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Characterization of volatile components in hop pellets using in-tube extraction GC-MS analysis

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Abstract

Hops (Humulus lupulus L.) are added to beer to impart bitterness and aroma. Our group of researchers in the field of hops, has investigated the bitter acids profile and volatile profile of hop cones for Hüller Bitterer and Magnum varieties. The main objective of these investigations was to identify volatile compounds from hop products (pellets) and determine differences between the hop varieties by cluster analysis. The pellets sample were harvested during 2011 and 2012. The hydrodistillation was used to obtain hop pellets essential oils. The Cluster analysis highlighted the main group and the minor components from essential oils and their evolution in the analyzed samples (pellets and essential oils). Using the ITEX/GC-MS technique, 48 volatiles were separated from hop pellets, with 46 of them being identified. The main components of hop essential oils included monoterpenes (β-myrcene) and sesquiterpenes (β-caryophyllene and α-humulene). The advantages of headspace ITEX/GC-MS are small sample sizes, increased sensitivity, solvent-free extraction and relatively low cost. The performed analysis can be an easy-to-use tool evaluating different chemotypes of hops, with relevance for the hop industry. The assessment of the results by cluster analysis provides phytochemical composition data, which are indispensible for standardization and quality control of plant raw materials required in food or pharmaceutical industry.

Keywords: Humulus Lupulus L., hop pellets, essential oils, cluster analysis, ITEX/GC-MS

1. Introduction

Hops (Humulus lupulus L.) are vital for the brewing industry, as they contribute significantly to the organoleptic qualities of beer, including taste and flavor (PAVLOVIC & al. [1]). Since ancient times, hops have been used in folkloric medicine for their claimed antiseptic, hypnotic, sedative, antidiuretic, anti-inflammatory, aphrodisiac and stomachic properties (VAN CLEEMPUT & al. [2]). In the last ten years the estrogenic properties as well as the potential cancer chemopreventive activities of hop have been investigated and some bioactive compounds, including xanthohumol, humulone, 8-prenylnaringenin and lupulone (STEVENS & al. [3]; ZANOLI & al. [4]; ERKKOLA & al. [5]; TYRRELL & al. [6]) have received much attention.

Hops extracts are currently marketed as botanical dietary supplements for the relief of hot flashes in menopausal women as an alternative to hormone replacement therapy; they have also been used for treating insomnia and anxiety (LIU & al. [7]). Dried hops cones (with 9-11% moisture) typically contain 0.5%-3% oil by mass (ERI & al. [8]). The characteristic profile of chemical components in hop cones and products (pellts, extract, etc.) was used by

different researchers to identify cultivars (DE COOMAN & al. [9]; JORGE & al. [10]; PATZAK & al. [11]; NANCE & al. [12]). Geographical location, climate and agronomical factors are the main factors that affect volatile composition of hops (GONÇALVES & al. [13]). The protocols most commonly used for the extraction and identification of volatile compounds from different matrices are those based on gas chromatography and mass spectrometry techniques (ERI & al. [8]; JORGE & al. [10]; GONÇALVES & al. [13]; BERTNOTIENE & al. [14]; COLDEA & al. [15]; VAN OPSTAELE & al. [16]; VAN OPSTAELE & al. [17]; COLDEA & al. [18]; LIGOR & al. [19]). "In-tube extraction" (ITEX) is a relatively new technique (commercially available since 2006, by CTC Analytics AG, Zwingen, Switzerland) similar to purge-and-trap systems (SOCACI & al. [20]).

This technique requires no or minimal sample preparation, allowing a simple, efficient and rapid enrichment of volatile or semi-volatile compounds during the headspace extraction (SOCACI & al. [21]) and can be coupled with gas-chromatography-mass-spectrometry for further separation and identification of compounds. Shortly, the sample introduced in a sealed vial is heated under continuous agitation until equilibrium is reached. A special syringe equipped with a trap, containing an adsorbent material (Tenax, Carbosieve, etc.), will then pierce the vial septum and the volatiles from the headspace phase will be adsorbed on the fibre. After the thermo-desorption of volatiles directly into GC injector, the hot ITEX trap is cleaned with inert flush gas before the next sampling (SANDRA & al. [22]). The ITEX/GC-MS technique was previously successfully applied for fingerprinting the volatile profile of other food matrices, including beer, and the obtained data further used in discrimination (LAAKS & al. [23]; ZAPATA & al. [24]; PĂUCEAN & al. [25]; LAAKS & al. [26]; SEMENIUC & al. [27]).

