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Characterisation of the key-aroma compounds among the volatile constituents in different hemp strains (*Cannabis sativa* L.)

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Abstract

The key-aroma compounds and the volatile constituents of the flowers of three different industrial hemp varieties (KC Virtus, Felina 32 and Santhica 70) grown during a field study in Switzerland, have been analysed by means of Gas Chromatography-Olfactometry (GC-O) and Gas Chromatography – Mass Spectrometry (GC-MS) in fresh flower samples at different time points of plant growth. The GC-O analysis revealed 33 different odour-active compounds, whereas most of these compounds were detectable in all three hemp cultivars, with different odour intensities. Not only terpenes but also compounds from other substance classes were detected as odour-active constituents. The GC-MS analysis of the terpene constitution in the same samples revealed cultivar-specific differences among the main volatile constituents. Furthermore, changes in the terpene profile could be observed during plant growth. Overall, it could be shown that the flowers of industrial hemp varieties can be regarded as a valuable source of aroma compounds for future food and beverage applications.

Keywords: key-aroma compounds, volatiles, industrial hemp varieties, gas chromatography-olfactometry, gas chromatography-mass spectrometry

Introduction

Hemp (*Cannabis sativa* L.) has been cultivated and used in folk medicine and for textile production since ancient times [1]. After its recent revival as a plant for medical and recreational purposes [2], the attention of the food industry towards industrial hemp with low contents of Δ-9-tetrahydrocannabinol (0.2 % w/w) is growing, due to its high contents in unsaturated fatty acids, proteins, antioxidants and, last but not least, aromatic substances such as terpenes [3-5]. The volatile composition of different Cannabis sativa species was studied intensively in many previous investigations by methodologies such as gas chromatography–mass spectrometry (GC-MS) [4, 6-8]. However, studies analysing the odour-active constituents in the aroma profiles of hemp, especially in industrial hemp varieties, by methodologies such as gas chromatography–olfactometry (GC-O), are scarcely available in literature. Therefore, the aim of this investigation was to characterise the key-aroma compounds among the volatile constituents in different industrial hemp varieties (cultivated in Switzerland, containing less than 0.2 % w/w of Δ-9-tetrahydrocannabinol) in the freshly harvested materials, by methodologies such as GC-O and GC-MS. Furthermore, the evolution of some selected volatile marker compounds during plant growth was investigated.

Experimental

The industrial hemp varieties KC Virtus, Felina 32, Santhica 70 (all cultivated in Switzerland, each containing less than 0.2 % w/w of Δ-9-tetrahydrocannabinol) were analysed for their odour-active constituents by GC-O analysis in the fresh flower samples harvested at the end of flowering or the full flowering stage. For this analysis, the hemp flowers were directly frozen in liquid nitrogen after harvest and ground into a fine powder; 25 g of the obtained hemp flower powder was immediately extracted with 250 ml freshly distilled dichloromethane for 2 hours. The extracted volatiles were separated from the non-volatiles by means of the solvent assisted flavour evaporation (SAFE) [9]. The obtained aroma distillate was concentrated and analysed by GC-O, applying the instrumental settings as previously reported [10]. Furthermore, the major terpene compounds in the flowers of the above-mentioned hemp samples were identified by GC-MS and quantified by gas chromatography with flame ionization detection (GC-FID) as previously described [8]. Additionally, the evolution of the terpene profile during the plant growth was studied as recently described [8].

Results and discussion

The GC-O analysis revealed 33 odour-active compounds in the flowers of three different hemp cultivars, in the fresh materials after harvest. Most of the odorants were detectable in all three hemp cultivars. However, differences in the intensities of the odorants were perceivable among the samples. Interestingly, not only terpenes but also compounds from other odour classes such as lipid degradation products, methoxypyrazines, sulphur

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compounds, one ester and even one pyrroline could be detected among the odour-active constituents during the GC-O analysis.

