Characterization of chemotype-dependent terpenoids profile in cannabis by headspace gas-chromatography coupled to time-of-flight mass spectrometry

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Abstract

A headspace method called full evaporation technique (FET) coupled to capillary gas chromatography with a mass detector operating in time-of-flight mode (HS-GC/MS-TOF) was developed to characterize the volatile components, especially the terpene fraction in *Cannabis sativa L.* inflorescences. This analytical approach allows to reach a high equilibration temperature, able to obtain a complete quantification of the volatile components, providing simple sample preparation, specific qualitative detection, high sensitivity, precise and accurate quantitative determination. The method was applied to 20 cannabis THC-dominant (I) and 13 CBD-dominant (III) chemotypes. The obtained results were then compared with a series of standard solutions consisting of 35 terpenoids and the mass spectra present in a computer library (NIST). The method has an accuracy of more than 90% and a limit of detection of 5 ppm for all analytes. The main terpenoids in cannabis are namely (% Chemotypes III vs. I of the total terpene content): β-Caryophyllene (25 vs. 19.3), β-Mircene (18.2 vs. 20.0), Terpinolene (12.1 vs. 7.0), α-Humulene (6.5 vs. 8.5), D-Limonene (6.2 vs. 7.2), α-Pinene (5.8 vs. 4.9), β-Pinene (5.0 vs. 5.8) and cis-β-Ocimene (4.3 vs. 5.2), whose presence is confirmed in both plant chemotypes and account for more than 80% of the total terpenoids amount. The terpenoids which can clearly distinguish the chemotype are α-Terpineol, linalool, DL-menthol, α-Cedrene, and Borneol. This application provides important data on the secondary volatile components of the plant, which may be useful for a better understanding of the therapeutic properties of the cannabis phyto-complex. It gives the possibility of establishing the aroma profile of different Cannabis batches, allowing possible similarities between samples and identifying any artificial adulteration such as hexyl butyrate ester and it provides the opportunity to highlight some target compounds characteristic of the different chemotypes.

Keywords: *Cannabis sativa*; Terpenes; Headspace gas-chromatography; Medicinal plant; Phytochemistry;

1. Introduction

The history of cannabis, the common name for *Cannabis Sativa L*, heads back to thousands of years ago. Though this name evokes in most people drug abuse and trafficking, cannabis stem has been used for ages as a source of fibers to produce textiles, ropes and construction materials, and its inflorescences have been employed as a medicine in many countries. As a matter of fact, its recreational use developed way after, especially in the cannabis indigenous areas (i.e., India and China). During the past century, cannabis production has been undermined by many western governments as a reaction to its widespread illicit use, which led to the concealment of this plant features. Nonetheless, cannabis trafficking carried on and intensified, despite the synthesis of many synthetic analogues [1–4]. In recent years only, cannabis pharmacological benefits were reassessed, and its curative properties are now exploited for the symptomatic treatment of several diseases (anorexia, AIDS, Multiple Sclerosis, chemotherapy-induced nausea) [5–7]. The most abundant and known components of cannabis are delta-9 tetrahydrocannabinol (THC) and cannabidiol (CBD). At least 85 more compounds exist in a typical C21 terpene phenolic skeleton in addition to these two: the *cannabinoids*. Cannabinoids are located in almost every part of the plant. However, they are more concentrated in the inflorescences, and almost all of them interact with receptors located in the central and peripheral nervous system (CB1 and CB2). THC is well known for its psychoactive effect, it is an illegal substance prohibited in most countries and it is the most considered active principle, along with CBD, in pharmacological therapy with cannabis and cannabinoids [7–9].

Terpenes are hydrocarbon chains classified by the number of isoprene units (C5H8) they contain. Furthermore, the isoprene chain in terpenoids is modified by the adding of different functional groups, such as alcohols, ethers, ketones, aldehydes and/or esters. Cannabis contains roughly 140 terpenes and terpenoids, mostly monoterpenes and sesquiterpenes, which give the plant its characteristic scent. Many studies underline the importance of the whole phyto-complex in an effective treatment versus the administration of the isolated active principles THC/CBD. This effect is usually called the *entourage effect*: a synergetic dynamism among all chemical compounds found in the cannabis plant, which are mainly cannabinoids and ,secondly, terpenes/terpenoids [7,9–12].

This project aims to define the terpenes profile in *Cannabis sativa L.* developing a method providing a targeted qualitative and quantitative analysis. Even if the literature is filled in analytical methods applying GC/MS for the study of terpenes profile [13,14] , the peculiarity

of the method here proposed is the headspace variant called full evaporation technique (FET). The autosampler is used to reach high equilibration temperature, essentially as an evaporator. This way, we got a snapshot of the inflorescence, deprived of all the terpenes content, that allowed us to obtain a complete quantification of the volatile components at that specific moment.

