"Hemping" the Drinks: Aromatizing Alcoholic Beverages with A Blend of *Cannabis sativa* L. Flowers

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Abstract

Cannabis sativa L. is a multi-purpose crop, traditionally used for fibre and seed production, whose cultivation is permitted in Europe for varieties complying with the Δ^9-tetrahydrocannabinol 0.2% threshold. To face the need for a more sustainable agriculture system, the circularization of the crop industries is of the utmost importance. For hemp, the present study proposes the use of the flowers, normally regarded to as crop residues, as further exploitable by-products. A French, 'Futura 75', and a Ukrainian, 'Uso 31', cultivar flowers were used for the extraction of the essential oil (EO) and as flavouring agents of an artisanal beer and liqueur. The compositions of the EOs and the beverage headspaces were characterized by GC-MS, then subjected to multivariate statistical analysis. The enrichment of the flavour bouquet was more evident for the liqueur, which retained more hemp-derived compounds. The beer maintained its volatile aroma compounds profile, slightly enriched with more balsamic notes.

Keywords: Futura 75; Uso 31; hemp beer; hemp liqueur; headspaces; essential oils; Chinook

Chemical compounds studied in this article: α-Pinene (PubChem CID: 6654); Myrcene (PubChem CID: 31253); Terpinolene (PubChem CID: 11463); β-Pinene (PubChem CID: 14896); β-Caryophyllene (PubChem CID: 5281515); α-Humulene (PubChem CID: 5281520); α-Muurolene (PubChem CID: 12306047); Ethyl octanoate (PubChem CID: 7799); *Iso*pentyl acetate (PubChem CID: 31276); Ethyl hexanoate (PubChem CID: 31265).

Introduction

Cannabis sativa L. has a well-established traditional use as a multi-purpose crop: its stems have been used since ancient times for fibre production, and its seeds are a high-value dietary product for humans and animals (Tang et al., 2016). The legal constraint of complying with the Δ^9-tetrahydrocannabinol (THC) 0.2% threshold established by the EU legislation (European Commission, 2000) has addressed breeding techniques towards an increase in fibre and seed yields (Salentijn, Zhang, Amaducci, Yang, & Trindade, 2015), which represent almost the only products exploited from the hemp cultivation. As a result, the non-stem aerial parts, namely leaves and inflorescences, represent most of the threshing residue: the aim of the present study was the evaluation of different methods to exploit this quantitatively relevant biomass. Indeed, in order to comply with a greener approach, the hemp agriculture should consider *C. sativa* flowers as a further usable by-product rather than a waste material.

The glandular trichomes, in which the hemp essential oil (EO) is secreted and stored, are mostly located on the pistillate flowers of these inflorescences (Calzolari et al., 2017; Hillig & Mahlberg, 2004). The hemp EO is a niche, value-added product, which exhibited useful activities in diverse fields of application. Concerning the green approach, the hemp EO itself could contribute to a more sustainable agriculture system, as it showed a favourable profile of activity as a pest management agent. It exhibited allelopathic activity against germination and seedling growth (Synowiec et al., 2016), which is an exploitable property in the agriculture of weeds and crops. Moreover, it showed a relevant and targeted toxic activity towards *Physella acuta* snail, an invasive species plaguing rice-fields, and an intermediate host for human parasitic trematodes and nematodes (Bedini et al., 2016). This EO also showed a favourable biopesticide activity as management tools of mosquito vectors, houseflies and moth pests (Benelli et al., 2017). Particularly relevant, in terms of

52 insecticidal properties, is hemp EO activity towards the most invasive mosquito species, *Aedes albopictus*: it
53 shows a very aggressive daytime biting behaviour, worsening its role as vector of many parasites and
54 pathogens, such as yellow fever and Dengue (Bedini et al., 2016).

55 *C*. *sativa* EO, though, has a peculiar and generally well-liked aromatic profile, whose odour bouquet heavily
56 relies on the cultivar. The cultivar-induced differences are not to be underestimated, because a study showed
57 the untrained panelists' ability to not only discriminate between the buds odour from different strains of *C*.
58 *sativa*, but also to do so consistently (Gilbert & DiVerdi, 2018). Different varieties EOs, as well, have been
59 subjected to consumers' sensorial evaluation: high ratings in terms of perceived pleasantness were correlated
60 to higher abundances of monoterpenes and, in general, mixed strain EOs were perceived as better smelling
61 (Mediavilla & Steinemann, 1997). Other than the cultivar, however, the geographical area of cultivation and
62 the agronomic techniques used showed an influence on the EO composition of this species (Ascrizzi,
63 Ceccarini, Tavarini, Flamini, & Angelini, 2019).