Hop flowers are produced in various products such as pellets, hop extracts and volatile oils, mainly used in the brewing industry (VÂRBAN & al. [28]). The international hops trade liberalization forced most growers of hop to process cones to ensure conservability of active principles, reducing the volumes of transport, in order to standardize dosing easier and ensure consistency of quality of the finished beer. The chemical composition of products depends on the hop variety, processing technology adopted and the performance of processing equipment (MUDURA & al. [29]).

Our group of researchers in the field of hops, has investigated the bitter acids profile (SALANȚĂ & al. [30]) and volatile profile of hop cones for Hüller Bitterer and Magnum varieties (SALANȚĂ & al. [31]; SALANȚĂ & al. [32]; SALANȚĂ & al. [33]). The main objective of these investigations was to identify volatile compounds from hop products (pellets) and determine differences between the hop varieties by cluster analysis.

2. Materials and Methods

Plant material. Two different varieties of the Humulus lupulus L., were taken into study. Cultivars Magnum (MG), classified as high α-acid and Hüller Bitterer (HB) classified as aroma (low α-acid), cultivated in the pedo-climatic areas of Transylvania, in the Seleuş farms from Mureş county (Romania), were harvested in 2011 and 2012 years crop. The pellets samples (type-90) were obtained from the pelletization station of Seleuş farm. Hop essential oils were extracted from the hop pellet samples harvested in 2011 and 2012, respectively. All hop samples were labelled and stored at -20ºC until the analysis. Prior to analysis they were left to reach room temperature.

Essential oil extraction. The samples of hop essential oil were isolated by hydrodistillation technique, using a Clevenger type apparatus (SALANȚĂ & al. [31]; SALANȚĂ & al. [32]; SALANȚĂ & al. [33]). As follows, 50 g of ground hop pellets

(ground in a coffee mill) were weighed into a distillation flask and 700 ml distilled water were added. The distillation time was 3.5 hours since the distillation begins. The obtained essential oil was collected and measured. Yield was calculated as ml of essential oil per 100g plant material free of moisture.

Extraction of volatile compounds. The extraction of volatile compounds was performed using the ITEX technique, as described our previous study (SALANȚĂ & al. [31]). The optimal extraction was achieved using 1g of hop pellet samples (p) as well as 1µL of hop essential oil samples (peo). Both types of samples were incubated at 60oC for 20 minutes. The enrichment of ITEX fibre (ITEX-2 TrapTXTA, (G23)-Siliconert 2000, Tenax TA 80/100 mesh, Switzerland) with the extracted volatile compounds was performed during 30 strokes. All samples were analysed in duplicate.