As mentioned, there is a growing interest in the cannabis non-cannabinoid content, as every single compound could contribute to the full pharmacological benefit. Moreover, terpenes profiling could be interesting for both botanical researchers, to evaluate analogies and differences between cannabis varieties, and cannabis producers, because terpenes are the main responsible for the aromatic plant bouquet varieties, which leads to different appreciation by the consumers.

2. Materials and methods

2.1 Chemicals and reagents

The terpenes standard Cannabis Terpene Mix A (CRM40755, Supelco), Cannabis Terpene Mix B (CRM40937, Supelco), trans-beta-ocimene, cis-beta-ocimene, beta-myrcene were purchased from Sigma-Aldrich (St.Louis, MO, USA). The internal standards used were 4-Vinyl-1-cyclohexene (Sigma-Aldrich, St.Louis, MO, USA) and Linalool D5 (HPC Standards GmbH, Cunnersdorf, DE). Analytical grade solvents were acquired from Sigma-Aldrich (St.Louis, MO, USA). The IS mix was made by mixing in methanol 4-Vinyl-1-cyclohexene and D5-Linalool at the concentration of 2 mg/mL.

2.2 Sample preparation

Standard solutions (10 µL) are transferred to a 20 mL headspace vial with 10 µL of IS solution (4-Vinyl-1-cyclohexene and D5-Linalool at 2 mg/mL) and processed for the analysis as described below.

Cannabis inflorescences were selected among THC-dominant or chemotype I samples (n.20), confiscated by the judicial authority at the airport of Milano Malpensa in Northern Italy, and industrial hemp or chemotype III samples (n.13), mainly containing CBD. Raw cannabis inflorescences (100 mg) are transferred directly into a 20 mL headspace vial. Then, 10 µL of IS solution (4-Vinyl-1-cyclohexene and D5-Linalool at 2 mg/mL) and 10 µL of methanol were added. It should be noted that, in order to establish reproducible conditions, the volume of methanol in standard or Cannabis samples should always account for the same amount because the internal pressure generated by the vaporisation process must be comparable. The vial was sealed with a silicone rubber/Teflon cap, placed in the autosampler, and incubated for 15 min at 160°C to allow the full evaporation of terpenoids. All the samples were also screened for their cannabinoids concentration to confirm their chemotype, as already described [15,16].

2.3 FET-HS-GC/MS-TOF

The analyses were performed on a Dani Master HS-GC system, with a split-splitless injection system and a Dani Master Time-of-flight (TOF) Plus detector (Dani Instruments, Cologno Monzese, Italy) operated in electron ionization (EI) mode (70 eV). The GC was equipped with a DB-5MSUI (5% diphenyl/95% dimethyl polysiloxane, 20 m x 0.18 mm i.d.) capillary column (Agilent Technologies, Santa Clara, CA, USA). The GC/MS conditions: split ratio 40:1; solvent delay 3 s; injector temperature, 250°C; interface transfer line,

200°C; ion source, 200°C, volume loop: 2 mL; oven temperature program, initial 40°C, 5°C/min up to 200°C. Split liner: straight direct inject liner ID (mm) 1.5. Helium was used as the carrier gas at a flow rate of 0.5 mL/min. The MS detector was operated in full scan mode, acquiring ions from *m/z* 40 to 300, with a rate of 5 spectra/s. The total analysis time was 35 min.

2.4 Terpenoids identification and quantitative analysis

Identification of terpenes was performed by (1) comparison with a series of standard solutions consisting of 35 terpenoids and/or (2) matching mass spectra with NIST library (ver.2017). The quantitative data were determined by comparing the extracted base peak areas for each analyte, corrected for internal standards responses, against a calibration curve (Table 1). Quantitative data were expressed in absolute concentration (ppm) and calculated as a percentage of the total.

2.5 Analytical performances

The performances of the *FET-HS-GC/MS-TOF* method were studied taking into consideration limit of detection (LOD), limit of quantification (LOQ), precision and accuracy. LOQ was considered as the lowest concentration that met at least a signal-to-noise ratio >5 whereas LOD as the value that encounters a signal-to-noise ratio>3. Accuracy was expressed as the percent bias (% bias), while precision was measured as coefficient of variation (CV%). A CV% below 15% and % bias between 80 and 120% were considered suitable.

2.6 Statistical analysis

Descriptive statistics by t-test was investigated by using GraphPad Prism 7.0 (GraphPad Software, Inc, La Jolla, CA). In order to find out potential discriminating features between the cannabis chemotypes, multivariate analysis was performed using the software MetaboAnalyst 4.0. Data were checked for integrity, filtered by interquartile range, log-transformed (generalized log transformation), and auto-scaled. Partial Least Square Discriminant Analysis (PLS-DA) was used for considering all variables in the dataset simultaneously and the derived Variable Importance in Projection (VIP) scores, superior to >1.5, were used to disclose terpenoids with discriminatory power. Q2 was used to estimate the predictive ability of the PLS-DA model, and was calculated via 10-fold cross-validation. Significance was set to a p-value inferior to 0.05, and normally distributed data were presented as mean±SD.