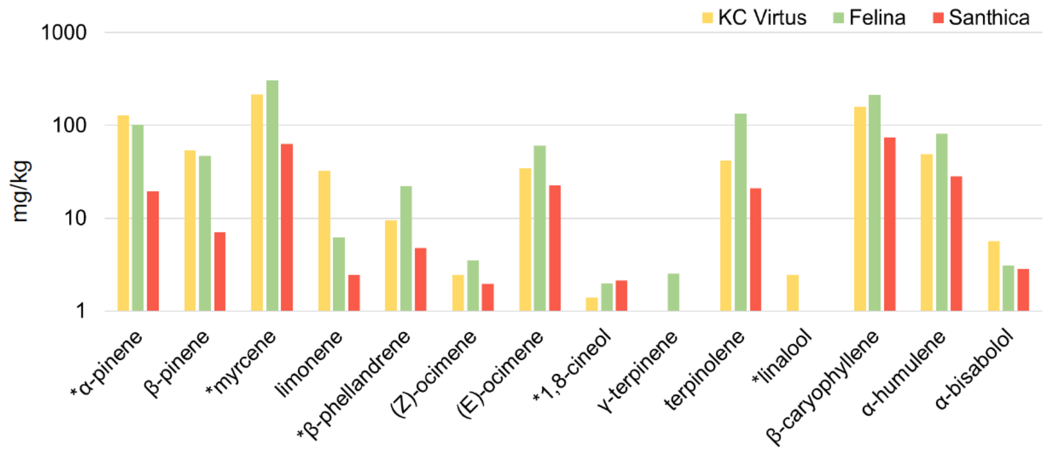
Table 1: Odour-active compounds determined by GC-O analysis in the flowers of three different hemp cultivars (fresh material).

no.	compound	odour-quality	RI-FFAP	odour intensity perceived during GC-O		
				KC Virtus	Felina	Santhica
1	α-pinene[a]	pine-like	1035	++	++	+
2	3-methyl-2-butene-1-thiol[b]	sulphury	1102	++	++	++
3	(Z)-3-hexenal[b*]	green	1138	++	++	++
4	α -pinene[a]	metallic	1156	+++	+++	++
5	1,8-cineol[a]	eucalyptus-like	1215	+	+	+
6	(Z)-4-heptenal[b*]	biscuit-like, fatty	1236	-	-	++
7	β-phellandrene[a]	terpene-like	1243	+	++	+
8	1-octen-3-one[b]	mushroom-like	1293	-	+	+
9	2-acetyl-1-pyrroline[b*]	nutty	1326	+	+	++
10	3-mercapto-2-pentanone[b*]	sulphury, catty	1347	++	++	++
11	4-mercapto-4-methyl-2-pentanone[b*]	sulphury, green	1365	++	-	-
12	(Z)-1,5-octadien-3-one[b*]	metallic, geranium-like	1367	-	+	+
13	3-methoxy-2,5-dimethylpyrazine[b*]	mouldy, earthy	1418	+	+	+
14	1-nonen-3-one[b*]	mushroom-like	1444	-	-	+
15	methional[a*]	cooked potato	1448	++	++	++
16	2-sec-butyl-3-methoxypyrazine[b*]	earthy	1483	+	++	-
17	(Z)-2-nonenal[b*]	cardboard-like	1487	++	-	+++
18	2-isobutyl-3-methoxypyrazine[b*]	bell-pepper-like	1516	++	-	-
19	(E)-2-nonenal[b]	cardboard-like	1515	+	+	++
20	linalool[a]	flowery, bergamot-like	1553	++	-	-
21	butanoic acid[a]	cheese	1619	-	+	+
22	phenylacetaldehyde[a]	flowery	1634	+	+	-
23	unknown	blackcurrant-like	1644	+	+	+
24	(E,E)-2,4-nonadienal[b]	fatty	1698	+	+	++
25	3-methyl-2,4-nonandione[b*]	hay, fruity	1710	+	+	-
26	(E,E,Z)-2,4-nonatrienal[b*]	oat flakes-like	1873	++	++	++
27	unknown	fruity, flowery	1912	+	+	+
28	trans-4,5-epoxy-(E)-2-decenal[b*]	metallic	2000	+	+	+
29	unknown	fruity, spicy	2060	+	+	++
30	ethyl 3-phenyl-(E)-2-propenoate[b*]	cinnamon-like	2130	+	-	++
31	sotolone[b*]	seasoning	2200	-	+	+
32	unknown	fruity, spicy	2417	+	-	-
33	unknown	sweet	2530	+	-	-

RI-FFAP: retention index on FFAP; +++ odorant perceivable in very high intensity; ++ odorant perceivable in high intensity; + perceivable odorant; - odorant was not perceivable; criteria of identification: a) odorant was identified by retention index on FFAP, MS-spectrum and comparison with reference substance concerning the before-mentioned criteria and the odour quality perceived during GC-O analysis; b) odorant was tentatively identified by retention index on FFAP and compared with the reference substance concerning its retention index on FFAP and the odour quality perceived during GC-O analysis; compounds marked with * were analysed for the first time in *Cannabis sativa* flowers.