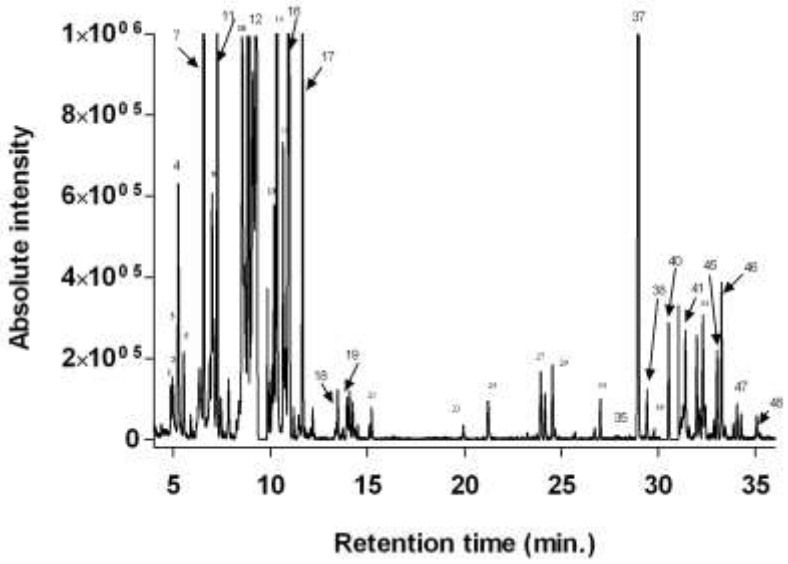
GC-MS analysis. The separation and identification of volatile compounds was carried out on a Shimadzu GC-MS QP-2010 (Shimadzu Scientific Instruments, Kyoto, Japan) model gas chromatograph-mass spectrometer equipped with a CombiPAL AOC-5000 autosampler (CTC Analytics, Zwingen, Switzerland). A Zebrone ZB - 5ms column of 50m x 0.32 mm i.d. and 0.25 µm film thickness was used for the separation. The program for column oven temperature was: 60ºC (held for 3 min), raised to 160ºC at 3ºC/ min and held for 10 min. The other parameters of the method were: injector temperature 250ºC; split ratio 1:20, carrier gas-helium 1.39mL/min, detector MS, ion source temperature 250ºC, interface temperature 250ºC, MS mode EI, scan range 50-400u. The identification of volatile compounds was carried out by comparing the obtained mass spectra with NIST27 and NIST147 library information and verified by comparison with retention indices drawn from www.pherobase.com or www.flavornet.org (for columns with a similar stationary phase to the ZB-5ms column).

Statistical analysis. For the characterisation of hop varieties, the obtained chromatographic matrix was subjected to cluster analysis (CA) with the Euclidean distances. Chemometric analysis was performed using Matlab (version 7.2.0232/2006).

3. Results and discussion

Fingerprinting of volatile components by ITEX/GC-MS analysis. In beer industry, hop is no longer used for a long time as a flower. Products used today are hop pellets, hop extracts, preisomerized or isomerised products. In these conditions to maintain varietal purity is sometimes difficult even for advanced processing units. In these circumstances for correct information to the recipient we have identified specific tools for the traceability of the variety of product, from harvest to processing products (MUDURA & al. [29]). The volatile composition of hop cones was established in our previous studies (SALANȚĂ & al. [31]; SALANȚĂ & al. [32]; SALANȚĂ & al. [33]). The present study aims to use the specific volatile profile of each hop pellets variety as a tool for their traceability. The percentage of essential oils, which was obtained by hydrodistillation varied from 1.35% to 1.91% for Hüller Bitterer variety, and from 1,71% to 1,94% for Magnum variety, no significant differences being found between years of harvest. The extraction of volatile compounds was performed using the ITEX technique whereas separation and identification was performed using gas-chromatography-mass spectrometry. The results of GC-MS analysis confirmed that pellets essential oil as well as pellets contained a complex mixture of constituents. Accordingly, 48 compounds were separated and of these 46 were identified. Characteristic ITEX/GC-MS chromatograms of each studied hop variety are presented in Figure 1, showing the specific volatile profile of Hüller Bitterer (A) and Magnum (B) hop varieties.

(A)



(B)

