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Short communication

Comparison of terpene profile in fresh and dry flowers of cultivated commercial strains and wildtype of Cannabis

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Introduction

Terpenes are hydrocarbons, made up of isoprene units, while terpenoids are an oxidized and denatured form of terpenes containing an additional functional group with oxygen (Al-Taweel and Perveen, 2018). This oxidation occurs during the drying and curing processes when the plant is exposed to open air (Lowe et al., 2021). In various chemotypes of Cannabis over 200 terpenes have been identified (Lowe et al., 2021). Most of the research, regarding terpenoids in Cannabis is conducted on dry flower material, however there is scarcely any data on terpenoid profiling in fresh flowers. Therefore, the aim of this study is to compare volatile terpenoid profiles of fresh and dry flowers by HS/GC/MS analysis.

Materials and methods

Seed material

Seeds from 4 different *Cannabis* strains (M1- Bubba Kush x OG Kush, M2 - NYC Diesel, M3 - Great White Shark and S1 - Charlotte Angels) were generously donated by a licenced cannabis company. Seeds of wildgrowing *Cannabis* were collected at 5 different locations (seed maturity growing stage) in R. N. Macedonia (Kochani: W1 - Gorni and Dolni Podlog, W2 - Dolni Balvan and W6 - Mojanci; Strumica: W3 - Prosenikovo and Saraj, W4 - Bosilovo).

Cultivation of plant material

Seeds were placed between two layers of dampened cotton ball with distilled water in a Petri dish sealed with parafilm. The seeds imbibed in a period of 3-5 days. Seedling stage lasted 3 weeks, vegetative and flowering stage were different for each strain/variety. At 7th week of vegetative growth plants were transferred at outdoor experimental plot with limited nutrition. Fresh flower samples (weigh variation of samples 0.8 - 1.2 g) from upper third of the plants were collected two days prior harvest and put in headspace vials. The samples were stored at -20°C until further analysis. After harvest and drying of plant material (loss on drying below 10%), dry flower samples (weight 0.5 g) were placed in headspace vials for analysis.

HS/GC/MS analysis

Fresh and dry flower samples in headspace vials were analyzed on Agilent 7890A Gas Chromatography system equipped with FID detector and Agilent 5975C mass spectrometer. For separation HP-5ms capillary column (30m x 0.25 mm, 0.25 μm) was used with helium as carrier gas (1 mL/min). Analytical conditions were as follows: oven temperature at 0 min 60°C, rate 4.5 °C/min 4.5 °C to 250 °C (1 min hold time) with total runtime of 43 min. Syringe T was 85°C, incubation T 80 °C, incubation time 5 min, agitator speed 500 rpm. FID detector temperature was 270 °C. Mass spectrometry conditions were as follows: ionization voltage 70 eV, ion source temperature 230 °C, transfer line temperature 280 °C and mass range from 50 - 550 Da. The MS was operated in scan mode. Components were identified with comparison of their mass spectra with reference spectra from libraries such as NIST,

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Wiley and Adams (Adams, 2007) and were quantified using normalization method of peak areas with no correction factors.

Results and discussion

In this study a total of 62 terpenes were identified and quantified with content above 0.1%. Twenty terpenes comprise 91.55 – 99.05%, and were determined throughout all the samples. Nine of them were considered as predominant: myrcene, limonene, α-pinene, β-pinene, terpinolene, (E)-β-ocimene, trans-(E)-caryophyllene and camphene. Six components: myrcene (81.25%, 78.49%), limonene (9.32%, 9.63%), α-pinene (1.86%, 5.44%), β-pinene (1.67%, 1.49%), trans-(E)-caryophyllene (1.55%, 1.49%) and linalool (0.89%, 0.59%) were identified as predominant in both fresh and dry flowers from the commercial strain Charlotte Angels, respectively. The same components were predominant in the Bubba Kush x OG Kush strain (fresh vs. dry flowers respectively) only in different ration compared to Charlotte Angels: myrcene (39.44%, 30.12%), limonene (36.45%, 18.68%), α-pinene (4.55%, 12.82%), β-pinene (4.77%, 6.61%), trans-(E)-caryophyllene (3.41%, 8.06%) and linalool (3.39%, 4.40%). On the other hand, in Great White Shark strain, the following composition was observed in fresh vs. dry flowers, respectively: α-pinene (54.31%, 62.81%), myrcene (23.72%, 19.76%), β-pinene (7.67%, 6.76%), limonene (6.77%, 3.30%) trans-(E)-caryophyllene (0.43%, 1.18%) and camphene (1.14%, 1.93%). In NYC Diesel: α-pinene (40.04%, 59.40%), myrcene (36.76%, 16.12%), limonene (8.64%, 5.10%), β-pinene (7.17%, 6.77%), trans-(E)-caryophyllene (1.60%, 4.43%) and (E)-β-ocimene (1.54%, 0.59%) were identified in fresh vs. dry flowers, respectively. The same six terpene components were also identified as predominant components in the fresh and dried flowers of cultivated wildtypes (W1-W4), respectively: W1 – myrcene (56.46%, 52.11%), α-pinene (30.30%, 36.19%), limonene (4.64%, 4.26%), β-pinene (4.47%, 3.41%), terpinolene (2.76%, 1.66%), and camphene (0.58%, 0.77%), W2 - myrcene (62.07%, 45.09%), α-pinene (14.88%, 33.53%), (E)-β-ocimene (9.17%, 7.73%), limonene (6.97%, 5.82%), β-pinene (3.22%, 4.09%), trans-(E)-caryophyllene (1.79%, 1.42%), W3 - myrcene (48.52%, 44.81%), α-pinene (22.52%, 27.18%), limonene (11.65%, 9.68%), (E)-β-ocimene (10.16%, 12.91%), β-pinene (4.19%, 2.483%), camphene (0.71%, 1.16%), and W4 - α-pinene (54.71%, 59.48%), myrcene (29.21%, 19.76%), β-pinene (9.66%, 6.52%), limonene (2.87%, 1.94%), trans-(E)-caryophyllene (1.19%, 0.448%) and camphene (1.12%, 1.41%). No significant changes in predominant terpene profile and content were noted comparing W6 fresh and dry flower. There are 2 separate studies that report changes in terpene profiles of fresh and dry cannabis flower essential oil samples due to influence of drying process that partially complies with the results reported in this research (Ross and ElSohly,1996; Kwasnica et al., 2021). Ross and ElSohly (1996) observed that drying the plant material results in a significantly greater loss of monoterpenes than of sesquiterpenes but qualitatively speaking, none of the major components of the oil (> 0.1%) completely disappeared with drying. In a more recent study by Kwasnica et al. (2021), similar predominant terpenes as our study with different order of predominance were identified (β-myrcene (26.66%), β-(E)-caryophyllene (17.50%), limonene (10.45%), α-humulene (7.26%), caryophyllene oxide (3.79%), β-pinene (2.51%), terpinolene (2.50%) and α-pinene (2.16%)). They quantified the terpenes after implementing different drying processes, pointing out the most effective drying process as convective drying at 50°C with least significant quantitative changes in predominant terpenes from fresh and dried cannabis material.

Conclusion

This is first study of its kind to compare volatile terpene profiles of fresh and dry flowers of cultivated Cannabis in North Macedonia by HS GC-MS. Changes in terpene content were observed between dry and fresh flowers of both cultivated wildtype and commercial Cannabis strains, indicating influence of drying process of the material.

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