3. Results and discussion

The proposed HS-GC/MS-TOF method allowed us to study the profile of the volatile components of different cannabis samples of both THC-dominant (chemotype I) and CBD-dominant (chemotype III) varieties. Our study focuses only on these two chemotypes (I vs III) since chemotype II is not acquirable in Italy by academic institutions for research purposes. The obtained results were then compared with a series of standard solutions consisting of 35 terpenoids and with the mass spectra present in a computer library (NIST ver. 2017). The calculation was performed using the internal standard method. The method has an accuracy of more than 90% and a limit of detection of 5 ppm for all analytes, which was reached by the use of FET (Supplementary Table 1). The use of FET, in our experience, outclassed either static and dynamic headspace or solid-phase microextraction (SPME). These alternatives were discharged since the pure standards do not provide trustable quantitative results, therefore they cannot be adequately analyzed. Essentially, in dynamic headspace the terpenoids standards chromatograms displayed inadequate peak-intensities (Supplementary Figure 1) whereas SPME showed a lack in linearity response, probably due to the interaction of the solvent with the fibre coating and the filling of the headspace trap (Supplementary Figure 2). Moreover, FET was able to fully volatilize all the terpenoids contained in the cannabis inflorescence. Some samples, previously processed with FET, showed no detectable terpenoids, except for some traces of sesquiterpenoids.

Analysis of the plant material revealed, in addition to the presence or the absence of the selected n.35 analytes, an elution region (between 20 and 27 min) particularly rich in sesquiterpenes, which could only be identified with the aid of the library. An example of separation and identification of unknown terpenoids in a cannabis sample is reported in Figure 1 and Supplementary Figure 3. Some unknown terpenoids comprise those reported in Figure 1 as (21) α-Guaiene, (23) Valencene, (24) γ-Selinene, (25) α-Bulsenene, (26) β-Panasinsene, (27) 7-epi-α-Selinene, (29) Guaiol, (31) 10-epi-γ-Eudesmol, and (33) α-Patchoulene. They are known to be minor sesquiterpenoids typical of cannabis species.

It should be noted that in some samples of cannabis light (chemotype III) the presence of exogenous molecules has also been detected, probably added by the producers with the aim of modifying the bouquet of the product. The dried inflorescences were coated with a mist of essential oils and food additives, which can give the product a particular scent that confers the commercial name under which the product itself is sold (i.e., Cannabis type

"pineapple" or "orange"). In this way, an example is the presence of hexyl butyrate ester, a compound with a fruity, pineapple-like scent (Rt 14.2*, m/z* 71, 43, 56, 84) in the sample reported in Figure 1.

In this preliminary study the terpenoids from n.20 samples of psychoactive cannabis (chemotype I) and n.13 cannabis light or industrial hemp (chemotype III) have been analyzed. In these samples, the 35 terpenes present in the calibration of the method were quantified. This type of comparison became necessary after the large variability in the content of volatile components was assessed. It was hypothesized that these differences were due to the natural variability existing between the different cultivars of the plant. These considerations and the need to carry out comparisons between different chemotypes or cultivars, in any case, led us also to evaluate the percentage distribution of terpenes in addition to their absolute content.

It is quite challenging to give an exhaustive list of the main terpenoids in cannabis since they are natural compounds susceptible to extreme variability: Firenzuoli *et al.* [17] reported n.41 main structures, n.29 were found in Valussi *et al.* [18]. We agree with them [17–20] that the main terpenoids in cannabis are namely (% Chemotypes III vs. I of the total terpene content): β-Caryophyllene (25 vs. 19.3), β-Mircene (18.2 vs. 20.0), Terpinolene (12.1 vs. 7.0), α-Humulene (6.5 vs. 8.5), D-Limonene (6.2 vs. 7.2), α-Pinene (5.8 vs. 4.9), β-Pinene (5.0 vs. 5.8) and cis-β-Ocimene (4.3 vs. 5.2), whose presence is confirmed in both plant chemotypes and account for more than 80% of the total terpenoids amount (Figure 2).

However, monoterpenes dictate the terpenoids composition [21] in all the analyzed samples (Figure 3A, Chemotypes III vs. I, 3769 ± 1855 vs. 3818 ±1486 ppm). Moreover, the plant is naturally richer in alkenes (5117 ± 2456 vs. 4560 ± 1537 ppm) and alcohols (733 ± 452 vs. 930 ± 316 ppm), whereas ketones, esters, and phenol, if present, are negligible quantities (Figure 3B). Linear (1484 ± 946 vs. 1849 ± 892 ppm) and monocyclic (1891 ± 1471 vs. 1603 ± 623 ppm) compounds are distributed similarly, while bicyclic structures (2493 ± 939 vs. 2070 ± 629 ppm) display slightly higher prevalence (Figure 3C).

