Aroma Profiling and Chemical Characterization of Swedish Heritage Hops in Beer Brewing

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Aromprofilering och kemisk karakterisering av svenska kulturhumlesorter i ölbryggning

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Abstract

Swedish heritage hop varieties represent a relatively unexplored resource in modern brewing, particularly regarding their aroma potential and chemical composition. The aim of the present study was to investigate selected Swedish heritage hops in beer brewing and evaluate differences in hop-derived compounds following dry hopping during fermentation. A single wort was divided into twelve fermentation vessels representing six treatments conducted in biological duplicate, including five dry-hopped beers and one non-dry-hopped control. Brewing performance was monitored throughout fermentation and maturation. α- and β-acids in hop material and finished beer were quantified using HPLC, while sensory evaluation was performed using a semi-trained consumer panel assessing hop-related aroma attributes. Selected hop-derived terpenes were analyzed in hop material, post dry-hop beer and finished beer using GC-FID.

The investigated hop varieties displayed clear differences in α- and β-acid composition. Korsta exhibited the highest β-acid content and the lowest α/β-acid ratio among the investigated varieties, while several Swedish cultivars showed elevated residual α-acid concentrations in beer compared with the non-dry-hopped control, indicating transfer of hop-derived compounds during dry hopping. Estimated apparent dry-hop α-acid recovery ranged between approximately 4–9%, while the Magnum kettle addition showed an apparent utilization of approximately 29%, consistent with previously reported brewing ranges.

Sensory analysis revealed substantial variation among assessors and no statistically significant differences between treatments were detected by one-way ANOVA ($p > 0.05$), although tendencies were observed for citrus-related aroma and overall hop intensity. GC-FID analysis indicated that hydrocarbon terpenes such as β-myrcene, trans-caryophyllene and γ-terpinene were primarily detected in hop material, whereas linalool and geraniol persisted throughout the brewing process and were detected in finished beer samples. Limonene was also detected in several beer samples, indicating partial transfer of hop-derived monoterpenes into the final product.

The results demonstrate that Swedish heritage hop varieties possess distinct chemical characteristics and contribute measurable differences in hop-derived compounds following dry hopping. The findings support continued investigation of Swedish heritage hops as a brewing resource and highlight the importance of compound-specific behavior during aroma transfer from hop material to finished beer.

Keywords: Swedish heritage hops, dry hopping, beer brewing, hop aroma, terpene analysis, HPLC, GC-FID, α-acids, β-acids, sensory evaluation, hop chemistry

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Abbreviations

Abbreviation	Description
ABV	Alcohol by volume
ANOVA	Analysis of variance
DH	Dry hopping
DME	Dry malt extract
EKG	East Kent Golding
FG	Final gravity
GB	Green beer
GC-FID	Gas chromatography–flame ionization detection
GC–MS	Gas chromatography–mass spectrometry
GRI	Grimsarbo
HPLC	High-performance liquid chromatography
HUL	Hulla Norrgård
IBU	International bitterness units
ICE-4	International calibration extract 4
KOR	Korsta
LOD	Limit of detection
MAG	Magnum
OG	Original gravity
PBW	Powdered Brewery Wash
POM	Program for Diversity of Cultivated Plants
RATA	Rate-all-that-apply
SG	Specific gravity
SPA	Spångby
SPE	Solid-phase extraction

1. Introduction

1.1 Beer aroma and the role of hops

Beer brewing is a complex food chemical process involving raw material selection, controlled temperature regimes, and fermentation. Balancing the four primary constituents' water, malted grains, hops, and yeast as defined by the 1516 German Reinheitsgebot, also known as the Bavarian Beer Purity Law.
The characteristic bitterness and various fruity aromas found in beer derive from essential oils found in the lupulin glands of hops (*Humulus lupulus*).

