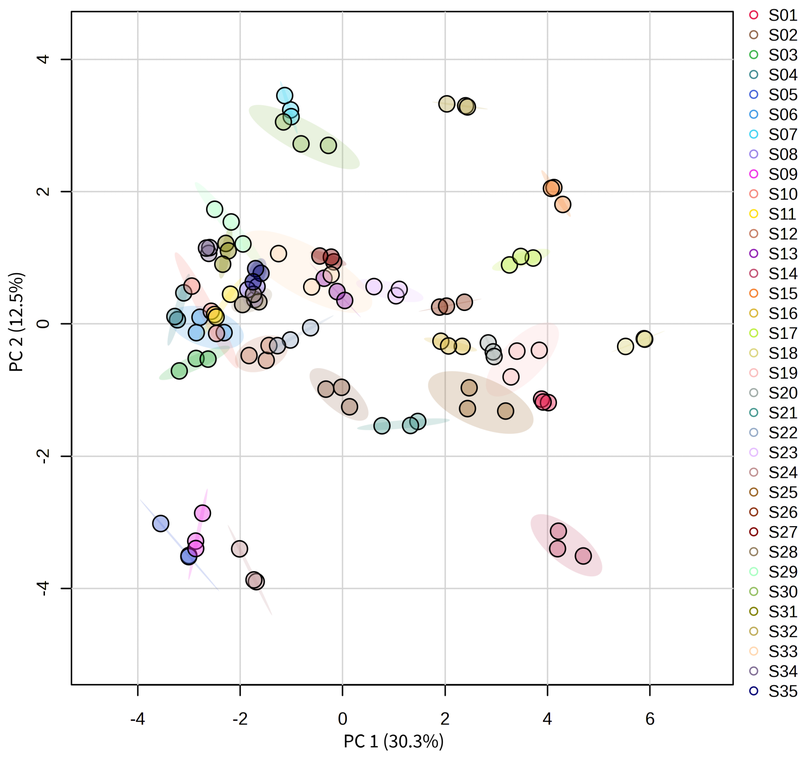

Fig.4: PCA plot of 35 CBD-type cannabis varieties S01-S35

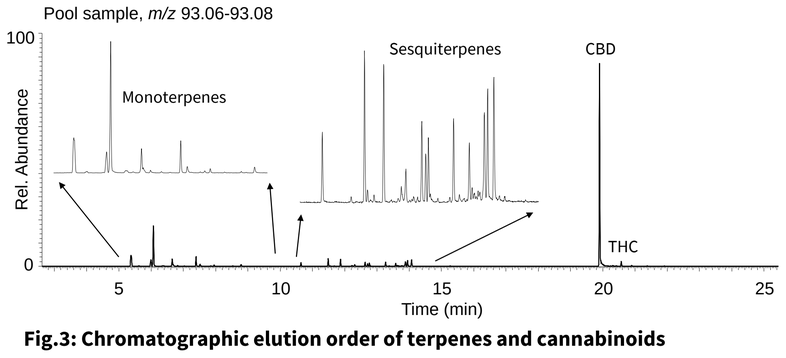

Fig.3: Chromatographic elution order of terpenes and cannabinoids

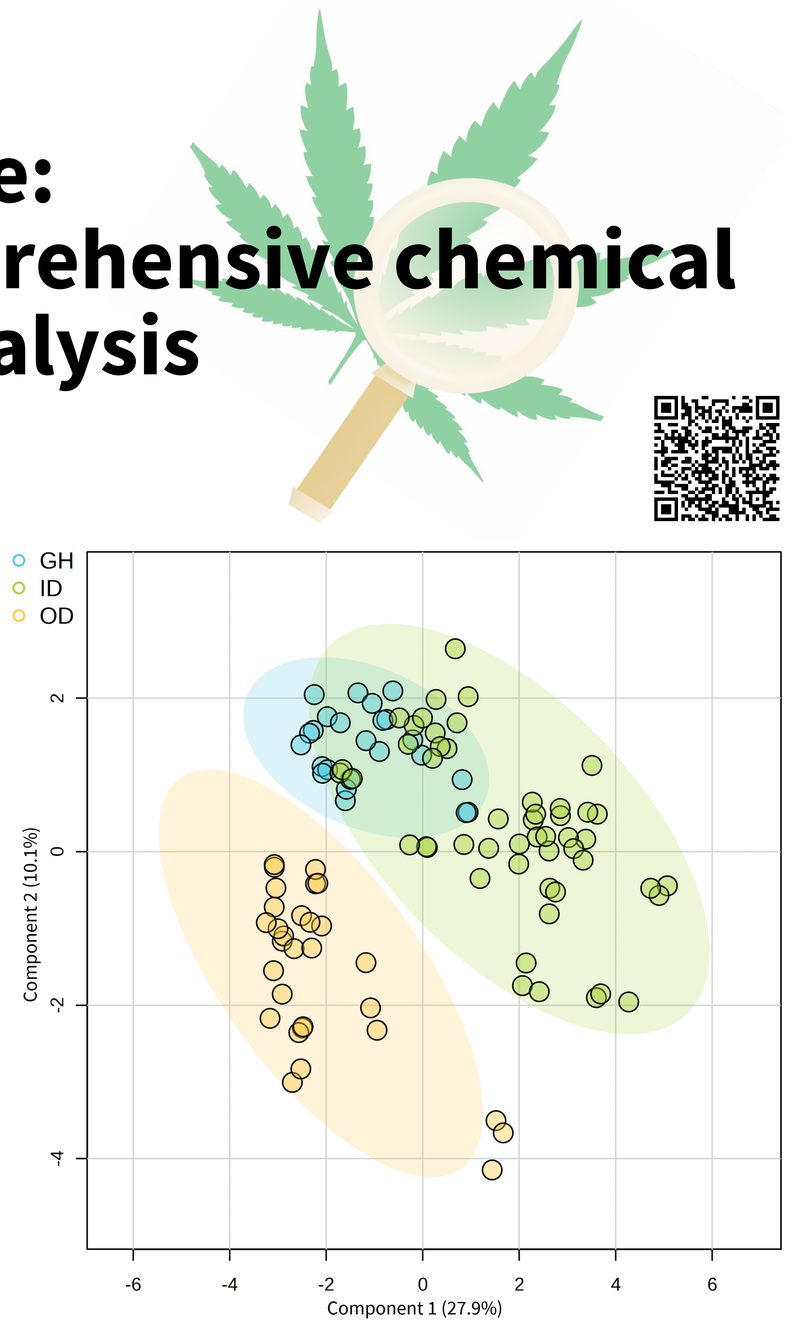

Fig.5: PLS-DA plot of the growing conditions greenhouse (GH), indoor (ID) and outdoor (OD)

Table 2 Important features in PLS-DA and level of identification acc. to MSI

RT [min]	m/z [Da]	Identification	Level
6.50	93.0700	3-carene	1
6.83	91.0543	beta-ocimene	1
7.03	91.0543	gamma-terpinene	1
7.39	91.0543	terpinolene	1
8.13	79.0543	not identified	
8.59	69.0336	monoterpene	3
8.63	111.0800	monoterpene	3
9.12	119.0856	not identified	
12.39	139.1118	sesquicineole?	2
13.19	91.0543	caryophyllene oxide	1
13.46	96.0570	not identified	
13.71	111.0805	sesquiterpene	3
14.02	111.0805	sesquiterpene	3
14.07	119.0856	alpha-bisabolol	1
15.38	71.0500	not identified	
15.39	58.0414	not identified	
19.18	189.091	cannabinoid	3
20.34	57.0699	pentacosane	1
21.61	57.0699	heptacosane	1

Discussion

The analysis of the cannabis metabolome by GC-HRMS enabled the classification of cannabis varieties beyond the principal phenotypes and may serve as a tool to explore e.g. the influences of growing conditions on the plant phenotype, batch-to-batch differences for pharmaceutical quality control, or the origin of cannabis samples for forensic purposes.

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