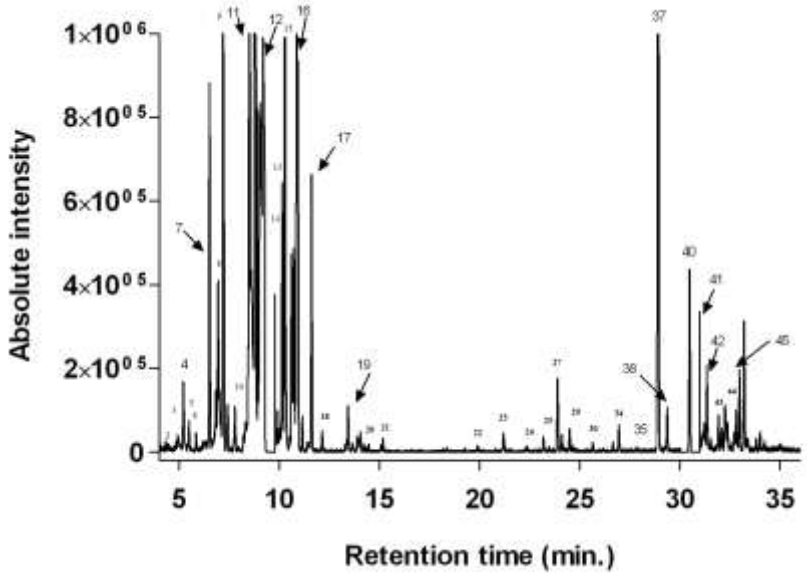


Figure 1. Chromatograms (TIC) of ITEX/GC-MS analysis of volatiles from Hüller Bitterer variety (A) and Magnum variety (B). The numbering of the peaks refers to the Table 1

The volatiles identified in the highest concentration in all hop cultivars were: linalool **(4)**, β-pinene **(11)**, β-myrcene **(12)**, β-caryophyllene **(35)** and α-humulene **(38)**. The hop aroma components are almost entirely terpenes oils, sulfur compounds, or derivatives thereof. The hop constituents determined using GC-MS included mainly esters: 1-methylbutyl propanoate **(10)**, 2-methylbutyl 2-methylpropanoate **(13)**, 2-methylbutyl 3-methylpropanoate **(14)**, methyl 4-decenoate **(29)**, methyl heptanoate (**15**), methyl 6-methyl heptanoate (**21**), methyl octanoate (**25**); ketones: 2-nonanone (**22**), 2-undecanone (**28**), 2-dodecanone (**34**); alcohols: 1-butanol, linalool (**4**), and terpenes: α-pinene (**7**), β- pinene (11), limonene **(16)**, β-phellandrene (17), β-*cis*-ocimene **(19)**; our findings corroborating with the previously

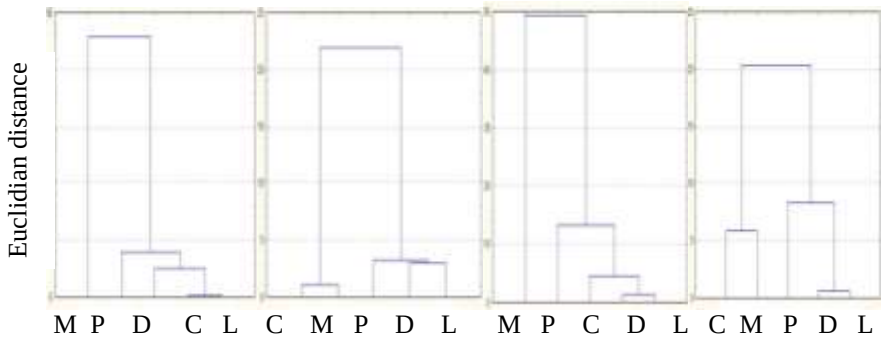
reported studies (VAN OPSTAELE & al. [16]; VAN OPSTAELE & al. [17]; STEINHAUS & al. [34]; TAKOI & al. [35]).

Due to the thermal treatment during the peletization process or during hydrodistillation extraction of essential oils, from qualitative point of view, the volatile profile of pellets and the corresponding essential oils may differ. Thus some oxygenated compounds can be formed in pellets and essential oil while other volatile aroma compounds may be lost. Nevertheless, even though some minor compounds were found only in pellets or in essential oils samples, the main ones are the same in all types of samples. Each variety of hop has its own typical essential oil pattern which is an important marker for the determination of hop chemotypes, ecotypes or evaluation of hop quality (JELÍNEK & al. [36]). Volatiles detected in pellets and essential oils samples, are listed in Table 1 (2011 and 2012 harvest) and expressed as percentages of total peak area. For 1st year harvest, the concentrations of the main components of hop essential oils samples varied between 1.41 % and 6.26 % for linalool; 1.13 % and 4.49 % for α -pinene; 20.19 % and 50.53 % for β-myrcene; 6.09 % and 15.59 % for β-pinene; 1.07 % and 3.3 % for β-phellandrene; 1.22 % and 2.93 % for limonene; 6.04 % and 22.72 % for β-caryophyllene; 0.44 % and 1.93 % for γ-muurolene; 0.1 % and 1.88 % for β-selinene; 0.39 % and 1.27 % for γ-cadinene; 0.55 % and 1.92 % for σ-cadinene. The composition of essential oils was relatively stable in both cultivars, no significant differences being found between years of harvest.