Bitterness found in beer develops through heat-induced isomerization of α-acids and oxidation products of β-acids. Beyond bitterness, the fruity aromas come from terpenes and terpenoids that impart sensory attributes such as citrus, pine, tropical fruit, and floral notes, which are characteristic of hop-forward beer styles such as modern India Pale Ale (IPA). The development of such styles has been facilitated by targeted hop breeding programs, particularly in the United States, New Zealand, and Australia, aimed at enhancing aroma intensity and diversity.
In addition, hop-derived compounds enhance foam stability and exhibit antimicrobial activity against Gram-positive bacteria, including lactic acid bacteria, primarily through membrane disruption playing a role in storage and food safety (Michiu et al. 2019; Young et al. 2023; Chen & Lv 2025).

Historical evidence suggests that hops were already being used in brewing by the ninth century in regions corresponding to present-day Germany and the Czech Republic, with documented cultivation dating to 859 AD and widespread adoption in brewing practices by the twelfth century (Yu et al. 2024). In Sweden, hop cultivation was legally mandated between 1414 and 1860, and feral hop plants originating from historical cultivation sites can still be found today (Karlsson Strese et al. 2014). Despite the introduction of hop cuttings from the Saaz region during the nineteenth century, Swedish hop populations have retained distinct genetic characteristics, forming a cluster that differs from other European and Nordic hop groups.

A study by (Mackegård 2021) evaluated the agronomic performance of ten Swedish heritage hop cultivars in an experimental field trial in Krusenberg outside Uppsala. The study assessed phenological development, yield, α- and β-acid content, and essential oil composition. Several cultivars exhibited relatively early maturation under local growing conditions. Among the most promising varieties were Korsta and Hulla Norrgård, which combined early development with

comparatively high α-acid levels and elevated total essential oil content, including a high proportion of myrcene.

In parallel with academic field trials the Swedish heritage hops gained attention by national innovation initiatives. One of these was the EIP-AGRI Operational Group project *"Hope for Swedish Hops" (2018–2022)*, coordinated by *RISE,* aimed to characterize the sensory properties of Swedish hop varieties and evaluate their performance through test brewing with sensory tests paired with a multi-year analytical datasets on Swedish hop varieties, including α- and β-acid content and essential oil composition *(2019-2021).* National initiatives as mentioned above points to a growing interest in sensory identity and practical value in the brewing industry of locally cultivated hops.

While agronomic performance and raw hop composition of Swedish heritage hop varieties have been documented, their behavior during brewing and the transfer of hop-derived aroma compounds into beer remain less explored. The concentration of essential oils and individual terpenes in raw hop cones is not necessarily directly reflected in the aroma profile of the finished beer. Brewing processes such as boiling, whirlpooling, dry hopping and fermentation can influence the extraction, retention and transformation of volatile compounds. Consequently, hop-derived aroma compounds are affected by factors including evaporation, oxidation, limited solubility and interactions with yeast during fermentation together determine their contribution to the final beer aroma.

1.2 Hypothesis and aim

Based on previous agronomic findings and documented variation in the chemical composition of Swedish heritage hop varieties, it was hypothesized that differences in hop composition would result in differences in hop-derived compounds in finished beer following dry hopping.

The aim of this study was to characterize selected Swedish heritage hop varieties and evaluate their potential in beer brewing applications. Specifically, the study aimed to quantify α- and β-acids using HPLC, investigate hop-derived terpene compounds throughout the brewing process using GC-FID, and relate the analytical results to sensory evaluation of the finished beers.

2. Background/Theory

2.1 Hops and essential oil

Hop-derived aroma compounds are primarily localized within glandular trichomes (lupulin glands) situated in the inner tissues of the hop cone. These structures function as specialized storage compartments for secondary metabolites that play roles in plant defense and protection against environmental stressors but are not essential for primary metabolism. The concentration of these compounds is higher in female hop plants, reflecting their role in protecting reproductive structures (Wang et al. 2008; Ramos et al. 2024).