Among the terpenes, α-pinene, myrcene, 1,8-cineol, β-phellandrene and linalool were analysed as odour-active constituents during GC-O analysis. Myrcene showed the highest odour intensity, especially in KC Virtus and Felina flowers. Similar trends were also observable for the odour-active terpenes such as α-pinene, 1,8-cineole and linalool, which were detected in KC Virtus and Felina with higher intensities. This observation corresponds well with the overall odour impression of these hemp cultivars, which could be described as fresh and citric. In contrast to this, Santhica showed somewhat higher intensities of the compounds, perceivable with fatty (no. 6, 17, 19 and 24), nutty (no. 9) and cinnamon (no. 30) odour notes, corresponding with the observation that the overall odour impression of this hemp cultivar was perceived as fatty, spicy and balsamic. Interestingly, this study indicates that the overall odour impression of the flowers of different hemp cultivars is not only composed of terpenes but also of odour-active compounds from other substance classes.

In addition to the GC-O analysis, the main terpene compounds were identified and quantified as previously reported [8]. The following terpenes, as presented in Figure 1, were identified as the most abundant compounds in the volatile constitution of the flowers of the analysed hemp strains. The results of the quantitative analysis of these major terpenes are displayed in Figure 1.



n=3; standard deviations ≤ +/- 10 %; * compound detected as odour-active during GC-O analysis (see Table 1)

Figure 1: *Major terpenes and their quantities in flowers of different hemp strains.*

Higher amounts for almost all analysed terpenes could be detected for the hemp cultivars KC Virtus and Felina. In contrast to that, the concentrations of the quantified terpenes were drastically lower in Santhica. Interestingly, linalool was only detectable in KC Virtus, and γ-terpinene only in Felina.

To investigate if the harvest period had an influence on the terpene profile, the major terpenes were analysed in flowers of KC Virtus cultivar at different points of time. Figure 2 presents the evolution of the monoterpenes, the sesquiterpenes and the total terpene content during plant growth, as previously reported [8].

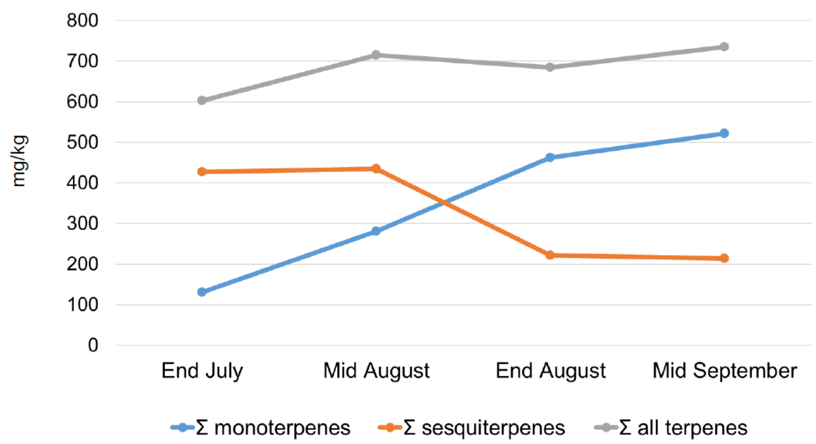


Figure 2: *Terpene evolution during plant growth in flowers of KC Virtus.*

Overall, our analysis revealed that the total amount of the terpene compounds increased during plant growth. It was observable that the monoterpene content was lower than the sesquiterpene content at the early flowering stages and increased during the cultivation period. In contrast to that, the sesquiterpenes decreased during the cultivation period.

Conclusion

Our study shows that the odour-active constitution of different varieties of industrial hemp flowers is not only composed of terpenes but also other key odorants of different substance classes, such as lipid degradation products, methoxypyrazines, sulphur compounds, one ester and even one pyrrole. Nevertheless, the terpenes constitute the main part of the volatile fraction, whereby the monoterpenes α-pinene, myrcene, β-phellandrene, 1,8-cineole and linalool could be analysed as odour-active constituents during GC-O analysis. In contrast to that, the sesquiterpenes β-caryophyllene, humulene and α-bisabolol were not perceivable during GC-O, indicating that these components are less important for the odour profiles of the analysed hemp flowers. Furthermore, species-related differences could be perceived in the odour constitution of the flowers of different hemp cultivars, and an alternation of the terpene composition was observable in the flowers during plant growth. However, the compounds potentially identified as odour-active by GC-O analysis will have to be verified by further quantitation experiments and the calculation of their odour activity values. It should be noted that possible changes in the odour constitution related to the drying process have to be kept in mind and should be analysed in subsequent investigations.

Overall, the findings of the study show that flowers of industrial hemp varieties, as a side product of hemp fibres and the oil production, can be regarded as a valuable source of aroma compounds, to be valorised in the development of future food and beverage applications.

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