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Posted Date: 10 December 2025

doi: 10.20944/preprints202512.0744.v1

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 doi:10.20944/preprints202512.0744.v1

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Effects of Drying Methods on Terpene Content in Hops and Beer

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† The work presented here is part of the master's thesis of Andrea Fasolo.

Abstract

Dried hops are used in beer production for imparting bitterness and characteristic aroma. Herein, Cascade hop cones are dried using the following two methods: conventional hot-air drying at 52 °C and innovative low-temperature drying at 30 °C via heat-pump technology. The dried hops are used either as whole cones or processed into pellets for brewing. The terpenoid composition of fresh hops, dried cones, pellets and the resulting beers is analysed using headspace solid-phase microextraction coupled with gas chromatography–quadrupole mass spectrometry. Twenty-three mono- and sesqui-terpenes are identified in fresh hops, while 23–26 compounds are detected in dried hops and their corresponding beers, depending on the drying method. Beers brewed with cold-dried cones exhibit a higher concentration of terpenes, especially oxygenated terpenoids and sesqui-terpenes. By contrast, hot-dried pellets exhibit major proportion of monoterpenes and fewer sesquiterpenes. However, cold-dried pellets result in higher levels of oxygenated sesquiterpenes in the final product. These results suggest that hop-drying temperature and physical form markedly affect the aromatic profile of beer. Furthermore, variations in hop terpenoids can influence aroma development through yeast biotransformation during fermentation.

Keywords: hot oven-drying; heap-pump drying; hop; beer; terpenic compounds

1. Introduction

The female cones of hops (*Humulus lupulus* L.), a flowering plant in the Cannabaceae family, play a vital role in brewing by contributing to the characteristic bitterness and distinctive flavour of beer. This flavour is imparted by lupulin, a golden, resinous substance produced by the female flowers which are rich in bitter α-acids, aromatic compounds and polyphenols [1]. The signature 'hoppy' aroma of beer is attributed to essential oils in lupulin, which comprise a complex blend of volatile compounds, mainly hydrocarbons such as monoterpenes and sesquiterpenes. These are particularly abundant in aromatic hop varieties [2].

Hops can be added to the brewing process as dried cones, pellets or extracts at various stages of production; their addition enables brewers to fine-tune the aroma profile of the beer according to their preference. During fermentation, active yeast can further change the aroma by converting certain terpenes such as transforming geraniol into β-citronellol and thereby modify the hop-derived profile [3].

Following harvest, hops can be dried to prevent microbial spoilage and mould growth during storage. In addition to preservation, drying highly influences the sensory quality of the hops and the beer brewed from them [4, 5, 6]. Although optimal drying conditions vary with hop variety and environmental factors, standard practice is to reduce the moisture content to 8%–10% at temperatures below 60 °C, thereby minimising the loss of volatile aromatic compounds [7].

Heat-pump drying, referred to as cold drying, is an emerging method in food processing that offers considerable advantages for delicate botanical materials. Owing to its operation at lower temperatures (typically 30 °C–40 °C or even as low as 18 °C–20 °C), this technique preserves the colour, structure, aroma and nutritional profile of hops while reducing the degradation of essential compounds [8, 9].

 doi:10.20944/preprints202512.0744.v1

Hops are dried using a heat-pump dryer and a conventional hot-air dryer. Subsequent analyses help evaluating differences in terpenoid composition as well as the aromatic attributes of beers brewed using hops processed by each drying method.

2. Materials and Methods

2.1. Hop Samples

Three batches of fresh hop cones (*H. lupulus* L., Cascade variety) were collected from a single site in Piozzo (Cuneo, Italy). The raw material was provided by the Baladin Beer company (Piozzo, Cuneo, Italy). Fresh hops had an average moisture content of ~72%. Hot drying was performed at 65 °C using a traditional dryer, while cold drying was performed using a heat-pump drying system at 30 °C (North West Technology, Boves, Cuneo, Italy). The dried cone showed 7%–8% of humidity. The hot-dried cones (HDC) and cold-dried cones (CDC) were immediately pelletised after drying to obtain Type 90 hot-dried hop pellets (HDP) and cold-dried hop pellets (CDP), respectively.

2.2. Beer Production

Cones and pellets were used for the production of a single malt ALE-style beer. Brewing was performed in a G30v3 semi-automatic system (Grainfather®, Bevie Handcraft, Europe). Barley malt of the Pilsner type (Mr. Malt®, Pasian di Prato, Udine, Italy) and SafAle S-33 yeast (Fermentis®, France) were used.

For beer production, 5 kg of malt was milled and mashed with 17 L of water at 45 °C for 12 min (β-glucan rest), followed by successive rests at 52 °C (12 min, protein rest), 65 °C (60 min, β-amylase phase), 72 °C (30 min, α-amylase phase) and 78 °C (10 min, mash-out). Sparging was performed with 12 L of 60 °C water. The wort was boiled for 60 min to reach a density of 1.0459 g/mL; then, it was cooled to 20 °C and transferred to a 30-L fermenter. Primary fermentation lasted for 5 days at 23 °C.

Dry-hopping was performed by transferring the fermented beer into 1.5-L bottles and adding 2 g/L of the respective hop form. Secondary fermentation was induced by adding sucrose (6 g/L) and storing bottles at 25 °C for 7 days. Maturation was completed at 4 °C for 21 days. No filtration was applied.