Freshly dried hop cones typical composition is typically 15-30 %w/w resins (alpha and beta acids) and 0.5-3 %w/w essential oils. Correlation between total hop essentials oils and aroma is not directly correlated. Even though the location of the most abundant aroma contributing group terpenes is found there. Aroma intensity varies between cultivars due to concentration, composition and synergistic interaction between the terpenes (Eyres & Dufour 2009; Verhagen 2010; Dietz et al. 2021a).

The compounds present in the essential oil can be divided into hydrocarbons, oxygen-containing compounds, and Sulfur compounds. Monoterpene hydrocarbons in essential oils are in the range of 9.4 to 52% and sesquiterpene hydrocarbons in the range of 29.1 to 70% (Nance & Setzer 2011). The vide compositional variation between cultivars and harvest point at the importance of evaluating individual compounds when predicting aroma outcomes than total essential oil content.

2.2 Terpenes

Terpenes constitute a major fraction of hop essential oil and form the chemical basis for hop aroma potential. Hop essential oils consist predominantly of hydrocarbons (approximately 50–80%), mainly monoterpenes and sesquiterpenes, while the remaining fraction comprises oxygenated compounds at average 30%. The relative proportion of these classes varies considerably between cultivars and strongly influences aroma character (Inui et al. 2013; Bizaj et al. 2022)

Hydrocarbon monoterpenes such as myrcene are often abundant in raw hop material but are highly volatile and poorly soluble in aqueous media, resulting in limited retention during wort boiling and fermentation (Eyres & Dufour 2009). In contrast, oxygenated terpenes (terpenoids), characterized by functional groups such as hydroxyl (-OH) moieties, exhibit greater polarity and improved sensory stability in beer. Common oxygenated terpenes across hop varieties include linalool (fresh, floral, slightly woody), geraniol (rose-like, citrus, fruity), and α-terpineol (woody, lilac-like) (Sharp et al. 2017a; Dietz et al. 2021a). Due to their increased polarity, these compounds are more frequently detected in finished beer and are considered key contributors to floral and citrus-like hop aroma.

Steyer et al. (2013) demonstrated that oxygenated terpenes were retained in measurable concentrations following both early and late hop additions during boiling, indicating greater stability compared to hydrocarbon monoterpenes under brewing conditions. Such findings highlight the importance of oxygenated terpenes as central aroma contributors in hop-forward beer styles.

Sesquiterpenes, including α-humulene and β-caryophyllene, consist of 15 carbon atoms formed from three isoprene units (C_5H_8). Although less volatile than monoterpenes, they remain relatively hydrophobic and display limited solubility in beer. During boiling and fermentation, sesquiterpenes may undergo oxidation and structural modification, forming more polar derivatives that can influence aroma contribution and stability in the beer matrix (Praet et al. 2016).

From a biosynthetic perspective, terpene diversity originates from isoprene-based precursors, primarily isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). Sequential condensation forms geranyl diphosphate (C_{10}) and farnesyl diphosphate (C_{15}), which are converted by terpene synthases into structurally diverse mono- and sesquiterpenes (Wang et al. 2008; Ninkuu et al. 2021). This biochemical variability underlies the compositional differences observed between hop cultivars.

During brewing, physical and biochemical processes substantially modify terpene composition. Volatile hydrocarbons may be lost through evaporation or CO_2 stripping, while yeast metabolism can modify terpene alcohols and release glycosidically bound aroma precursors. Consequently, the terpene profile in finished beer often differs markedly from that observed in raw hop material (Sharp et al. 2017a; Haslbeck et al. 2018; Han et al. 2023).

In brewing practice, total essential oil content is frequently used as an indicator of aroma intensity. However, reliance on total oil alone is insufficient, as aroma expression depends on the relative abundance and behavior of individual compounds. Lafontaine et al. (2018) demonstrated that variation in specific terpenes accounted for a greater proportion of aroma variability than total oil concentration alone, highlighting the importance of targeted compound analysis when evaluating hop aroma potential.