The singles terpenoids concentrations can also be visualized among cannabis chemotypes by a heatmap in Supplementary Figure 4 or in Table 2, in which some compounds showed different behavior according to the plant chemotype. Multivariate analysis (PLS-DA, that was cross-validated with Q2=0.86) in Figure 4A shows a clear-cut separation of the chemotypes (in the first component, the separation account for 14%). Finally, we use the

VIP values from PLS-DA that describes a quantitative approximation of the discriminatory strength of each individual feature. The terpenoids which can clearly distinguish the chemotype ($p<0.05$ and VIP> 1.5) are visualized as box-plot graphs in Figure 4 (panel B-F). They are α-Terpineol (VIP 2.41, Figure 4B), linalool (VIP 2.03, Figure 4C), DL-menthol (VIP 2.03, Figure 4D), α-Cedrene (VIP 1.63, Figure 4E) and Borneol (VIP 1.53, Figure 4E).

Terpenes, together with classical cannabinoids THC or CBD, are believed to evoke the so-called *'entourage effect',* suggesting that they are capable of enhancing or modulating these cannabinoids activity. However, it is still debated whether different cannabis chemotypes induce their distinct effects, probably by a dynamic interplay of both cannabinoids and by the help of terpenes [12,22]. The phytocannabinoid-terpenoid synergetic interactions were deeply investigated with respect to the treatment of pain, inflammation, depression, anxiety, addiction, epilepsy, cancer, and various infections [23,24]. By contrast, some authors evoke the possibility that the cannabis terpene mixture did not have any significant effect on cannabinoid anti-proliferative action [25]. The phyto-complex of cannabis is needed to be further investigated since cannabis-based products are of growing interest in clinical research and personalized medicine [26,27]. In this view, the recognized cannabis chemotypes may be further subcategorized based on the content of relevant mono- and sesquiterpenes [20,28], which is deeply linked to the plant genome [29,30].

4. Conclusion

The aim of this experimental work was to develop a method for the characterization of the volatile compounds and especially the terpene fraction in *Cannabis sativa L.* inflorescences. A headspace method was coupled to the capillary gas chromatography with a mass detector operating in time-of-flight mode. This analytical approach provides rapid sample preparation, specific qualitative detection, high sensitivity, precise and accurate quantitative determination. The method was then applied to cannabis THC-dominant (I) and CBD-dominant (III) chemotypes. This application provides (1) important data on the volatile secondary components of the plant, which may be useful for better understanding the therapeutic properties of the cannabis phyto-complex; (2) the possibility of establishing the aroma profile of different Cannabis batches, allowing possible similarities between samples and, at the same time, identifying any artificial adulteration; (3) the opportunity to individuate some target compounds that seem to differ in the different chemotypes. The latter point is exciting as it could provide information on the phyto-complex before the balsamic time and strategies for cultivar and chemotype classification. We hope in a future study on a much larger homogeneous set of samples so that, by increasing the population of homogeneous data, we can attempt to produce more meaningful perspectives.

Author Contributions

Conceptualization: MDC, GR; Investigation: LM, SA, EVM, CM and EC; Formal analysis: MDC; Drafting of the manuscript: MDC; Supervision: GR, CB, MP,VG; Writing-review and editing: MDC, GR; All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest

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Figures.