2.3. Volatile Compound Analysis in Hops

An untargeted volatile compound analysis of fresh and dried hops was performed using headspace solid-phase microextraction (HS-SPME), followed by gas chromatography–quadrupole mass spectrometry (GC–qMS). A CAR/PDMS/DVB fibre (1 cm, 50/30 µm; Supelco, USA) was used with an SPME autosampler (PAL System, Combi PAL, Switzerland). For each sample, 0.2 g of hop material (cones or pellets) was combined with 1 mL of distilled water and 10 µL of internal standard (1,3,5-triisopropylbenzene, 94.4 ppm). Samples were equilibrated at 50 °C for 15 min, which was followed by fibre exposure to the headspace for 30 min at 50 °C. Desorption was performed in split mode (split ratio 15:1) at 260 °C for 2 min.

GC–qMS analysis was conducted using a Shimadzu GC-2010 gas chromatograph coupled with a QP-2010 Plus quadrupole mass spectrometer (Shimadzu, Japan), using a Rtx-5 capillary column (20 m × 0.10 mm, 0.1 µm; Restek, USA). The oven programme was set to 40 °C (1 min), ramped at 5 °C/min to 130 °C, then at 4 °C/min to 250 °C (held for 5 min) and finally at 27 °C/min to 300 °C (held for 5 min). Helium was used as a carrier gas at 31.2-cm/s linear velocity. Injection port, ion source and interface temperatures were set at 260 °C, 200 °C and 245 °C, respectively. Detection was performed via electron ionisation (70 eV), which scanned from *m*/*z* 33 to 300. Compounds were tentatively identified using the NIST 17 library and confirmed with analytical standards where available. Semi-quantification (mg/kg) was performed using *m*/*z* 189 as the quantifier ion relative to the internal standard. All measurements were conducted in triplicate.

 doi:10.20944/preprints202512.0744.v1

2.4. Volatile Compound Analysis in Beer

Volatile analysis of beers was conducted via HS-SPME–GC/MS [10]. Samples were degassed using ultrasonic pulses (1 s intervals). For extraction, 4 g of beer was placed in a 20-mL vial with 1 g of NaCl and 10 µL of internal standard (1,3,5-triisopropylbenzene, 107 mg/L).

Desorption was performed in splitless mode at 260 °C for 2 min. GC–qMS analysis was performed using a Shimadzu GC-2010 with a QP-2010 Plus detector and a Stabilwax-MS column (30 m × 0.25 mm, 0.25 µm; Restek, USA). The oven was held at 42 °C for 10 min, ramped at 3 °C/min to 150 °C, then at 20 °C/min to 250 °C with a 5 min hold. Helium was used as the carrier gas (34.7 cm/s). Detection was performed via EI (70 eV), scanning from *m/z* 33 to 350. Tentative identification was based on the NIST 17 MS library, and it confirmed with standards when available. Semi-quantification (µg/kg) was based on the selected quantifier ion (*m/z* 189) of the internal standard. All analyses were performed in triplicate.

2.5. Physico-Chemical Analysis of Beer

Beer samples were degassed in an ultrasonic bath (LBS 1, Falc, Treviglio, Italy) and subsequently centrifuged (MPW-352RH, Warsaw, Poland) at 4,000 rpm for 15 min.

The pH was measured using an inoLab® 720 pH metre (WTW, Germany); turbidity was measured in NTU using a 2100P turbidimeter (HACH®, USA) and colour was measured using a CM-5 spectrophotometer (Konica Minolta®, Japan) in a 1cm cuvette. The results were expressed in EBC units using the formula: EBC = absorbance at 720 nm × 25. Alcohol content (% v/v), polyphenol content (mg/L) and bitterness index (IBU) were measured using the BeerLab® Jr (CDR, Florence, Italy).

2.6. Standard Products

Analytical standards used for volatile compound identification and quantification included β-myrcene (>90%), linalool (>98%, racemic mixture), citral (geranial and neral mixture), nerol (>98%), β-citronellol (>92%, racemic mixture), geraniol (98%), geranyl acetate (>95%, racemic mixture), β-caryophyllene (>80%), caryophyllene oxide (>99%) and α-humulene, all of which were purchased from Merck (Supelco, Milano, Italy). All standards were of analytical grade and used without further purification.

2.7. Statistical Analysis

All statistical analyses were conducted using STATISTICA software for Windows (version 7.0; StatSoft Inc., Tulsa, OK, USA). One-way analysis of variance followed by Duncan's post hoc test was used to assess significant differences in the chemical composition of hop samples. A significance level of $p < 0.05$ was applied.

3. Results

3.1. Hop Cones and Pellets

Notably, 23, 25 and 24 volatile terpenoids, which were classified as monoterpenes or sesquiterpenes, were detected in fresh, HDP and CDP, respectively (Table 1). Two sesquiterpenes were not identified.

Table 1. Concentration (ppm) of terpene compounds identified in fresh hops, HDC and CDC, based on the results of variance analysis.