Hüller Bitterer aroma variety presented low levels of β-myrcene comparative with bitter sample Magnum. In the analysed samples, β-myrcene and linalool were found in higher quantity in pellets obtained from 2011 harvest. The monoterpene myrcene is produced in the young cones immediately, and is typically the largest constituent of the essential oil (as much as 70% by volume) (EYRES & al. [37]). Linalool is a key contributor to the hoppy flavour and a source of the floral note in beer (HANKE [38]). On the one side, there is a good correlation between linalool content and perceived fruity-flowery flavour and on the other hand, linalool exceeds its threshold and, therefore, contributes actively to the aroma of hoppy beers. This compound has an active aroma contribution (38). Compounds such as aromadendrene **(37)**, β-guaiene **(46)**, valencene **(47)** and γ-elemene **(50),** were detected only in samples of aroma variety, instead camphene **(9)** was specific for bitter samples.

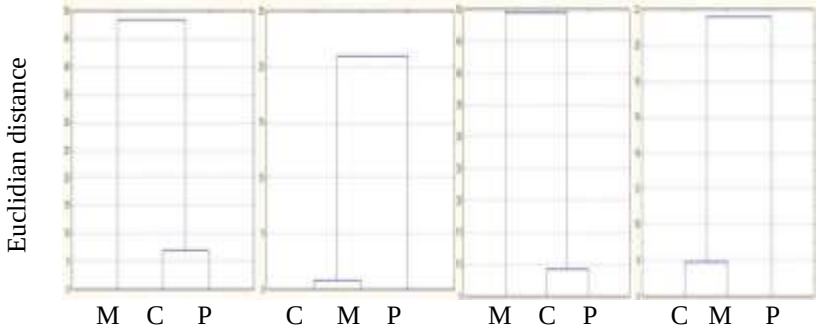
For Magnum variety, which is considered to be one of the most important commercially, the content of camphene varies between 0.15 and 0.26 %, similar concentrations (0.05- 0.26 %) with those reported in our previous researches on hop cones (SALANȚĂ & al. [31]). The proportion of β-pinene (8.08-15.59 %) and β-phellandrene (1.28-3.3 %) was higher in samples from Magnum variety compared with Hüller Bitterer variety. A distinctive characteristic for the aroma variety was the relative high content of γ-murolene (0.53-1.93 %) and β-selinene (0.41-1.88 %). Comparing the results obtained from cones (SALANȚĂ & al. [31]) and pellets samples it can be observed a difference between the content of volatile compounds, which implies changing qualitative markers during the processing of hops and aggressive drying in specialized installations.

Cluster analysis. Using cluster analysis there have been highlighted both major and minor volatile compounds present in hop varieties. The dendrograms obtained for the major volatile compounds identified and quantified in the experimental period (2011 and 2012 harvest) (Figure 2 and 3), reveal as a major compound β-myrcene for samples of pellets, and β-caryophyllene for samples of volatile oil extracted from pellets; at the opposite pole is linalool for all samples from 2011 year crop and β-pinene for samples from 2012 year crop.



a. HB pellets b. essential oil from HB pellets c. MG pellets d. essential oil from MG pellets