64 In the present study, the essential oils extracted from the inflorescences of two *C*. *sativa* cultivars ('Futura
65 75' and 'Uso 31') harvested in August 2016 have been characterized. 'Futura 75' is one of the most imported
66 French cultivar. It is monoecious, with a THC well below the consented level and a later on-set of the
67 flowering time. Its production is mainly aimed at fibre production (Tang et al., 2016): the strength
68 characteristics of its fibres, both thermally modified or unmodified, has been assessed in the reinforcement of
69 epoxy-hemp composites (Väisänen, Batello, Lappalainen, & Tomppo, 2018). It is very adaptable to different
70 latitudes, as it shows low sensitivity to differences in the photoperiod (Salentijn et al., 2015), but it strongly
71 affected by water shortage (Cosentino, Riggi, Testa, Scordia, & Copani, 2013). 'USO 31' is a monoecious
72 Ukrainian cultivar, introduced in the European market by FNPC (Salentijn et al., 2015). It shows an early
73 onset of the maturation and it is suitable for fibre production (Sankari, 2000): its fibre and bast yields (both
74 long and short) are among the highest between the different varieties cultivated in China, with high cellulose
75 and moderate pectin content (Ji & Jiang, 2011). This cultivar also has a good salinity tolerance, as well as a
76 good resistance against wireworm (Song, Li, Wu, Fang, & Zhang, 2007).

77 Hemp EO peculiar aroma bouquet and the botanical proximity of *C*. *sativa* with another plant of the
78 Cannabaceae family, *Humulus lupulus* L., make this species an ideal flavouring agent in the beer brewing. A

79 mix of these two hemp variety flowers has been used to flavour an artisanal beer, 'Hempitaly', produced in
80 the geothermal EGP (Enel Green Power) complex of Larderello (Pisa, Italy).

81 The brewing method used in the production of the 'Hempitaly' beer is the 'all-grain', in which malt grains
82 are used, instead of the concentrated malt extract. The addition of the *C. sativa* flowers have been added: i) at
83 the beginning of the rinsing of the threshers, directly on them, as in the 'mash-hemping' technique; ii) at the
84 end of the boiling phase; iii) during the last 10 minutes of the whirlpooling phase. The development of the
85 beer aroma is a complex process, as the flavour compounds (both already aroma-active and precursors) are
86 extracted into the wort, but can then be metabolized (and, also, inactivated) by the yeasts, later in the
87 brewing process (Briggs, Brookes, Stevens, & Boulton, 2004). Moreover, the beer aroma is most certainly
88 attributable to a synergistic effect of several compounds, rather than a single component, thus its character
89 also depends on the contribution of minor compounds (Nickerson & Van Engel, 1992). The hydrodistillation
90 of the 'Chinook' hop pellets used in the brewing of this beer has been performed to analyse the obtained EO,
91 as well as its head space volatile emission, to better "isolate" the *Cannabis sativa* L. flowers contribution
92 only, as these two species are closely related.

93 A mix of the two hemp cultivars in the same proportion selected for the 'Hempitaly' beer has been used to
94 produce a hemp-flavoured liqueur. Its headspace has been analysed to evaluate the influence on the aromatic
95 volatile emission of an alcoholic beverage obtained with a different method and with a diverse matrix-effect,
96 using the same plant material as flavouring agent.

97 The present work aimed at proposing the further exploitation of hemp flowers, now generally considered
98 crop residues, as sources of a high value-added product, the essential oil, and as beverage flavouring agents.

99 ## 1. Materials and methods

100 ### *1.1. Plant material*

101 The *Cannabis sativa* L. cultivars 'Futura 75' and 'Uso 31' were produced by Azienda Agricola Carmazzi
102 (Torre del Lago, Lucca, Italy). These two hemp varieties comply with the 0.2% w/w of Δ^9-
103 tetrahydrocannabinol (Δ^9-THC) limit (Regulation EC No. 1124/2008, Annex XII) and are, thus, permitted

for cultivation in Italy. The sowing was performed in May 2016. The sowing density was 20-30 specimens per m^2, with 50 cm of distance between the rows.

1.2. Brewing process

The grist (ground malt grains) for 'Hempitaly' recipes was composed of 88% of Pilsner and 6% Vienna as base malts, with the addition of 3% Weizen malt for sourish profile and 3% Carapils malt for more body. The mashing process of the ground grains (grains:water in a 1:3 ratio) was performed in a multi-step system. Once the mixture had reached 45°C, the temperature program proceeded as follows:

1) 45°C for 10 minutes (protease enzymes react to hydrolyze low-weight protein as nourishment for yeast);
2) 50°C for 20 minutes (amylolytic activity);
3) 62°C for 20 minutes (β-amylase activity, pH 5.0-5.5, maximum activity);
4) 65°C for 20 minutes (β-amylase activity, pH 5.0-5.5, enzymatic synergy point between amylases);
5) 70°C for 20 minutes (α-amylase activity, pH 5.6-5.8, maximum activity);
6) 78°C for 5 minutes (enzymatical inactivation phase).

After 15 minutes of cooling, the filtering took place, with the washing of the threshes and the collection of the wort in a sanitized fermenter: this process was repeated 6 times, with water at pH 6. At this point, the 'mash-hemping' technique was used: a mix of fresh inflorescences of *C. sativa* 'Futura 75' and 'Uso 31' varieties in a 2:1 ratio was added to the threshes and washed through.