	Fresh hop	**HDC**	**CDC**	***p*-Value**
β-Myrcene (m)	130.715±1.505a	280.042 ± 54.063b	344.584 ± 99.850b	0.05
Limonene (m)	nd	nd	nd	-
cis-β-Ocimene (m)	0.138±0.81	2.209 ± 0.376	2.215 ± 0.450	ns
Linalool (m)	2.116±2.063	3.556 ± 0.725	3.449 ± 0.927	ns

 doi:10.20944/preprints202512.0744.v1

Neral (m)	0.184±0.114a	1.366 ± 0.273b	0.494 ± 0.096a	0.001
Geraniol (m)	nd	0.600 ± 0.055	nd	-
Geranial (m)	0.181±208.7a	3.187 ± 0.640c	1.255 ± 0.198b	0.001
Methyl geraniate (m)	3.251±2.468	3.481 ± 0.673	4.132 ± 1.438	ns
α-Cubebene (s)	0.687±0.239	0.908 ± 0.252	1.153 ± 0.522	ns
α-Copaene (s)	1.529±0.486	2.425 ± 0.867	3.009 ± 1.309	ns
Geranyl acetate (m)	0.839±0.380	2.969 ± 0.820	5.608 ± 3.551	ns
β-Caryophyllene (s)	27.187±8.091	47.135 ± 15.566	51.721 ± 22.397	ns
Sesquiterpene isomer 1 (s)	2.127±0.734	2.828 ± 1.026	3.618 ± 1.708	ns
α-Bergamotene (s)	2.373±0.848	3.645 ± 1.322	4.823 ± 2.620	ns
α-Humulene (s)	47.753±14.669	100.328 ± 35.136	105.708 ± 56.596	ns
(*E*)-β-Farnesene (s)	6.261±2.236a	13.140 ± 3.310a	23.911 ± 5.277b	0.01
Selinene isomer 1 (s)	5.871±1.641	9.216 ± 3.063	8.921 ± 3.518	ns
Sesquiterpene isomer 2 (s)	5.104±4.202	11.095 ± 3.930	10.154 ± 4.340	ns
α-Muurolene (s)	1.185±0.392	1.245 ± 0.361	2.121 ± 1.091	ns
α-Farnesene (s)	0.643±0.160	1.567 ± 0.666	1.883 ± 1.149	ns
γ-Muurolene (s)	nd	3.091 ± 1.151	6.420 ± 3.489	ns
β-Cadinene (s)	0.4700±1.356	6.059 ± 2.254	7.920 ± 4.035	ns
α-Cadinene (s)	0.572±0.222	0.658 ± 0.243	0.861 ± 0.476	ns
Caryophyllene oxide (s)	1.586±2.233	0.912 ± 0.423	0.303 ± 0.159	ns
Humulene epoxide II (s)	2.722±3.781	1.580 ± 0.743	0.588 ± 0.384	ns
⌾-Cadinol (s)	0.177±0.155	0.135 ± 0.046	0.133 ± 0.094	ns
Sum Monoterpenoids	137.390±4.623a	297.409 ± 54.083b	361.738 ± 99.929c	0.05
Sum Sesquiterpenoids	109.767±32.585	205.967 ± 39.038	233.248 ± 61.700	ns

Values are expressed as mean ± standard deviation (n=9); HDC – hot dried cones; CDC – cold dried cones; sesquiterpene isomer 1 (*m/z*: 91, 105, 161, 204); sesquiterpene isomer 2 (*m/z*: 93, 105, 119, 133, 161, 175, 189, 204); mean values marked with different letters in the same line are significantly different ($p < 0.05$); m – monoterpene; s – sesquiterpene; ns – not significant; nd – not detected.

Eleven of these terpenoids were detected in Cascade dried cone hops [7], many of which were terpenoids with high-intensity aromas such as β-myrcene, *trans*-α-bergamotene and linalool, even if found in lower concentrations in the present study.

In all hop cones, β-myrcene was the most abundant monoterpene, with ~54.5%, 56.1% and 58.7%, respectively, followed by α-humulene, β-caryophyllene and (*E*)-β-farnesene among the sesquiterpenes, all of which were consistent with the established aroma markers of different hops [10] or hop extracts [4]. After drying, a considerable increase of 163% and 112% was observed in the hop of monoterpenes and sesquiterpenes content, particularly for CDC. Moreover, four volatile compounds, β-myrcene, neral, geranial and (*E*)-β-farnesene, considerably differed among the three products.

Neral and geranial were present in the highest amounts in HDC, while β-myrcene and (*E*)-β-farnesene were the most abundant in CDC. Geraniol was detected only in the HDC samples.

A considerable difference between fresh and dried cones was observed for the total monoterpene content, which was ~20% higher in CDC than in HDC. By contrast, no notable differences were observed in the levels of sesquiterpenes, despite its level being ~14% higher in CDC than in HDC.

In HDP and CDP, 26 terpene compounds that are commonly found in beer were detected (Table 2) [7,10-12]. In fresh and dried cones, ~23 terpenoids were identified (Table 1).

Table 2. Concentration (ppm) of terpene compounds in dried hops and corresponding pellets, along with results of variance analysis.