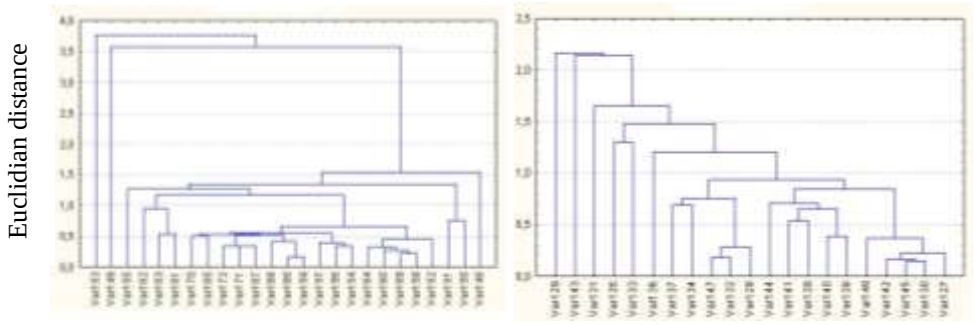
Figure 2. Dendograms of major compounds found in Hüller Bitterer (a,b) and Magnum (c,d) hop varieties samples / 2011 year crop (M-β myrcene, P-β pinene, D-limonene, C-caryophyllene, L-linalool)



a. HB pellets b. essential oil from HB pellets c. MG pellets d. essential oil from MG pellets

Figure 3. Dendograms of major compounds found in Hüller Bitterer (a,b) and Magnum (c,d) hop varieties samples / 2012 year crop (M-β myrcene, C-caryophyllene, P-β pinene)

The dendrograms of the major compounds have the same classification in the case of all types of samples, resulting in a group of similarity. The major compounds found in all samples are represented by: β-myrcene, β-caryophyllene and β-pinene. In case of minor components, an example of dendrograms is presented in Figure 4. Predominant compounds are represented by 2-methyl-3-methylbutyl propanoate, linalool, α-pinene, while γ-murolene and β-selinene belong to the category of those identified in a minimum quantity.



a. HB samples b. MG samples

Figure 4. Dendogram of minor compounds found in Hüller Bitterer (a) and Magnum (b) hop pellets / 2012 year crop

The volatile composition of the pellets samples is well differentiated from the volatile composition of the essential oil extracted by hydrodistillation. Chemometric method have shown that the qualitative volatile composition of hop are generally characteristic of all varieties, while quantitatively specific to each variety, and thus the profile of the volatile fraction can be used for variety authentication and discrimination.

4. Conclusion

The results showed that the headspace ITEX/GC-MS technique is suitable, fast and easy to apply for the extraction and analysis of volatile compounds from hop products (pellets), 48 volatiles were separated, with the cost/sample and analysis time being reduced. Evaluation of results by cluster analysis provides phytochemical composition data, which are indispensible for standardization and quality control of plant raw materials required in food or pharmaceutical industry.

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Table 1. Mean relative concentrations (expressed as % from total peak areas) and standard deviations (SD) of volatile compounds from pellets and essential oils of hop varieties analyzed by the headspace ITEX/GC–MS technique* / 2011 and 2012 harvest