The wort-boiling phase was performed for 1 hour, together with the bitter and aroma hopping. The IBU (International Bitterness Units) value for the 'HempItaly' recipe was between 35-35% of α-acids. The 'Chinook' hop pellets (Birramia, Enterprise s.r.l., Querceta, Lucca, Italy) were used for the aroma attributes: they were added in the last 10-15 minutes of the boiling phase to transfer scent and aroma. Moreover, fresh hemp inflorescences in the same ratio reported for the 'mash-hemping' were added in the last 10 minutes of the boiling phase.

The must was then cooled during the whirlpooling phase with a counter flow heat exchanger. Fresh hemp inflorescences were added during this phase, as well. The cooling phase was performed using a plate-heat

exchanger, where the hot mash and the coolant (tap water) circulate in the opposite direction. The mash was then oxygenated to favor the beginning of the fermentation, stirring for at least a couple of minutes. Finally, the yeast (Fermentis SafAle™ US-05, Lesaffre, Cedex, France) were inoculated and the mix was stirred again. The mix was closed in the fermenter for 12 days at 20 °C, with a gradual temperature decrement down to 4 °C.

After priming, the bottling and priming processes were performed: the bottles were stored at 22-25 °C for 20 days; then, the nucleation of carbon dioxide was repeated by placing the bottles in a refrigerator at 4 °C for 4-5 days.

1.3. *Hemp-flavored liqueur*

The hemp inflorescences were macerated in pure ethyl alcohol in a food-grade stainless steel container to allow the extraction of aromatic compounds in the alcoholic solution. The alcohol extract was then filtered through a stainless-steel filter. Glucose syrup was added in order to dilute the alcohol extract to a volume of alcohol equal to 28%. After this dilution, bottling was carried out.

1.4. Essential oil extractions

The EO extraction was performed on fresh *Cannabis sativa* L. 'Futura 75' and 'Uso 31' inflorescences and on the *Humulus lupulus* L. 'Chinook' pellets with a standard Clevenger-type apparatus, with 2 hours extraction time. For both the hemp cultivars, the extraction yields were lower than 0.1% w/w. For the hop pellets, the extraction yield was 1.63% w/w.

1.5. Headspace analyses

For all the samples, the adsorption of the volatile analytes was performed with the Supelco DVB/CAR/PDMS fiber assembly (100 µm coating thickness) (SUPELCO, Bellefonte, PA, USA) preconditioned according to the manufacturer instructions. After the equilibration time, the septum of each vial was perforated by the holder (syringe) and the fibre was exposed to the headspace of the sample at room temperature. For both the beers and the liqueur, the sampling time was 5 minutes. Once the sampling was completed, the fibre was retracted into the holder and directly injected in the GC–MS apparatus for

155 separation and analysis. All the SPME sampling and desorption conditions were identical for all the samples.
156 Furthermore, blanks were performed before each first SPME extraction.

157 *1.6. Gas Chromatography – Mass Spectrometry Analyses*

158 Gas chromatography–electron impact mass spectrometry (GC–EI-MS) analyses were performed with a
159 Varian CP-3800 gas chromatograph equipped with a DB-5 capillary column (30 m × 0.25 mm; film
160 thickness 0.25 µm) and a Varian Saturn 2000 ion trap mass detector. Analytical conditions were as reported
161 in Ascrizzi et al. (2017): injector and transfer line temperatures 220 and 240 °C, respectively; oven
162 temperature programmed to rise from 60 to 240 °C at 3 °C min^{-1}; carrier gas helium at 1 ml/min; splitless
163 injection. The identification of the constituents was based on a comparison of the retention times with those
164 of the authentic samples, comparing their linear retention indices relative to the series of *n*-hydrocarbons.
165 Computer matching was also used against commercial (NIST 14 and ADAMS) and laboratory-developed
166 library mass spectra built up from pure substances and components of commercial essential oils of known
167 composition and MS literature data (Adams, 1995; Davies, 1990; Jennings & Shibamoto, 1982; Masada,
168 1976; Stenhagen, Abrahamsson, & McLafferty, 1974).

169 *1.7. Multivariate statistical analysis*

170 The percentage of dissimilarity contribution of the all the compounds in the two *C. sativa* cultivar EOs was
171 evaluated by the similarity percentage test (SIMPER) with the Bray-Curtis distance/similarity measure. The
172 statistical significance of the difference in the relative abundances of the compounds accounting for at least
173 1.00% in the dissimilarity rate of the emissions was evaluated using the F- or T-test, for compounds with
174 equal or unequal variances, respectively. The SIMPER, F- and T-tests were performed with the Past 3.20
175 Software (Hammer, Harper, & Ryan, 2001).

176 The principal component (PC) and hierarchical cluster (HC) analyses were carried out with the JMP software
177 package (SAS Institute, Cary, NC, USA). For the statistical evaluation of the composition of both the
178 extracted essential oils and the headspaces, a 145 × 6 covariance matrix (145 individual compounds x 6
179 samples = 870 data) was used. To perform the PCA, linear regressions were operated on mean-centered,
180 unscaled data to select the two highest principal components (PCs). This unsupervised method reduced the

dimensionality of the multivariate data of the matrix, whilst preserving most of the variance (Ascrizzi et al., 2018). The chosen PC1 and PC2 cover 65.70% and 23.10% of the variance, respectively, for a total explained variance of 88.80%. The HCA was performed using the Ward's method. The observation of the groups of samples by the HCA and the PCA methods can be applied even when there are no reference samples that can be used as a training set to establish the model.