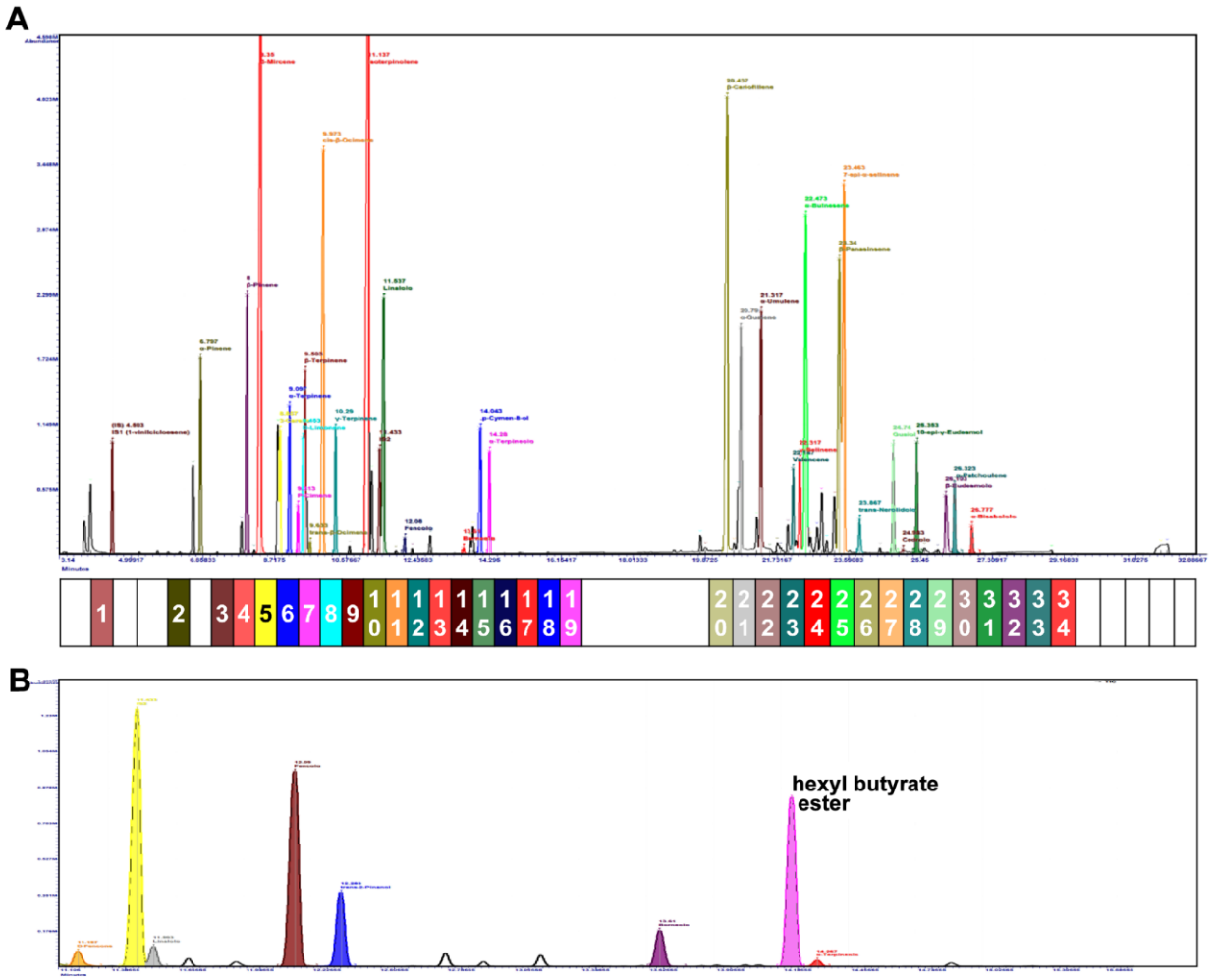

Figure 1. (A) Chromatographic separation of terpenoids in a cannabis sample. The peaks, in total ion scan m/z 40-300, were coded by number and colors as follows: (1) IS1 4-Vinyl-1-cyclohexene; (2) α-Pinene; (3) β-Pinene; (4) β-Mircene; (5) 3-Carene; (6) α-Terpinene; (7) P-Cimene; (8) D-Limonene;(9) β-Terpinene; (10) trans-β-Ocimene; (11) cis-β-Ocimene; (12) γ-Terpinene; (13)Terpinolene; (14) IS2 D5-Linalool; (15) Linalool; (16) Fenchol; (17) Borneol; (18) DL-Menthol; (19) α-Terpineol; (20) β-Caryophyllene; (21) α-Guaiene; (22) α-Humulene; (23) Valencene; (24) γ-Selinene; (25) α-Bulsenene; (26) β-Panasinsene; (27) 7-epi-α-Selinene; (28) trans-Nerolidol; (29) Guaiol; (30) Cedrol; (31) 10-epi-γ-Eudesmol; (32) β-Eudesmol; (33) α-Patchoulene; (34) α-Bisabolol. **(B)** Magnification of the adulterant hexyl butyrate ester: a compound with a fruity, pineapple-like scent (Rt 14.2, m/z 71, 43, 56, 84).