	HDC	**HDP**	***p*-Value**
β-Myrcene	280.042 ± 54.063	900.892 ± 370.545	0.05
Limonene	nd	21.716 ± 17.960	-
cis-β-Ocimene	2.209 ± 0.376	7.488 ± 2.707	0.05

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Linalool	3.556 ± 0.725	24.898 ± 12.077	0.05
Neral	1.366 ± 0.273	7.539 ± 4.856	ns
Geraniol	0.600 ± 0.055	nd	-
Geranial	3.187 ± 0.640	22.376 ± 10.617	0.05
Methyl geranate	3.481 ± 0.673	26.754 ± 11.823	0.05
α-Cubebene	0.908 ± 0.252	2.073 ± 0.589	0.05
α-Copaene	2.425 ± 0.867	13.049 ± 3.930	0.05
Geranyl acetate	2.969 ± 0.820	2.421 ± 0.667	ns
β-Caryophyllene	47.135 ± 15.566	254.528 ± 80.408	0.05
Sesquiterpene isomer 1	2.828 ± 1.026	14.311 ± 4.160	0.01
α-Bergamotene	3.645 ± 1.322	33.257 ± 13.443	0.05
α-Humulene	100.328 ± 35.136	433.077 ± 140.585	0.05
(*E*)-β-Farnesene	13.140 ± 3.310	285.894 ± 107.394	0.05
Selinene isomer 1	9.216 ± 3.063	68.229 ± 34.303	0.05
Sesquiterpene isomer 2	11.095 ± 3.930	35.830 ± 31.037	0.001
α-Muurolene	1.245 ± 0.361	10.391 ± 3.316	0.01
α-Farnesene	1.567 ± 0.666	11.729 ± 3.312	0.01
γ-Muurolene	3.091 ± 1.151	39.213 ± 12.885	0.01
β-Cadinene	6.059 ± 2.254	49.964 ± 15.694	0.01
α-Cadinene	0.658 ± 0.243	5.543 ± 1.699	0.01
Caryophyllene oxide	0.912 ± 0.423	2.221 ± 1.128	ns
Humulene epoxide II	1.580 ± 0.743	5.132 ± 2.492	ns
⍟-Cadinol	0.135 ± 0.046	1.298 ± 0.554	0.05
Sum Monoterpenoids	297.409 ± 54.083	1014.086 ± 371.559	0.05
Sum Sesquiterpenoids	205.967 ± 39.038	1265.748 ± 201.401	0.01
	CDC	**CDP**	***p*-Value**
β-Myrcene	344.584 ± 99.850	1086.325 ± 134.567	0.01
Limonene	nd	32.024 ± 4.313	-
cis-β-Ocimene	2.215 ± 0.450	18.648 ± 16.279	ns
Linalool	3.449 ± 0.927	27.356± 4.076	0.001
Neral	0.494 ± 0.096	4.051 ± 1.308	0.01
Geraniol	nd	6.433 ± 1.366	-
Geranial	1.255 ± 0.198	17.293 ± 3.517	0.01
Methyl geraniate	4.132 ± 1.438	34.648 ± 14.138	0.05
α-Cubebene	1.153 ± 0.522	2.597 ± 0.493	0.05
α-Copaene	3.009 ± 1.309	19.139 ± 3.390	0.01
Geranyl acetate	5.608 ± 3.551	44.037 ± 4.132	0.001
β-Caryophyllene	51.721 ± 22.397	294.374 ± 38.737	0.001
Sesquiterpene isomer 1	3.618 ± 1.708	15.885 ± 13.771	0.001
α-Bergamotene	4.823 ± 2.620	29.322 ± 4.681	0.01
α-Humulene	105.708 ± 56.596	495.007 ± 53.000	0.001
(*E*)-β-Farnesene	23.911 ± 5.277	644.447 ± 274.987	0.05
Selinene isomer 1	8.921 ± 3.518	85.931 ± 10.360	0.001
Sesquiterpene isomer 2	10.154 ± 4.340	76.387 ± 67.261	0.001
α-Muurolene	2.121 ± 1.091	19.415 ± 2.265	0.001
α-Farnesene	1.883 ± 1.149	19.613 ± 2.220	0.001
γ-Muurolene	6.420 ± 3.489	78.547 ± 8.184	0.001
β-Cadinene	7.920 ± 4.035	85.098 ± 9.513	0.001
α-Cadinene	0.861 ± 0.476	12.086 ± 1.250	0.001
Caryophyllene oxide	0.303 ± 0.159	6.186 ± 0.741	0.001
Humulene epoxide II	0.588 ± 0.384	15.638 ± 1.754	0.001
⍟-Cadinol	0.133 ± 0.094	3.038 ± 0.476	0.001
Sum Monoterpenoids	361.738 ± 99.929	1270.814 ± 136.533	0.01

 doi:10.20944/preprints202512.0744.v1

Sum Sesquiterpenoids	233.248 ± 61.700	1902.709 ± 291.47	0.001

Values are expressed as mean ± standard deviation (n=9); HDC – hot dried cones; HDP – hot dried pellets; CDC – cold dried cones; CDP – cold dried pellets; sesquiterpene isomer 1 (*m*/*z*: 91, 105, 161, 204); sesquiterpene isomer 2 (*m*/*z*: 93, 105, 119, 133, 161, 175, 189, 204); ns – not significant; nd – not detected.

The highest β-myrcene content in the two pellets with respect to the dried hops confirmed that this type of production preserved this molecule that could exhibit intense 'woody' and 'herbal' aroma, followed by linalool [13].