2. Results and discussion

2.1. Chemical composition of the extracted essential oils

The complete compositions of the essential oils (EOs) extracted from the aerial parts of the two *Cannabis sativa* cultivars and from the 'Chinook' hop pellets are reported in Table 1.

The EOs extracted from both the *C. sativa* cultivars exhibited a predominance of monoterpene hydrocarbons in their compositions: this chemical class of compounds accounted for 52.4 and 60.5% in 'Uso 31' and 'Futura 75', respectively. α-Pinene, myrcene, terpinolene, and β-pinene were the most abundant, with relative abundances over 5%. Sesquiterpene hydrocarbons followed, accounting for up to 29.7 and 19.1% in 'Uso 31' and 'Futura 75', respectively. For this chemical class, compositional differences were more evidenced in the compositional pattern: whilst β-caryophyllene and α-humulene were the most abundant volatile organic compounds (VOCs) in this group, 9-*epi*-(*E*)-caryophyllene was exclusive to 'Uso 31', with a relative abundance of 3.8%; *allo*aromadendrene, instead, was exclusive to 'Futura 75', where it reached 1.6%. Oxygenated sesquiterpenes, instead, were detected in very similar relative amounts, and the most relevant was caryophyllene oxide for both EOs. Overall, 19 compounds were detected exclusively in the 'Uso 31' composition, whilst 22 were only detected in the 'Futura 75' EO. As evidenced by the SIMPER analysis (Table 2), 3 monoterpene hydrocarbons (myrcene, α-pinene and terpinolene) and 3 sesquiterpene hydrocarbons (β-caryophyllene, 9-*epi*-(*E*)-caryophyllene and α-humulene) contributed to at least 1% of the dissimilarity in the composition of these EOs, for a total dissimilarity contribution of 55.20%.

The 'Chinook' hop pellets EO composition, instead, was dominated by sesquiterpene hydrocarbons (67.4%), with α-humulene (19.3%), α-muurolene (10.1%), β-caryophyllene (9.9%), and δ-cadinene (6.2%) as the most abundant. Among monoterpene hydrocarbons, which followed as the second most relevant (17.6%) chemical class in this EO, myrcene was the most important, accounting for up to 17.0%. Non-terpene derivatives

exhibited an important presence in this composition (5.5%): among them, esters were the most represented, with 2-methylbutyl *iso*butanoate, methyl 6-methyl heptanoate and methyl-4-decenoate exhibiting relative abundances over 0.5%. These compounds were detected exclusively in the hop EO, which showed the overall highest number (32) of unique compounds over the three EOs (22 for 'Futura 75', 19 for 'Uso 31').

2.2. Beverages headspaces

The complete compositions of the headspaces of the two artisanal beers (control and hemp) and of the artisanal hemp liqueur are reported in Table 3.

The volatile emission profiles of the two beers were dominated by non-terpene derivatives, which accounted for over 83% for their compositions. The main detected volatiles of this class were aldehydes: ethyl octanoate, *iso*pentyl acetate and ethyl hexanoate were the most represented. Esters confer pleasant flavour properties to the fermented beverages, especially the low-boiling ones (Christoph & Bauer-Christoph, 2007). The addition of the hemp flowers to the mixture, however, induced a slight decrement in these compounds, coupled with the increment in the monoterpene relative content. The above-mentioned aldehydes showed different behaviours in the headspaces after the addition of hemp flowers: ethyl octanoate and hexanoate, both conferring a fruity aroma contribution to the beer, decremented, while *iso*pentyl acetate, for which a more fragrant and sweeter odour contribution is reported, exhibited an increase. Myrcene, which was the monoterpene hydrocarbon with the highest relative concentration, incremented from 5.6% to 9.8% in the hemp beer. This profile clearly showed the impact of the hemp flowers, whose EO was rich in this compound, thus adding their contribution to that of the hop pellets. The aroma contribution of this compound is reported as sweet and balsamic.

The headspace emission of the hemp liqueur, instead, was dominated by monoterpene hydrocarbons, whose relative content accounted for up to 90.4%. Among these compounds, α-pinene (38.8%) and myrcene (28.0%) made up more than 50% of the total composition: both share a balsamic aroma contribution, reported as pine-like for the former, and sweet for the latter. A woody and herbal odour contribution to this liqueur bouquet is due to β-pinene, which followed as the third most abundant (12.4%) volatile organic compound detected in this headspace. β-Caryophyllene and limonene followed, with relative abundances of 5.8% and 5.5%, respectively. The former confers to this liqueur a spicy, clove-like odour note, while the

235 latter has a pleasant lemon-like, citrusy aroma. All the above-mentioned terpenes were the result of the hemp
236 flower contribution to this beverage aroma, since they were identified in the EO compositions of both the *C*.
237 *sativa* cultivars of the added blend, of which they are reported as predominant compounds. The volatile
238 emission profiles of the average commercial liqueurs, indeed, are reported as mainly composed by non-
239 terpene, especially esters such as ethyl octanoate and decanoate, which are by-products of the fermentation
240 of carbohydrates, with low relative contents of terpenes (Christoph & Bauer-Christoph, 2007; Vázquez-
241 Araújo, Rodríguez-Solana, Cortés-Diéguez, & Domínguez, 2013). The relative concentration of the latter,
242 instead, incremented when the liqueur was macerated with the addition of dried hop flowers: the headspaces
243 were enriched in the terpenes fraction (Vázquez-Araújo et al., 2013). As *Humulus lupulus* L. is closely
244 related to *C*. *sativa*, the above-mentioned study is in accordance with the findings of the present work.