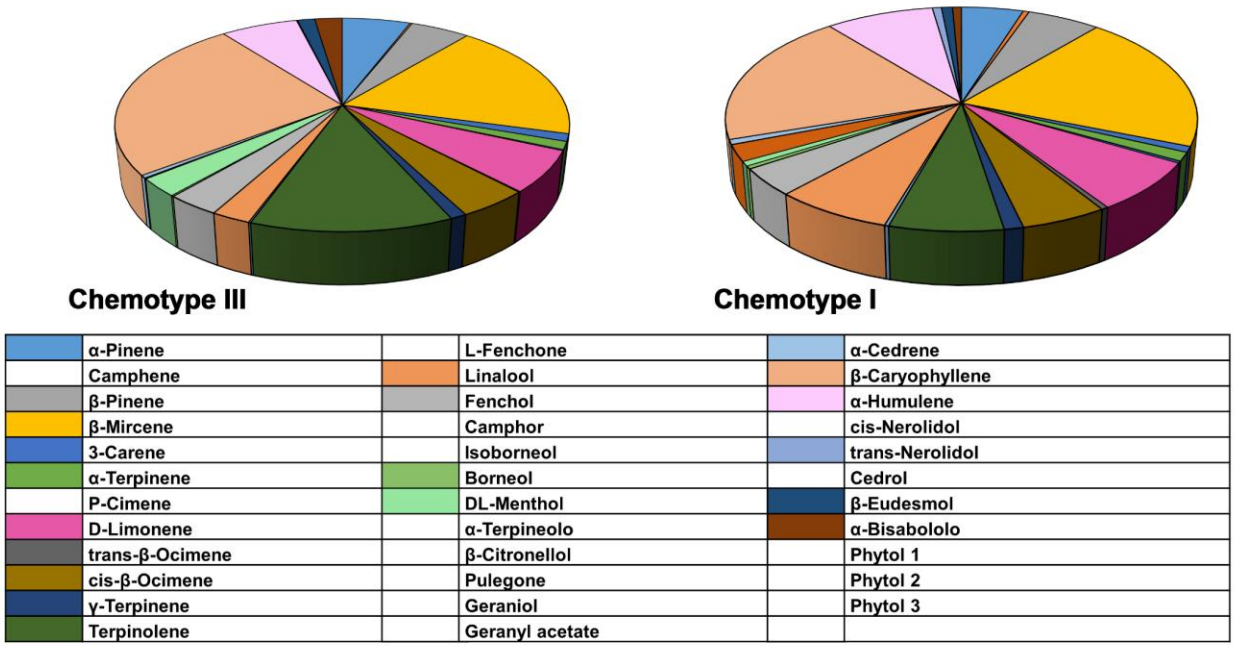

Figure 2. Distribution of terpenoids in different cannabis chemotypes. In the legend blank indicates terpenoids with a concentration inferior to 0.5% on dry weight.

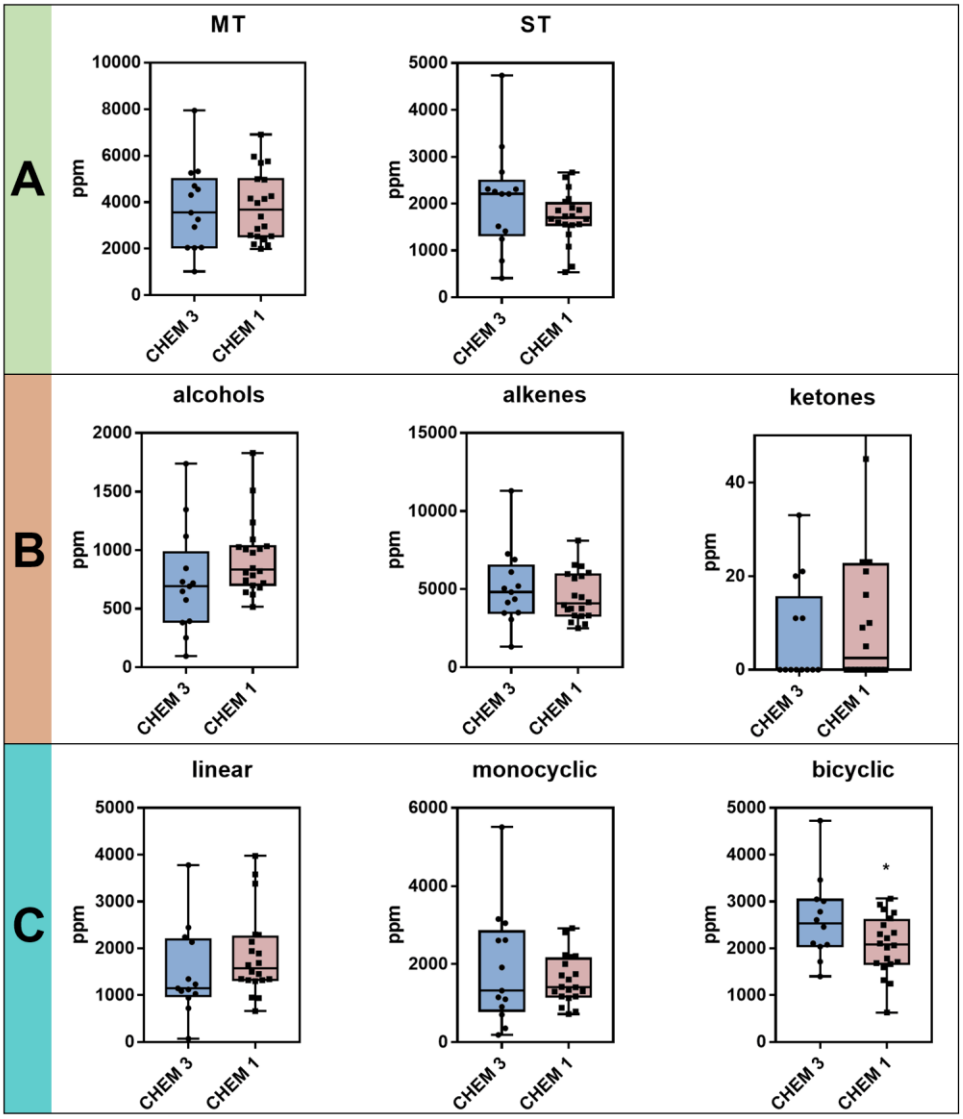

Figure 3. Box-plots of terpenoids concentration in different cannabis chemotypes. The left color-scale indicates the different classifications according to: (**A**) number of isoprene units: monoterpenes (MT) and sesquiterpenes (ST); (**B**) chemical classes: alcohols, ketones, alkenes and (**C**) presence or absence of rings: linear, monocyclic and bicyclic.