Generally, terpene concentrations in pellets were higher than those in dried cones. Limonene was detected only in pellets, while geraniol was preserved only in CDP.

A considerable increase of ~240% in monoterpenes and 514% in sesquiterpenes was observed in HDP when compared with HDC. Similarly, in CDP, the terpene concentration was markedly higher than in CDC, which was >251% for total monoterpenes and >751% for total sesquiterpenes (Table 2).

Generally, terpene concentrations increased during pellet production, likely owing to the compact structure of the pellets, which reduced contact with the environment and limited the dispersion of volatile components.

Geranyl acetate, sesquiterpene (isomer 1), sesquiterpene (isomer 2), α-muurolene, α-farnesene, γ-muurolene, β-cadinene, α-cadinene, caryophyllene oxide, humulene epoxide II and τ-cadinol showed considerable differences between HDP and CDP, with consistently being in higher concentrations in CDP (Table 3).

The concentrations of the latter two oxygenated sesquiterpenes were three times higher in CDP than in HDP, while geraniol was detected only in CDP. Oxidative decomposition of sesquiterpenes into terpenes during drying could generate a desirable 'floral' aroma through oxygenated terpenoids such as linalool and geraniol and thereby enhanced the complexity of hop-related aroma [7].

No marked differences in the total monoterpene and sesquiterpene contents were detected between the two pellet types. However, the mean concentrations were consistently higher in CDP, with increases of ~25% and 50%, respectively (Table 3).

Table 3. Concentration (ppm) of terpene compounds identified in pellets produced from hops dried at HDP and CDP temperatures, along with results of variance analysis.

	HDP	**CDP**	***p*-Value**
β-Myrcene	900.892 ± 370.545	1086.325 ± 134.567	ns
Limonene	21.716 ± 17.960	32.024 ± 4.313	ns
cis-β-Ocimene	7.488 ± 2.707	18.648 ± 16.279	ns
Linalool	24.898 ± 12.077	27.356± 4.076	ns
Neral	7.539 ± 4.856	4.051 ± 1.308	ns
Geraniol	nd	6.433 ± 1.366	-
Geranial	22.376 ± 10.617	17.293 ± 3.517	ns
Methyl geraniate	26.754 ± 11.823	34.648 ± 14.138	ns
α-Cubebene	2.073 ± 0.589	2.597 ± 0.493	ns
α-Copaene	13.049 ± 3.930	19.139 ± 3.390	ns
Geranyl acetate	2.421 ± 0.667	44.037 ± 4.132	0.001
β-Caryophyllene	254.528 ± 80.408	294.374 ± 38.737	ns
Sesquiterpene isomer 1	14.311 ± 4.160	15.885 ± 13.771	0.05
α-Bergamotene	33.257 ± 13.443	29.322 ± 4.681	ns
α-Humulene	433.077 ± 140.585	495.007 ± 53.000	ns
(*E*)-β-Farnesene	285.894 ± 107.394	644.447 ± 274.987	ns
Selinene isomer 1	68.229 ± 34.303	85.931 ± 10.360	ns
Sesquiterpene isomer 2	35.830 ± 31.037	76.387 ± 67.261	0.001
α-Muurolene	10.391 ± 3.316	19.415 ± 2.265	0.05
α-Farnesene	11.729 ± 3.312	19.613 ± 2.220	0.05
γ-Muurolene	39.213 ± 12.885	78.547 ± 8.184	0.01

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β-Cadinene	49.964 ± 15.694	85.098 ± 9.513	0.05
α-Cadinene	5.543 ± 1.699	12.086 ± 1.250	0.01
Caryophyllene oxide	2.221 ± 1.128	6.186 ± 0.741	0.01
Humulene epoxide II	5.132 ± 2.492	15.638 ± 1.754	0.01
◎-Cadinol	1.298 ± 0.554	3.038 ± 0.476	0.05
Sum Monoterpenoids	1014.086 ± 371.559	1270.814 ±136.53	ns
Sum Sesquiterpenoids	1265.748 ± 201.401	1902.709 ± 291.47	ns

Values are expressed as mean ± standard deviation (n=9); HDP – hot dried pellets; CDP – cold dried pellets; sesquiterpene isomer 1 (*m*/*z*: 91, 105, 161, 204); sesquiterpene isomer 2 (*m*/*z*: 93, 105, 119, 133, 161, 175, 189, 204).

In CDP pellets, we detected geraniol, whose presence was reported [7, 14] in Cascade hop essential oils. In hop pellets from the Saaz variety, 16 terpenoids were identified (5 monoterpenoids and 11 sesquiterpenoids) [15]. Moreover, Duarte et al. (2020) [16] reported 12 terpenoids—β-myrcene, limonene, linalool, geraniol, α-copaene, β-caryophyllene, α-bergamotene, α-humulene, γ-muurolene, selinene isomers, α-muurolene and α-farnesene—in Cascade hop pellets following ultrasound-assisted solvent extraction with hexane, with β-myrcene being the most abundant one, which was consistent with our findings.

3.2. Beers

The chemical characteristics of the beers are presented in Table 4.

Table 4. Physico-chemical composition of beers produced from dried cones and pellets, along with the results of significance analysis.