No.	Compound name	HB p	±SD	HB peo	±SD	MG p	±SD	MG peo	±SD	HB p	±SD	HB peo	±SD	MG p	±SD	MG peo	±SD
									2011								2012
1.	2-Hexenal	-	-	-	-	-	-	0.19	±0.06	-	-	-	-	-	-	0.13	±0.04
2.	2-Methylpropyl propionate	1.19	±0.04	0.45	±0.06	0.41	±0.04	0.20	±0.04	1.50	±0.05	0.61	±0.04	0.30	±0.03	0.14	±0.04
3.	1-Butanol, 3-methyl-, formate	0.31	±0.10	0.14	±0.06	0.11	±0.04	0.1	±0.01	0.24	±0.04	0.17	±0.03	0.11	±0.02	0.10	±0.04
4.	Linalool	6.26	±0.03	3.93	±0.01	1.99	±0.04	1.74	±0.09	5.55	±0.03	3.89	±0.11	1.49	±0.01	1.41	±0.03
5.	Methyl hexanoate	0.18	±0.05	0.15	±0.03	0.22	±0.02	0.19	±0.04	0.82	±0.00	0.04	±0.04	0.20	±0.02	0.14	±0.00
6.	1,3-Nonadiene	1.13	±0.01	0.31	±0.02	0.95	±0.05	0.22	±0.02	1.00	±0.00	0.20	±0.01	0.75	±0.03	0.18	±0.00
7.	α-Pinene	2.85	±0.04	1.13	±0.01	3.10	±0.14	2.71	±0.01	2.99	±0.01	1.14	±0.02	4.49	±0.04	2.04	±0.08
8.	Thiopivalic acid	0.06	±0.02	0.11	±0.01	0.21	±0.17	0.20	±0.06	0.05	±0.00	0.15	±0.04	0.18	±0.00	0.19	±0.07
9.	Camphene	-	-	-	-	0.25	±0.01	0.15	±0.04	-	-	-	-	0.21	±0.04	0.26	±0.00
10.	1-Methylbutyl-propanoate	4.16	±0.23	3.77	±0.03	3.88	±0.11	5.10	±0.08	2.99	±0.10	1.61	±0.04	2.6	±0.00	3.92	±0.14
11.	β-Pinene	11.84	±0.15	6.09	±0.04	15.59	±0.28	8.08	±0.11	11.52	±0.05	7.96	±0.25	14.15	±0.13	8.19	±0.04
12.	β-Myrcene	44.22	±0.31	22.20	±0.56	50.53	±0.55	26.56	±0.14	42.67	±0.44	20.19	±0.26	48.09	±0.50	28.27	±0.14
13.	2-Methylbutyl 2-methylpropanoate	1.10	±0.11	0.8	±0.06	1.35	±0.14	1.64	±0.03	0.63	±0.05	0.70	±0.08	1.84	±0.11	1.42	±0.13
14.	2-Methylbutyl 3-methylpropanoate	6.94	±0.06	5.95	±0.37	2.77	±0.15	3.43	±0.15	4.40	±0.13	3.24	±0.17	2.81	±0.17	2.22	±0.85
15.	Methyl heptanoate	1.11	±0.15	1.05	±0.04	0.68	±0.16	0.90	±0.34	1.63	±0.00	1.29	±0.04	1.52	±0.01	0.86	±0.00
16.	Limonene	2.44	±0.04	1.65	±0.09	2.93	±0.01	2.18	±0.04	1.31	±0.01	1.50	±0.21	0.45	±0.01	1.22	±0.01
17.	β-Phellandrene	1.81	±0.09	1.07	±0.10	1.90	±0.09	1.28	±0.07	2.19	±0.04	1.83	±0.05	3.3	±0.01	2.99	±0.02
18.	β-trans-Ocimene	0.08	±0.01	1.04	±0.09	0.09	±0.01	0.16	±0.01	1.12	±0.00	1.08	±0.06	2.09	±0.03	2.4	±0.11
19.	β-cis-Ocimene	1.91	±0.06	0.64	±0.08	0.61	±0.13	0.89	±0.06	1.02	±0.00	1.10	±0.02	0.15	±0.01	0.10	±0.01
20.	Not identified	0.06	±0.04	0.06	±0.01	0.05	±0	0.09	±0.01	0.04	±0.01	0.06	±0.21	0.05	±0.01	0.07	±0.01