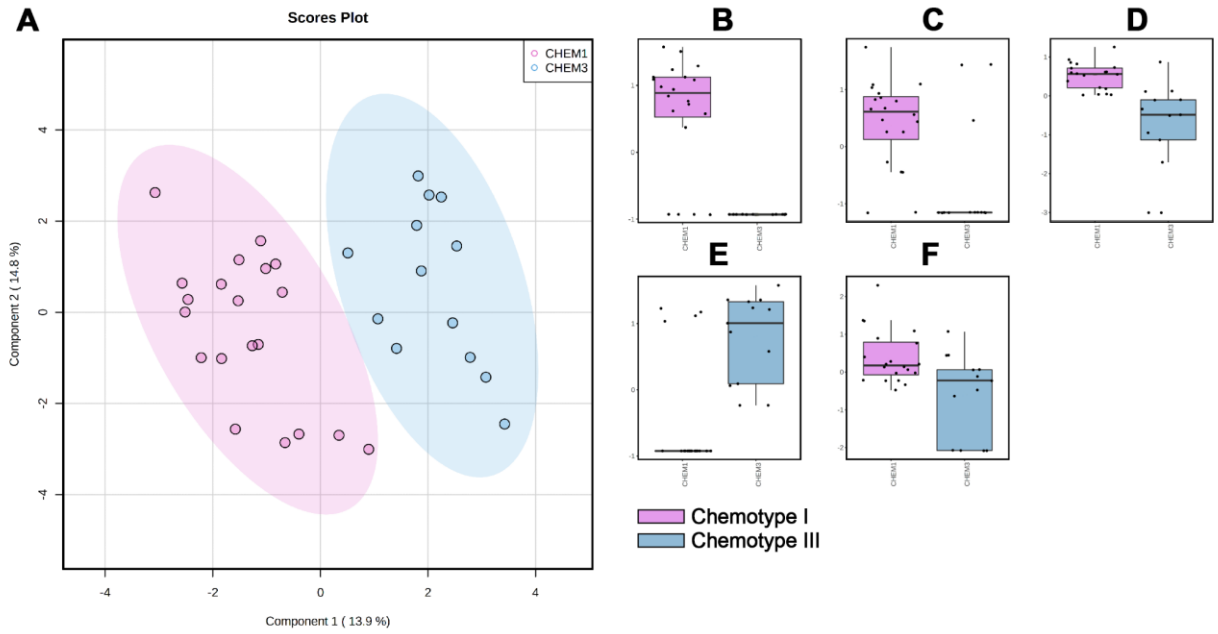

Figure 4. (A) Multivariate analysis visualized as principal discriminant analysis of terpenoids in cannabis chemotypes I (THC-dominant, pink) vs. III (CBD-dominant, light blue). Discriminant terpenoids ($p<0.05$) between chemotypes visualized as box-plot graphs after their concentration was log-transformed and auto-scaled: **(B)** α-Terpineol (VIP 2.41); **(C)** linalool (VIP 2.03); **(D)** DL-menthol (VIP 2.03); **(E)** α-Cedrene (VIP 1.63) and **(F)** Borneol (VIP 1.53). The boxes stretch from the 25th to the 75th percentile; the line across the boxes indicates the median value; the lines stretching from the boxes indicate extreme values. Outliers are displayed as separate points.

Tables.

Table 1. Chemical identity, class, chromatographic and mass spectrometry properties of the n.35 terpenoids investigated. IS were italicized: 4-Vinyl-1-cyclohexene was used for the quantification of alkenes, whereas D5-Linalool for alcohols.