	BHDP	**BCDP**	**BHDC**	**BCDC**	***p*-Value**
pH	4.84 ± 0.05^{b}	4.81 ± 0.02^{b}	4.16 ± 0.02^{a}	4.11 ± 0.05^{a}	0.01
Ethanol [% v/v]	4.8 ± 0.2	4.8 ± 0.2	4.8 ± 0.2	4.9 ± 0.1	ns
IBU	8.3 ± 1.2^{a}	9.7 ± 0.6^{b}	7.6 ± 0.7^{a}	6.1 ± 0.6^{a}	0.01
DPPH [mg caffeic acid/L]	167 ± 6^{b}	166 ± 4^{b}	132 ± 9^{a}	125 ± 4 ^{a}	0.01
Turbidity [NTU]	11.7 ± 0.4^{b}	16.0 ± 0.3^{b}	8.1 ± 0.3^{a}	9.2 ± 0.3^{a}	0.01
EBC	4	4	4	4	ns

Values are expressed as mean ± standard deviation (n=9); BHDP – beer hot dried pellets; BCDP – beer cold dried pellets; BHDC – beer hot dried cones; BCDC – beer cold dried cones; mean values marked with different letters in the same line are significantly different ($p < 0.05$); ns – not significant.

Although no differences were observed among the beers in terms of ethanol content and EBC, considerable differences were reported for pH, IBU, polyphenol content and turbidity.

In particular, the values for pH, IBU, DPPH and turbidity were higher in beers produced with pellets. These differences could be attributed to the distinct hop structures of pellets, which facilitated component diffusion into the beer. The IBU value was particularly high in CDP (9.7), when compared with that with HDP pellets (8.3), and it was similar to cone-dried hops (6.1–7.6).

In addition, beers produced using pellet hops had a higher polyphenolic content (166–167 mg/L) than those obtained from cone hops (125–132 mg/L). The different hop-drying treatments did not considerably influence the characteristics of the beers, such as alcohol content, colour or pH, keeping them close to the average values of dry-hopped beers, except for the IBU, which was markedly below the expected range (15–40) [12]. This result was predictable, as the absence of bittering hops during the boiling phas rich in α-acids did not allow the achievement of the expected average IBU.

In beer, 21–26 terpenoid compounds were detected, with their highest number found in beer produced from hot-dried pellets (BHDP) and the lowest in beer produced using cold-dried pellets (BCDP) (Table 5).

 doi:10.20944/preprints202512.0744.v1

Table 5. The concentration (ppb) of terpene compounds identified in beers produced using hop cones or pellets obtained by hot- or cold-drying, with results of variance analysis. For each compound, the presence (P) or absence (A) in the corresponding cone or pellet hops is reported.