245 *2.3. Multivariate statistical analyses*

246 The dendrogram obtained by the hierarchical cluster analysis (HCA) performed on the complete
247 compositions of the EOs and headspaces of all the studied samples is reported in Figure 1.

248 A first classification identified two macro-clusters, of which the first one was further divided in two groups
249 (red and green), while the second was composed of only one group (blue). This first clustering already
250 evidenced the closest compositional relation of the hemp liqueur to the EOs, as they were grouped in the
251 same macro-cluster. All the EOs were sub-grouped together in the green cluster: the two EOs extracted from
252 the hemp flowers showed high similarity in their compositions, and were also very similar to hop EO, as
253 these species are closely related. Both the beer headspaces, instead, were clustered by themselves in the blue
254 macro-cluster, confirming the compositional differences evidenced by the GC-MS analyses.

255 The score plot obtained by the principal component analysis (PCA) performed on the complete compositions
256 of the EOs and headspaces of all the studied samples is reported in Figure 2.

257 The position of the samples on the score plot further evidenced the distribution of the samples based on their
258 compositions. In the same fashion of the HCA analysis, the most relevant distribution divided the samples
259 placing them into either the right or the left quadrants. The beer headspaces were positioned in the upper
260 right quadrant (PC1 and PC2>0), while all the other samples were placed in the left quadrants (PC1<0). In

261 particular, all the EOs were reported in the lower left quadrant (PC1 and PC2<0), with the hemp ones in a
262 closer distribution. The hemp liqueur, instead, was positioned by itself in the upper left quadrant (PC1<0,
263 PC2>0). Its position is intermediate between the other fermented samples (the beer headspaces) and the
264 hemp EOs.

265 ## 3. Conclusion

266 The hemp industry, today, is mainly addressed to the production of seeds for flours and fixed oils extraction,
267 as well as for the production of hemp fibres obtained from the hemp shives. The flowers, or what remains of
268 them once the seeds have been harvested, are generally discarded as a crop residue. The present work aimed
269 at demonstrating the possibility of further exploiting these residues: as the need for a circular economy, with
270 a more sustainable profile, arises, hemp flowers might and should be considered an exploitable by-product.
271 The extraction of their essential oil, indeed, represents a source of a high value-added product; moreover,
272 their use as beverage flavouring agents was presented as viable in two products, beer and liqueur,
273 characterized by two different matrices. The enrichment of the beer headspace did not alter the overall beer-
274 flavour. Compared to the beer, instead, the liqueur retained more hemp-derived compounds, exhibiting them
275 in its headspace and, thus, in its aroma bouquet.

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362 **Tables**

363 **Table 1.** Complete compositions of the two *Cannabis sativa* L. cultivars and of the pellets of *Humulus*
364 *lupulus* L. 'Chinook' essential oils.

Constituents	**I.r.i.**[a]	**Relative abundance (%) ± SD**		
		***Cannabis sativa* L.**	***Cannabis sativa* L. 'Futura**	***Humulus lupulus* L.**
		'Uso 31'	**75'**	**'Chinook'**
santolina triene	909	0.1±0.01	0.5±0.06	-[b]
α-thujene	931	0.1±0.08	0.2±0.04	-
α-pinene	941	11.9±0.32	17.8±0.54	-
camphene	954	0.1±0.13	-	-
pentyl propanoate	974	-	-	0.0±0.06
sabinene	976	0.2±0.06	0.2±0.03	-
β-pinene	982	5.1±0.33	6.1±0.30	0.3±0.10
myrcene	993	10.3±0.57	16.7±1.10	17.0±2.38
α-phellandrene	1005	0.3±0.06	0.2±0.00	-
δ-3-carene	1011	0.4±0.06	0.3±0.01	-
2-methylbutyl isobutanoate	1015	-	-	1.7±0.23
α-terpinene	1018	0.3±0.04	0.3±0.01	-
p-cymene	1027	0.2±0.03	0.2±0.03	-
limonene	1032	2.4±0.37	3.1±0.35	0.3±0.08
(*Z*)-β-ocimene	1042	1.4±0.13	0.7±0.00	-
(*E*)-β-ocimene	1052	5.7±0.72	4.1±0.45	-
γ-terpinene	1062	0.2±0.02	0.3±0.04	-
cis-sabinene hydrate	1070	0.2±0.01	0.2±0.04	-
terpinolene	1088	13.9±0.5	9.8±0.34	-
methyl 6-methyl heptanoate	1089	-	-	0.5±0.11
*iso*pentyl 2-methyl butanoate	1100	-	-	0.3±0.06
linalool	1101	-	0.1±0.01	0.3±0.01
nonanal	1102	-	0.3±0.03	-
pentyl isovalerate	1106	-	-	0.4±0.07
exo-fenchol	1119	-	0.2±0.01	-
trans-pinene hydrate	1123	-	0.1±0.13	-