n.	Analyte	Classifications			RT	CAS	Base peak (m/z)	MW
1	IS1 (4-Vinyl-1-cyclohexene)				4.51	100-40-3	54	108
2	α-Pinene	MT	Bicyclic	Alkenes	6.8	80-56-8	93	136
3	Camphene	MT	Bicyclic	Alkenes	7.25	79-92-5	93	136
4	β-Pinene	MT	Bicyclic	Alkenes	8	127-91-3	93	136
5	β-Mircene	MT	Linear	Alkenes	8.32	123-35-3	93	136
6	3-Carene	MT	Bicyclic	Alkenes	8.86	13466-78-9	93	136
7	α-Terpinene	MT	Monocyclic	Alkenes	9.11	99-86-5	121	136
8	P-Cimene	MT	Monocyclic	Phenyl	9.33	99-87-6	119	134
9	D-Limonene	MT	Monocyclic	Alkenes	9.46	5989-27-5	68	136
10	trans-β-Ocimene	MT	Linear	Alkenes	9.64	3779-61-1	93	136
11	cis-β-Ocimene	MT	Linear	Alkenes	9.95	13877-91-3	93	136
12	γ-Terpinene	MT	Monocyclic	Alkenes	10.3	99-85-4	93	136
13	Terpinolene	MT	Monocyclic	Alkenes	11.09	586-62-9	93	136
14	L-Fenchone	MT	Bicyclic	Ketones	11.2	7787-20-4	81	152
15	IS 2 (D5-Linalool)				11.44	159592-39-9	74	159
16	Linalool	MT	Linear	Alcohols	11.5	78-70-6	93	154
17	Fenchol	MT	Bicyclic	Alcohols	12.09	2217-02-09	81	154
18	Camphor	MT	Bicyclic	Ketones	12.9	464-48-2	95	152
19	Isoborneol	MT	Bicyclic	Alcohols	13.37	124-76-5	95	154
20	Borneol	MT	Bicyclic	Alcohols	13.63	464-43-7	95	154
21	DL-Menthol	MT	Monocyclic	Alcohols	13.78	89-78-1	81	156
22	α-Terpineol	MT	Monocyclic	Alcohols	14.29	10482-56-1	59	154
23	β-Citronellol	MT	Linear	Alcohols	15.15	106-22-9	69	156
24	Pulegone	MT	Monocyclic	Ketones	15.49	89-82-7	81	152
25	Geraniol	MT	Linear	Alcohols	15.81	106-24-1	69	154
26	Geranyl acetate	MT	Linear	Esters	19.28	105-87-3	69	196
27	α-Cedrene	ST	Bicyclic	Alkenes	20.32	469-61-4	119	204
28	β-Caryophyllene	ST	Bicyclic	Alkenes	20.4	87-44-5	93	204
29	α-Humulene	ST	Monocyclic	Alkenes	21.31	6753-98-6	93	204
30	cis-Nerolidol	ST	Linear	Alcohols	23.12	7212-44-4	69	222
31	trans-Nerolidol	ST	Linear	Alcohols	23.87	40716-66-3	69	222
32	Cedrol	ST	Bicyclic	Alcohols	25.05	77-53-2	95	222
33	β-Eudesmol	ST	Bicyclic	Alcohols	26.1	473-15-4	59	222
34	α-Bisabolol	ST	Monocyclic	Alcohols	26.78	23089-26-1	119	285
35	Phytol 1	DT	Linear	Alcohols	30.02	7541-49-3	68	128
36	Phytol 2	DT	Linear	Alcohols	30.52	7541-49-3	82	128
37	Phytol 3	DT	Linear	Alcohols	30.9	7541-49-3	81	128

MT: monoterpenes; DT: diterpenes and ST: sesquiterpenes

Table 2. Terpenoids concentration (ppm, mean±SD) in Cannabis chemotypes III and I. Significance was estimated by unpaired t-test (*p<0.05; **** p<0.0001)

Analyte	CHEM III	CHEM I	p-value
α-Pinene	340±225	268±136	
Camphene	10±8	26±29	
β-Pinene	295±153	318±155	
β-Mircene	1070±829	1104±665	
3-Carene	65±66	45±60	
α-Terpinene	68±92	69±84	
P-Cimene	10±12	16±23	
D-Limonene	362±272	398±272	
trans-β-Ocimene	7±12	23±28	
cis-β-Ocimene	254±248	285±397	
γ-Terpinene	57±76	64±71	
Terpinolene	712±919	378±453	
L-Fenchone	7±11	13±20	
Linalool	138±148	393±173	****
Fenchol	191±142	219±139	
Camphor	0±0	1±2	
Isoborneol	0±0	0±0	
Borneol	9±9	23±29	
DL-Menthol	158±147	35±73	****
α-Terpineol	0±0	131±90	****
β-Citronellol	3±12	0±3	
Pulegone	0±0	0±0	
Geraniol	4±16	0±2	
Geranyl acetate	0±0	0±0	
α-Cedrene	23±54	38±63	
β-Caryophyllene	1463±774	1067±548	
α-Humulene	384±223	471±117	
cis-Nerolidol	0±0	0±0	
trans-Nerolidol	5±15	39±54	*
Cedrol	0±0	0±0	
β-Eudesmol	85±77	48±47	
α-Bisabolol	136±175	38±37	*
Phytol 1	0±0	0±0	
Phytol 2	0±0	0±0	
Phytol 3	0±0	0±0	
	5868±2664	**5521±1615**	