2.2.1 Behavior during brewing and fermentation

Hop extraction begins immediately when in contact with the wort and beer in fermentation and the proportion of extraction depends on the compound group. Where terpene alcohols belonging to the oxygenated compound class extracted more efficiently due to their polarity and solubility. Hauser et al. (2019a) evaluated the hop oil extraction during a 24-hour dry-hop, it showed that the hops contained 33-51% of the essential oil originally whereas 52-77% was hydrocarbons (β-myrcene). While the oxygenated compounds extraction efficiency into the beer medium was higher only 10% of initial concentration remained, even showing extraction exceeding 100% of extraction (linalool & geraniol) (Haslbeck et al. 2018) .

Dry-hopping is shown to affect the content of bitterness inducing agents in beer measured in content of iso-α-acids (isohumulones) and their oxidation products of α-acids (humulinones) (Hahn et al. 2018). As seen in 3-day dry-hopping study, initial iso-α-acids concentration decreased from 51 mg/L to 32 mg/L and the content of humulinones and α-acids increased from non-detectable to 13 mg/L each (JP Maye, R Smith 2016). Due to the poor solubility of α-acids and lower perceived bitterness of humulinones 66% ($\pm$13) of iso-α-acids (Algazzali & Shellhammer 2016).

Extraction efficiency is determined by pH, temperature, time, dosage and chemical composition of the wort. Lafontaine & Shellhammer (2018) observed that increasing dry-hop dosage increased both the concentrations of β-caryophyllene, α-humulene, terpinen-4-ol, α-terpineol, and geranial, and the intensity of herbal and tea-like aroma attributes, while having a negative effect on the extraction efficiency of the terpene alcohols geraniol, linalool and nerol. As the dry-hop dosage increased from 200 g/hl to 1600 g/hl the terpene alcohol extraction decreased from 23%, 13% and 6% to 7%, 3% and 1% respectively. A similar observation was observed by Kohles et al. (2021) with the exception of polyfunctional thiols.

The iso-α acids mirrored the terpene alcohols in the decreased extraction with higher hop dosage while the lower bitterness α-acids was extracted more efficiently (Lafontaine & Shellhammer 2018; Ocvirk & Kosir 2020). A study by (Haslbeck & Benjamin 2022) also pointed at this correlation going as far as saying bittering hops are characterized by a lower extraction of essential oils, hops dosage was decided by total essential oil content.

2.3 α-acids

α-acids constitute approximately 3–20 wt% of the dry weight of commercial hop cultivars, with an average content of around 10 wt%, depending on cultivar and growing conditions (Steenackers et al. 2015; Krofta et al. 2022; Nesvadba et al. 2024) The commercial value of hops is strongly influenced by this concentration. α-Acids are the precursors to the bittering compounds that are essential in beer; however, they only contribute to bitterness after undergoing heat-induced isomerization during wort boiling.

The sensory threshold for iso-α-acids in water is approximately 6 mg/L, although bitterness perception in beer is influenced by matrix effects and individual variability (Hughes & Simpson 1996; Kolpin & Shellhammer 2009). Beer bitterness is measured in International Bitterness Units (IBU), where 1 IBU corresponds approximately to 1 ppm (mg/L) of iso-α-acids. Generally, beers may be classified as lightly bitter (0–20 IBU), moderately bitter (20–40 IBU), or highly bitter (>40 IBU). Human perception generally does not distinguish bitterness above approximately 100 IBU. Furthermore, perceived bitterness is influenced by interactions with pH and malt sweetness in the beer (Kishimoto et al. 2022).

$$\text{IBU} = \frac{\alpha\text{-acid fraction} \times \text{hop mass (g)} \times \text{utilization} \times 1000}{\text{wort volume (L)}}$$

Figure 1. Formula used to estimate International Bitterness Units (IBU), where the α-acid fraction represents the α-acid content of the hops, hop mass is expressed in grams, utilization represents the proportion of α-acids that are isomerized and dissolved into the wort during boiling, and wort volume is expressed in liters. Adapted from Kunze (2004).