cis-*p*-menth-2-en-1-ol	1124	-	0.1±0.14	-
trans-pinocarveol	1139	-	0.4±0.01	-
trans-limonene oxide	1141	-	0.1±0.20	-
(*E*)-myroxide	1142	0.5±0.01	0.3±0.48	-
β-pinene oxide	1158	-	0.1±0.08	-
pinocarvone	1163	-	0.1±0.00	-
borneol	1167	-	0.2±0.01	-
*iso*pinocampheol	1178	0.4±0.05	0.4±0.55	-
4-terpineol	1179	-	0.4±0.51	-
p-cymen-8-ol	1183	0.1±0.08	-	-
α-terpineol	1192	-	0.2±0.02	-
methyl 6-methyl octanoate	1193	-	-	0.5±0.09
hexyl butyrate	1194	0.2±0.03	-	-
cis-carveol	1229	0.1±0.12	-	-
geraniol	1257	-	-	0.7±0.03
methyl 6-methyl-nonanoate	1287	-	-	0.4±0.04
2-undecanone	1294	-	-	0.2±0.02
methyl-4-decenoate	1312	-	-	1.3±0.15
methyl geranate	1325	-	-	1.0±0.13
α-cubebene	1351	-	-	0.1±0.02
α-ylangene	1372	-	-	0.3±0.01
α-copaene	1376	-	-	1.2±0.13
1-hexyl-1-hexanoate	1385	0.2±0.01	0.4±0.01	-
β-elemene	1392	0.3±0.02	-	-
sativene	1395	-	-	0.1±0.13
(*Z*)-caryophyllene	1405	0.1±0.01	0.3±0.01	-
α-gurjunene	1410	0.1±0.01	-	-
β-caryophyllene	1420	12.7±1.00	8.4±0.80	9.9±1.48
β-copaene	1429	-	-	1.0±0.14
trans-α-bergamotene	1438	1.5±0.02	1.1±0.10	-
(*Z*)-β-farnesene	1445	0.1±0.01	-	-
α-humulene	1456	4.8±0.44	2.8±0.23	19.3±2.27
*allo*aromadendrene	1461	-	1.6±0.18	-
cis-muurola-4(14),5-diene	1462	-	-	0.1±0.11

9-*epi*-(*E*)-caryophyllene	1467	3.8±0.23	-	-
trans-cadina-1(6),4-diene	1470	-	-	0.4±0.04
γ-muurolene	1477	-	-	3.2±0.52
germacrene D	1478	-	-	0.5±0.07
β-selinene	1485	1.2±1.00	1.2±0.06	1.9±0.33
cis-β-guaiene	1490	0.7±1.00	-	-
viridiflorene	1493	0.7±1.00	0.9±0.04	3.1±0.54
α-selinene	1495	0.7±0.97	-	-
α-muurolene	1498	-	-	10.1±10.71
δ-amorphene	1505	-	0.1±0.08	0.3±0.35
β-bisabolene	1509	0.2±0.02	0.2±0.00	-
trans-γ-cadinene	1513	0.2±0.01	0.2±0.00	4.0±0.81
7-*epi*-α-selinene	1517	0.3±0.36	0.4±0.01	-
β-sesquiphellandrene	1524	-	0.2±0.00	-
δ-cadinene	1525	0.3±0.03	-	6.2±0.93
(*E*)-α-bisabolene	1531	-	0.2±0.25	-
(*E*)-γ-bisabolene	1535	-	0.2±0.01	-
(*Z*)-nerolidol	1536	0.2±0.04	-	-
α-cadinene	1538	-	-	1.0±0.16
selina-3,7(11)-diene	1542	1.5±0.08	1.3±0.01	2.5±0.40
cis-sesquisabinene hydrate	1545	0.1±0.09	0.1±0.01	-
germacrene B	1554	0.4±0.09	0.3±0.01	0.2±0.03
guaia-3,9-diene	1556	-	-	2.1±0.38
longipinanol	1560	-	0.4±0.02	-
(*E*)-nerolidol	1565	0.6±0.08	0.4±0.11	0.1±0.12
caryophyllene alcohol	1568	-	-	0.1±0.07
germacrene D-4-ol	1575	0.1±0.08	-	-
trans-sesquisabinene hydrate	1579	0.2±0.22	-	-
caryophyllene oxide	1581	5.6±0.13	5.5±0.05	-
(*E*,*Z*)-α-bisabolene epoxide	1586	-	-	0.2±0.01
carotol	1594	0.1±0.08	-	-
guaiol	1595	0.6±0.81	-	-
5-*epi*-7-*epi*-α-eudesmol	1603	-	0.1±0.08	-
humulene epoxide II	1607	2.0±0.16	1.7±0.01	0.3±0.03