	BHDC		BCDC		BHDP		BCDP		*p*-Value
β-Myrcene	0.18 ± 0.03 a	**P**	0.63 ± 0.20 a	**P**	15.91 ± 7.07 b	**P**	nd	**P**	0.001
Limonene	0.26 ± 0.03	**A**	0.58 ± 0.28	**A**	0.36 ± 0.23	**P**	nd	**P**	ns
cis-Furan linalool oxide	1.02 ± 0.23	**A**	1.14 ± 0.48	**A**	nd	**A**	nd	**A**	ns
Linalool	5.47 ± 0.54 a	**P**	8.99 ± 5.03 a	**P**	13.84 ± 5.25 b	**P**	2.69 ± 0.19 a	**P**	0.01
β-Caryophyllene	nd	**P**	nd	**P**	4.65 ± 3.33	**P**	nd	**P**	-
4-Terpineol	0.24 ± 0.09	**A**	0.25 ± 0.12	**A**	0.39 ± 0.24	**A**	nd	**A**	ns
Cis-Verbenyl acetate	nd	**A**	0.33 ± 0.20	**A**	2.42 ± 0.92	**A**	nd	**A**	0.01
Sesquiterpene isomer 1	nd	**A**	nd	**A**	18.93 ± 12.21	**A**	nd	**A**	-
trans-β-Farnesene	nd	**A**	nd	**A**	3.32 ± 1.15	**A**	nd	**A**	-
γ-Muurolene	nd	**P**	nd	**P**	1.08 ± 0.43	**P**	nd	**P**	-
α-Terpineol	1.34 ± 0.07 b	**A**	1.58 ± 0.68 b	**A**	1.88 ± 0.92 b	**A**	0.35 ± 0.17 a	**A**	0.05
Selinene isomer 1	nd	**P**	nd	**P**	0.82 ± 0.58	**P**	nd	**P**	-
Mentha-1(7),8-diene	nd	**A**	nd	**A**	0.20 ± 0.07	**A**	nd	**A**	-
δ-Cadinene	nd	**A**	nd	**A**	1.75 ± 1.05	**A**	nd	**A**	-
cis-Geranyl acetate	nd	**A**	1.25 ± 0.54	**A**	nd	**A**	nd	**A**	-
Citronellol	3.15 ± 0.27 a	**A**	3.93 ± 2.04 a	**A**	21.12 ± 8.22 c	**A**	6.41 ± 0.85 b	**A**	0.001
Neryl formate	nd	**A**	0.52 ± 0.26	**A**	nd	**A**	nd	**A**	-
Nerol	0.48 ± 0.03 a	**A**	nd	**A**	1.34 ± 0.71 b	**A**	0.58 ± 0.12 a	**A**	0.05
Geraniol	5.40 ± 0.64 b	**A**	8.62 ± 3.12 c	**P**	8.23 ± 2.77 c	**A**	3.56 ± 0.24 a	**A**	0.05
Monoterpene isomer 1	0.28 ± 0.08 a	**A**	0.29 ± 0.16 a	**A**	nd	**A**	0.75 ± 0.10 b	**A**	0.001
p-Menth-8-en-2-ol	0.31 ± 0.06	**A**	0.51 ± 0.25	**A**	0.20 ± 0.08	**A**	0.38 ± 0.07	**A**	ns
Caryophyllene oxide	0.43 ± 0.03 a	**P**	nd	**P**	1.37 ± 0.49 b	**P**	5.32 ± 0.45 c	**P**	0.001
α-Humulene	2.80 ± 0.41 b	**A**	3.85 ± 1.50 c	**A**	1.79 ± 0.63 a	**P**	4.71 ± 0.40 c	**P**	0.01
p-Mentha-1,8-dien-7-ol	0.53 ± 0.06	**A**	0.57 ± 0.33	**A**	nd	**A**	0.28 ± 0.03	**A**	ns
Humulene epoxide II	1.19 ± 0.51 a	**P**	0.97 ± 0.22 a	**P**	0.88 ± 0.33 a	**P**	4.67 ± 0.48 b	**P**	0.001
Nerolidol	0.69 ± 0.03	**A**	0.61 ± 0.29	**A**	0.55 ± 0.19	**A**	0.89 ± 0.13	**A**	ns
Cubenol isomer 1	nd	**A**	nd	**A**	nd	**A**	0.70 ± 0.11	**A**	-
Cubenol isomer 2	nd	**A**	nd	**A**	nd	**A**	1.34 ± 0.07	**A**	-
Sesquiterpene isomer 2	nd	**A**	nd	**A**	nd	**A**	1.44 ± 0.07	**A**	-
⌾-Cadinol	0.60 ± 0.14 a	**P**	0.50 ± 0.20 a	**P**	1.85 ± 0.52 b	**P**	1.52 ± 0.19 b	**P**	0.001
γ-Cadinene	0.72 ± 0.24 a	**A**	1.11 ± 0.32 ab	**A**	nd	**A**	1.45 ± 0.17 b	**A**	0.05
α-Cadinol	0.31 ± 0.08 a	**A**	0.27 ± 0.20 a	**A**	0.59 ± 0.27 ab	**A**	0.95 ± 0.10 b	**P**	0.001
Selin-6-en-4a-ol	1.11 ± 0.17 a	**A**	1.01 ± 0.30 a	**A**	2.11 ± 0.83 b	**A**	2.03 ± 0.25 b	**A**	0.05
14-Hydroxy-α-humulene	8.43 ± 1.49 b	**A**	25.49 ± 5.39 c	**A**	3.52 ± 0.68 a	**A**	8.80 ± 1.00 b	**A**	0.001
trans-Farnesol	1.42 ± 0.23	**A**	1.20 ± 0.53	**A**	0.92 ± 0.30	**A**	0.73 ± 0.13	**A**	ns
Sum Monoterpenoids	19.13 ± 0.94 a		29.20 ± 6.36 b		65.90 ± 12.45 c		15.00 ± 0.94 a		0.01
Sum Sesquiterpenoids	17.69 ± 1.64 a		35.00 ± 5.46 b		44.14 ± 12.85 b		34.55 ± 1.40 b		0.05

Values are expressed as mean ± standard deviation (n=9); BHDC – beer hot dried cones; BCDC – beer cold dried cones; BHDP – beer hot dried pellets; BCDP – beer cold dried pellets; sesquiterpene isomer 1 (*m*/*z*: 91, 105, 161, 204); monoterpene isomer 1 (*m*/*z*: 41, 69, 121, 139); sesquiterpene isomer 2 (*m*/*z*: 93, 105, 119, 133, 161, 175, 189, 204); ns – not significant; nd – not detected; A – absence; P – presence .

 doi:10.20944/preprints202512.0744.v1

Marked differences were highlighted among the terpenic compounds of beers. In particular, β-myrcene, linalool, *cis*-verbenil-acetate, α-terpineol, citronellol, nerol, geraniol, monoterpene isomer 1, caryophyllene oxide, α-humulene, humulene epoxide II, τ-cadinol, γ-cadinene, α-cadinol, selin-6-en-4a-ol and 14-hydroxy-α-humulene showed the highest contents in BHDP, which collectively exhibited the highest values for the monoterpenes and sesquiterpenes. By contrast, caryophyllene oxide, α-humulene, humulene epoxide II and α-cadinol were the most abundant in BCDP, while 14-hydroxy-α-humulene was the highest in BCDC beers. Few compounds, such as β-caryophyllene, sesquiterpene isomer 1, *trans*-β-farnesene, γ-muurolene, selinene isomer 1, mentha-1(7),8-diene and δ-cadinene, were present only in BHDP.

Notably, traces of β-myrcene were present in beer produced using hot-dried cones (BHDC), while it was clearly present in the corresponding pellets; in fact, its concentration was the highest in BHDP. β-Myrcene, which was characteristic of the 'Cascade' hop variety used in this study [14], can impart floral and geranium-like notes to beer, and given its low flavour threshold, it can play an important role in hop aroma [10].