21.	Methyl 6-methyl heptanoate	0.19	±0.00	0.15	±0.01	0.21	±0.08	0.32	±0.25	-	-	0.52	±0.00	-	-	0.67	±0.00
22.	2-Nonanone	0.30	±0.02	0.56	±0.08	0.80	±0.05	1.04	±0.00	0.35	±0.01	1.08	±0.08	1.69	±0.05	1.72	±0.02
23.	n.i.	0.21	±0.03	1.24	±0.11	0.86	±0.06	0.58	±0.34	0.15	±0.01	0.90	±0.00	0.52	±0.10	0,7	±0.04
24.	Amyl isovalerate	0.55	±0.00	1.98	±0.09	0.45	±0.00	0.95	±0.02	0.75	±0.06	2.01	±0.04	0.06	±0.00	1.30	±0.01
25.	Methyl octanoate	0.10	±0.00	0.23	±0.08	-	-	0.27	±0.03	-	-	0,22	±0,02	-	-	0.40	±0.02
26.	Not identified	0.08	±0.11	2.80	±0.12	-	-	0.61	±0.33	-	-	0.76	±0.05	-	-	0.56	±0.04
27.	Methyl decanoate	-	-	0.35	±0.05	-	-	0.36	±0.07	-	-	0.69	±0.09	-	-	0,68	±0,00
28.	2-Undecanone	-	-	0.45	±0.01	-	-	1.25	±0.11	-	-	0.69	±0.09	0.99	±0.04	2.58	±0.12
29.	Methyl 4-decenoate	0.31	±0	2.99	±0.05	0.50	±0.02	4.80	±0.06	-	-	0.59	±0.06	-	-	2.47	±0.01
30.	Methyl nerolate	0.36	±0	3.15	±0.16	0.1	±0.01	1.33	±0.04	-	-	1.3	±0.1	-	-	1.67	±0.05
31.	α-Cubebene	-	-	-	-	0.14	±0.01	0.04	±0.02	1.31	±0.00	2.82	±0.12	0.19	±0.04	1.1	±0.02
32.	Ylangene	-	-	0.27	±0.03	-	-	0.27	±0.04	-	-	0.59	±0.06	-	-	-	-
33.	α-Copaene	0.18	±0.01	0.92	±0.06	-	-	0.91	±0.02	-	-	0.97	±0.09	-	-	0.46	±0.03
34.	2-Dodecanone	-	-	0.02	±0.04	-	-	0.08	±0.03	0.99	±0.02	3.02	±0.06	-	-	0,97	±0,08
35.	β-Caryophyllene	6.04	±0.28	20.38	±0.18	6.21	±0.28	22.44	±0.04	8.63	±0.10	20.81	±0.28	7.64	±0.69	22.72	±0.18
36.	Germacrene D	0.22	±0.01	0.61	±0.03	0.24	±0.04	0.61	±0.01	0.25	±0.02	1.14	±0.14	0.28	±0.02	0.20	±0.08
37.	Aromadendrene	-	-	0.19	±0.01	-	-	-	-	-	-	0.62	±0.04	-	-	-	-
38.	α-Humulene	0.41	±0.03	1.86	±0.04	0.78	±0.03	3.65	±0.04	1.1	±0.03	1.75	±0.06	0.75	±0.04	3.14	±0.11
39.	γ-Muurolene	0.53	±0.05	1.51	±0.09	0.44	±0.02	1.31	±0.03	0.77	±0.04	1.93	±0.04	0.59	±0.04	0.98	±0.04
40.	β-Selinene	0.43	±0.13	1.67	±0.07	0.1	±0.07	0.51	±0.01	0.41	±0.04	1.88	±0.04	0.27	±0.01	0.35	±0.04
41.	α-Guaiene	0.59	±0.18	2.24	±0.09	0.27	±0.07	-	-	0.69	±0.03	2.26	±0.09	0.35	±0.06	-	-
42.	α-Amorphene	0.11	±0.04	0.50	±0.04	0.05	±0.03	0.47	±0.02	-	-	0.88	±0.05	0.46	±0.03	-	-
43.	γ-Cadinene	0.42	±0.04	1.27	±0.15	0.41	±0.16	1.20	±0.04	0.46	±0.01	1.19	±0.05	0.62	±0.03	0.39	±0.03
44.	δ-Cadinene	0.55	±0.21	1.92	±0.04	0.82	±0.01	1.77	±0.11	1.28	±0.04	1,82	±0,06	1.85	±0.06	1.53	±0.01
45.	Not identified	-	-	-	-	-	-	-	-	-	-	1.2	±0.01	-	-	-	-
46.	β-Guaiene	0.14	±0.01	0.75	±0.08	-	-	-	-	0.17	±0.08	0.41	±0.05	-	-	-	-
47.	Valencene	0.45	±0.03	1.23	±0.04	-	-	-	-	0.44	±0.03	1.65	±0.13	-	-	-	-
48.	γ-Elemene	0.18	±0.03	0.20	±0.05	-	-	-	-	0.2	±0.01	1.64	±0.08	-	-	-	-

„HB"- Hüller Bitterer variety; „MG"- Magnum variety; „p"- hop pellets; „peo"- essential oils from pellets; n.i.- not identified

* All samples were analysed in duplicate

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