1,10-*di*-*epi*-cubenol	1614	-	-	0.4±0.01
10-*epi*-γ-eudesmol	1623	0.6±0.18	1.0±0.11	-
β-cedrene epoxide	1624	-	0.2±0.35	-
1-*epi*-cubenol	1628	0.4±0.55	-	0.4±0.00
γ-eudesmol	1630	-	-	0.5±0.01
α-acorenol	1631	0.2±0.23	0.2±0.31	-
caryophylla-4(14),8(15)-dien-5-ol	1636	0.7±0.10	1.3±0.11	-
epi-α-cadinol	1640	0.2±0.25	-	0.8±0.00
epi-α-muurolol	1642	-	-	0.1±0.10
cubenol	1643	-	0.4±0.51	-
selina-3,11-dien-6-α-ol	1644	-	0.4±0.04	-
β-eudesmol	1650	-	-	0.2±0.01
α-eudesmol	1652	-	0.4±0.06	-
α-cadinol	1653	-	-	0.9±0.01
7-*epi*-α-eudesmol	1654	0.1±0.19	-	-
bisabolol oxide II	1655	0.1±0.17	-	-
(*E*)-11-tetradecen-1-ol	1673	-	-	0.2±0.29
aromadendrene epoxide I	1674	1.2±0.49	0.8±0.16	-
epi-α-bisabolol	1686	-	0.4±0.08	-
juniper camphor	1692	0.4±0.18	0.2±0.00	0.1±0.08
mayurone	1710	0.1±0.08	-	-
(*E*)-nerolidol acetate	1713	0.1±0.16	-	-
(*E*,*E*)-farnesol	1722	-	-	1.0±0.16
cannabidiol	2431	0.6±0.90	1.9±2.23	-
Δ^9-tetrahydrocannabinol	2468	-	tr[c]	-
Monoterpene hydrocarbons		52.4±3.13	60.5±2.16	17.6±2.56
Oxygenated monoterpenes		1.3±0.13	3.0±0.20	2.0±0.15
Sesquiterpene hydrocarbons		29.7±1.10	19.1±1.59	67.4±2.35
Oxygenated sesquiterpenes		13.2±2.33	13.5±1.02	4.9±0.54
Cannabinoids		0.6±0.90	1.9±2.23	-
Non-terpene derivatives		0.4±0.01	0.6±0.01	5.5±0.42
Total identified (%)		97.5±0.88	98.7±0.68	97.4±0.23
Extraction yield (% w/w)		0.08	0.08	1.63

[a] Linear retention indices on a DB5 column; [b] Not detected; [c] Traces, <0.1%.

365

366 **Table 2.** Compounds detected in essential oils of the two hemp cultivars contributing for at least 1.00% to
367 the dissimilarity of the samples.

Compounds	**Dissimilarity contribution (%)**	**Cumulative dissimilarity contribution (%)**
myrcene	13.32	13.32
α-pinene	12.22	25.54
β-caryophyllene	8.985	34.53
terpinolene	8.466	43.0
9-*epi*-(*E*)-caryophyllene	7.927	50.92
α-humulene	4.275	55.2
*allo*aromadendrene	3.362	58.56
(*E*)-β-ocimene	3.279	61.84
cannabidiol	2.677	64.52
β-pinene	2.117	66.63
limonene	1.536	68.17
cis-β-guaiene	1.473	69.64
α-selinene	1.432	71.07
(*Z*)-β-ocimene	1.39	72.46
caryophylla-4(14),8(15)-dien-5-ol	1.37	73.83
guaiol	1.204	75.04

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369

370 **Table 3.** Complete headspace volatile profiles of the two artisanal beers (control and hemp) and of the hemp

371 liqueur.

Constituents	l.r.i.[a]	Relative abundance (%) ± SD			Aroma contribution[b]
		Control beer	Hemp beer	Hemp liqueur	
4-methyl heptane	767	-[c]	0.7±0.01	-	
isobutyl acetate	778	0.4±0.52	-	-	
ethyl butyrate	802	2.7±0.79	1.8±0.01	-	Sweet, fruity, pineapple-like aroma
ethyl isovalerate	854	0.6±0.04	0.2±0.00	-	
*iso*pentyl acetate	876	13.8±3.80	17.5±0.13	-	Fruity, sweet, fragrant odor
styrene	890	12.9±1.24	9.4±0.05	-	Balsamic
propyl butanoate	898	1.6±0.16	2.3±0.01	-	Sweet, fruit (apricot, pineapple)
santolina triene	909	-	-	0.8±0.01	
α-thujene	931	-	-	0.4±0.01	
α-pinene	941	-	-	38.8±0.01	Pine, turpentine-like characteristic aroma
camphene	954	-	-	0.8±0.01	
ethyl 4-methylpentanoate	969	0.1±0.06	-	-	
*iso*pentyl propionate	970	-	0.1±0.01	-	
sabinene	976	-	-	0.2±0.01	
β-pinene	982	-	-	12.4±0.01	Woody, herbal, spicy notes
myrcene	993	5.6±1.44	9.8±0.12	28.0±0.01	Sweet, balsamic
ethyl hexanoate	997	11.7±0.27	10.2±0.01	-	Fruity (pineapple-, banana-like)
δ-3-carene	1011	-	-	0.8±0.00	
*iso*pentyl isobutanoate	1014	0.6±0.44	0.9±0.04	-	
2-methylbutyl *iso*butanoate	1015	2.3±0.52	4.2±0.19	-	Fruity (tropical, banan-like)
p-cymene	1027	-	-	0.2±0.00	
limonene	1032	0.6±0.08	0.5±0.13	5.5±0.23	Pleasant, lemon-like
(*Z*)-β-ocimene	1042	-	-	0.5±0.23	
(*E*)-β-ocimene	1052	-	-	2.1±0.00	
ethyl 5-methyl hexanoate	1072	0.4±0.06	0.1±0.09	-	
terpinolene	1088	-	-	0.1±0.01	
*iso*pentyl-2-methyl butanoate	1099	0.5±0.01	-	-	
ethyl heptanoate	1099	0.3±0.05	0.1±0.01	-	