The difference in caryophyllene oxide was noteworthy, with the highest concentration in BCDP. Although this compound did not considerably effect the taste or mouthfeel properties of beer, its synergy with oxygenated sesquiterpenes could alter bitterness perception [4]. The humulene epoxide II concentration was approximately five times higher in the BCDP than in BCDC, while the concentration of 14-hydroxy-α-humulene was approximately three times higher in BCDC than in BCDP.

In general, the oxidised forms of terpene compounds, such as caryophyllene oxide, humulene epoxide II and 14-hydroxy-α-humulene, exhibited considerably higher concentrations in the BCDP than in BHDP. In particular, the concentration of 14-hydroxy-α-humulene was three times higher in the BCDP than in BHDP beers.

The concentration of citronellol was three times higher in the BHDP than in BCDP.

Upon comparing the terpenic profiles of beers with those of the corresponding hops (cones or pellets), after the brewing process, including fermentation and maturation, some molecules identified in hops, such as β-caryophyllene, γ-muurolene and selinene isomer 1, were absent in the resulting beers, while new molecules, such as limonene, geraniol, *cis*-furan linalool oxide, 4-terpineol, α-terpineol, citronellol, nerol, monoterpene isomer 1, *p*-menth-8-en-2-ol, α-humulene, *p*-mentha-1,8-dien-7-ol, nerolidol, γ-cadinene, α-cadinol, selin-6-en-4a-ol, 14-hydroxy-α-humulene and *trans*-farnesol, were detected.

Linalool and geraniol were approximately three- and two-fold higher, respectively, in BCDC than in the BCDP, while citronellol was approximately two-folds higher in the BCDP than in BCDC.

By comparing the terpenic profiles of BHDP with those of the corresponding HDP, new molecules were produced during the brewing process—mainly in the fermentation and maturation stages—such as 4-terpineol, *cis*-verbenyl acetate, *trans*-β-farnesene, α-terpineol, mentha-1(7),8-diene, δ-cadinene, citronellol, nerol, geraniol, *p*-menth-8-en-2-ol, nerolidol, α-cadinol, selin-6-en-4a-ol, 14-hydroxy-α-humulene and *trans*-farnesol. This suggests the occurrence of either biotransformation by yeast during fermentation or oxidative processes [17]. Similarly, in beers produced from cones and pellets obtained by cold drying, several terpenic compounds not detected in the respective hops were identified, such as α-terpineol, citronellol, nerol, geraniol, monoterpene isomer 1, *p*-menth-8-en-2-ol, *p*-mentha-1,8-dien-7-ol, nerolidol, cubenol isomer 1, cubenol isomer 2, γ-cadinene, selin-6-en-4a-ol, 14-hydroxy-α-humulene and *trans*-farnesol. Sesquiterpene isomer 1 was detected only in the BHDP beers and was absent in beers obtained using their corresponding pellets.

The concentration of monoterpenes was observed to be approximately three times higher in BHDP than in BHDC. A marked difference was observed between the beers obtained using hot-dried pellets (BHDP) and those obtained using HDC (BHDC).

Monacci et al. (2024) [18] described 20 terpene compounds in beers obtained from Cascade hops using different drying techniques, such as freeze-drying and hot-stove drying. Of these, nine terpenic compounds were common with the ones observed in our study, with marker concentration ranges being similar to those of our values. In Lager beers, Martins et al. (2018) [19] detected 34 terpene

 doi:10.20944/preprints202512.0744.v1

compounds, eight of which were similar to the ones found in our study. Two sesquiterpenic alcohols, nerolidol and τ-cadinol, were consistently present in all our beers.

4. Conclusions

Analyses highlighted a set of 23 mono- and sesqui-terpenic compounds in fresh hop inflorescences, which comprised seven monoterpenes and 16 sesquiterpenes. In dried cones, pellets and beers produced using these hops, the number of terpenic compounds varied from 23 to 26, depending on the drying process adopted (hot or cold).

Generally, the concentration of terpenic compounds is higher in dried cones than in fresh hops and this concentration further increases in pellets. In particular, pellets produced from hops dried at low temperature showed the highest concentrations of terpenic compounds.

In beers, the concentrations of terpenic compounds varied according to the form of hop used (cones or pellets) and the drying method used (hot or cold).

For beers produced using cone hops, a higher content of terpene molecules was observed when CDC was used, particularly for oxygenated terpenoids and sesquiterpenes.

In beers produced from pelletised hops, a higher number of monoterpenes and fewer sesquiterpenes were detected when high temperatures were used for hop drying. In this case as well, a higher concentration of oxygenated sesquiterpenes was observed in beers produced using CDP. According to the obtained results, the aromatic profile of a beer can be directly modified by changing the drying temperature of the hops and indirectly by altering the terpenic compounds that yeast could transform during fermentation.

Author Contributions: Conceptualization, M.G and G.Z.; methodology, M.G; formal analysis, M.G., A.F. and V.C.; writing—original draft preparation, M.G.; writing—review and editing, M.G. and G.Z. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Acknowledgments: We thank Birrificio Baladin (Piozzo, Cuneo, Italy) for their assistance and technical support

Conflicts of Interest: The authors declare no conflicts of interest.

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