The principal α-acids are humulone, adhumulone, cohumulone, posthumulone, and prehumulone, with humulone and adhumulone typically being the dominant homologues. The utilization of α-acids, defined as the proportion of isomerized and dissolved into the wort during boiling, is generally around 30–40%, and may be lower depending on brewing conditions. Utilization is influenced by factors such as boil duration, wort gravity, pH, and losses during hot trub separation. At higher pH values, isomerization efficiency increases; however, elevated pH may also negatively affect the stability of the resulting iso-α-acids (Kappler et al. 2010; Clippeleer et al. 2014a).

During isomerization, α-acids undergo structural rearrangement, becoming more polar and therefore more water-soluble. The resulting iso-α-acids exist as two stereoisomers, cis and trans, of which the cis form is more stable and typically more prominent in finished beer. The corresponding iso-α-acids include isohumulone, isoadhumulone, isocohumulone, isoposthumulone, and isoprehumulone (Clippeleer et al. 2014b; Evans & Doolittle 2024).

Iso-α-acids are light sensitive and can undergo photochemical degradation when exposed to ultraviolet light in the presence of riboflavin. This reaction leads to the formation of 3-methyl-2-butene-1-thiol (3-MBT), a highly potent sulfur compound responsible for the characteristic “light-struck” or “skunky” off-flavor in beer (Gros & Collin 2012).

Structurally, α-acids belong to the soft resin fraction of hops, characterized by solubility in cold methanol. They are diprenylated phloroglucinol derivatives consisting of a phloroglucinol core (1,3,5-trihydroxybenzene, $C_6H_3(OH)_3$) substituted with hydrophobic prenyl side chains derived from isoprene units, in addition to an acyl side chain. In contrast, the hard resin fraction, which contains more oxidized hop constituents, is soluble in diethyl ether (Steenackers et al. 2015; Paniagua-García et al. 2023).

2.3.1 β-acids

β-acids, also known as Lupulone (lupulone, colupulone and adlupulone), belong to the soft resin fraction of hops and typically constitute approximately 3–10 wt% of hop dry weight. Due to their low solubility in wort and beer, β-acids do not undergo significant isomerization during boiling and therefore contribute little to bitterness in freshly brewed beer. Compared to the α-acids the β-acids are generally lower in concentration with a ratio of 1.5-2 (Krofta et al. 2019; Duarte et al. 2022; Nesvadba et al. 2024).

However, lupulones are highly reactive toward oxygen and can oxidize to form compounds known as hulupones. Hulupones are more soluble than their parent β-acids and contribute bitterness, often described as sharper and more lingering compared to iso-α-acids. Sensory studies have shown that hulupones exhibit approximately 84% of the bitterness intensity of iso-α-acids (±10%) under controlled conditions (Algazzali & Shellhammer 2016).

2.4 Yeast-Mediated Transformation of Hop-Derived Terpenes

In hops, a proportion of terpene-derived aroma compounds occurs in glycosidically bound form. In these conjugates, terpene aglycones are linked to one or more sugar moieties, forming non-volatile and aroma-inactive precursors. Although these glycosides are generally more water-soluble than their corresponding aglycones, they do not contribute directly to beer aroma unless the glycosidic bond is hydrolyzed (Cibaka et al. 2017; Sharp et al. 2017b; Han et al. 2023).

During fermentation and dry hopping, yeast metabolism may influence hop-derived aroma through both enzymatic release of bound compounds and biotransformation of free terpenes already present in the beer. Previous studies have demonstrated that monoterpene alcohols such as geraniol can be converted into β-citronellol during fermentation, while linalool generally exhibits greater stability (Takoi et al. 2010). As a result, the terpene composition of finished beer may differ substantially from that of the original hop material.