linalool	1101	0.4±0.06	0.2±0.01	-	
pentyl *iso*valerate	1106	0.8±0.11	1.6±0.03	-	Fruity (apple)
phenylethyl alcohol	1110	1.0±0.81	0.8±0.37	-	Floral, rose-like
allo-ocimene	1129	-	-	0.1±0.08	
trans-pinocarveol	1139	-	0.1±0.08	-	
neo-*allo*-ocimene	1145	-	-	0.1±0.01	
methyl 2-methyl octanoate	1156	2.5±0.17	1.1±0.01	-	Fruity, wine- and brandy-like
ethyl octanoate	1196	32.2±3.33	24.7±0.25	-	Fruity, floral, wine-apricot notes
2-phenylethyl acetate	1258	-	0.2±0.00	-	
2,4-decadien-1-ol	1264	0.7±0.02	-	-	
ethyl nonanoate	1298	0.1±0.11	-	-	
methyl geranate	1325	0.2±0.01	0.3±0.01	-	
ethyl (*Z*)-4-decenoate	1382	0.3±0.06	0.3±0.01	-	
ethyl (*E*)-9-decenoate	1387	0.4±0.15	0.7±0.01	-	
ethyl decanoate	1395	2.4±1.03	6.5±0.01	-	Fruity, grape- and brandy-like
(*Z*)-caryophyllene	1405	-	-	0.1±0.01	
β-caryophyllene	1420	1.1±0.21	1.0±0.03	5.8±0.13	Woody, spicy (clove-like)
γ-elemene	1433	-	-	0.1±0.01	
trans-α-bergamotene	1438	-	-	0.6±0.07	
α-humulene	1456	3.0±0.32	2.3±0.03	1.3±0.01	Woody, spicy (clove-like)
*allo*aromadendrene	1461	-	-	0.6±0.01	
γ-muurolene	1477	0.3±0.03	0.2±0.02	-	
β-selinene	1485	0.1±0.08	0.4±0.12	0.6±0.01	
viridiflorene	1493	0.1±0.11	0.3±0.10	0.4±0.04	
trans-γ-cadinene	1513	0.1±0.12	-	-	
δ-cadinene	1525	0.2±0.31	0.3±0.01	-	
ethyl dodecanoate	1596	-	0.4±0.04	-	
Monoterpene hydrocarbons		6.1±1.52	10.3±0.01	90.4±0.10	
Oxygenated monoterpenes		0.6±0.04	0.6±0.10	-	
Sesquiterpene hydrocarbons		4.8±0.12	4.5±0.05	9.5±0.09	
Non-terpene derivatives		88.1±1.47	84.0±0.09	-	
Total identified (%)		99.6±0.03	99.4±0.04	99.99±0.01	

[a] Linear retention indices on a DB5 column; [b] Aroma descriptors as reported in Burdock (2010) and/or The Good Scents Company Database (http://www.thegoodscentscompany.com); [c] Not detected.

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Figures

Figure 1. Dendrogram of the hierarchical cluster analysis (HCA) performed on the complete headspace profiles of the beverages, and the essential oil compositions of the two *Cannabis sativa* cultivars and the hop pellets.

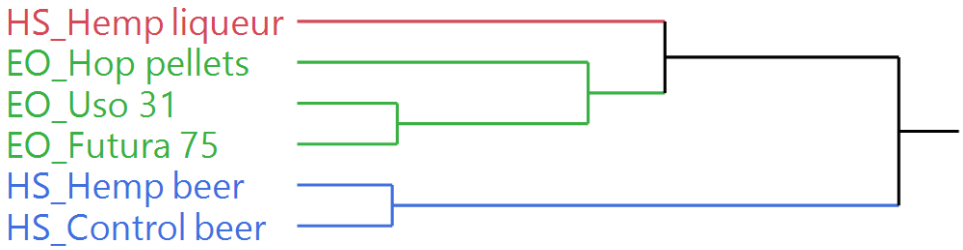

Figure 2. Score plot of the principal component analysis (PCA) performed on the complete headspace profiles of the beverages, and the essential oil compositions of the two *Cannabis sativa* cultivars and the hop pellets.