The extent of these transformations depends on factors including yeast strain, fermentation conditions, substrate availability and hop composition. Consequently, the aroma profile of beer is determined not only by the compounds extracted from the hops but also by the chemical and biological processes

occurring during fermentation. These processes contribute to the complexity of hop aroma expression and help explain why the terpene composition of finished beer often differs from that of the raw hop material (Sharp et al. 2017b).

2.5 Fate of hop-derived compounds during beer brewing

The extraction of hop-derived compounds begins immediately following hop addition to wort or beer. Depending on the stage of addition, different classes of compounds are extracted, transformed or lost during brewing. Hops are often categorized as bittering or aroma cultivars according to their typical use, with bittering cultivars generally selected for high α-acid content and aroma cultivars for their essential oil composition (Cibaka et al. 2017; Duarte et al. 2020).

Hot-side hopping during wort boiling primarily promotes the isomerization of α-acids into iso-α-acids, which contribute bitterness and microbial stability. However, boiling also results in substantial losses of volatile hop aroma compounds through evaporation. These losses are particularly pronounced for hydrocarbon terpenes such as myrcene, β-caryophyllene and α-humulene, which exhibit low polarity, limited solubility in beer and high volatility. In contrast, oxygenated terpenes such as linalool and geraniol generally display greater retention due to their increased polarity and improved solubility (Takoi et al. 2017; Lafontaine et al. 2018; Hauser et al. 2019).

Consequently, late and cold-side hop additions have become increasingly important for maximizing hop aroma while minimizing thermal losses. In hop-forward beer styles, hops are commonly added at multiple stages throughout the brewing process to achieve a broad and complex aroma profile.

Dry hopping refers to the addition of hops after the onset of fermentation, typically several days into fermentation, when the wort temperature is reduced and active boiling has ceased. Although commonly referred to as “dry hopping,” the process more accurately represents cold-side hopping, as hops are added directly to beer or fermenting wort. Dry hopping is generally not performed

during the most active phase of fermentation, as vigorous carbon dioxide evolution may promote the stripping and volatilization of aroma-active compounds (Haefliger & Jeckelmann 2013).

The most common dry hopping approach is static dry hopping, in which hops are added directly to the fermentation vessel without agitation. When applying dry hopping, practical considerations must be considered, as hop material can absorb beer and expand, thereby reducing available headspace and potentially increasing pressure within the fermentation vessel. This effect is particularly relevant when using dried or freeze-dried hop products (Lafontaine & Shellhammer 2018; Hauser et al. 2019).

The final composition of hop-derived compounds in beer therefore reflects a combination of extraction efficiency, volatilization, solubility, adsorption to yeast and hop material, and potential biotransformation during fermentation. Consequently, the aroma profile of finished beer does not necessarily mirror the composition of the original hop material. Understanding these processes is essential when evaluating the transfer and fate of hop-derived compounds from raw hops to finished beer (Salamon et al. 2022).

3. Method

3.1 Brewing setup

The base beer was designed as a neutral American Pale Ale model beer to provide a consistent beer matrix for comparing hop treatments. The recipe was designed with a target original gravity of approximately 1.065, a target alcohol content of 5.0–5.5% ABV, and a theoretical bitterness of approximately 35 IBU from a single Magnum boil addition.

Table 1. Target beer model and specifications.

Base style	Neutral American Pale Ale model beer
Target ABV	5.0–5.5%
Target OG	1.065
Target IBU	35
Batch size	80 L
Fermentation vessels	12
Approximate volume per vessel	6-7 L

3.2 Raw materials

Table 2. Brewing raw materials used for target beer model.

DME	Spraymalt Extra Light Muntons	11.6 kg
Yeast	BRY-97 American West Coast, Lallemand	60 g
Bittering Hops	Organic Magnum, Sweden, 11% α-acids	77 g
Aroma Hops	Whole cone hops, treatment-specific cultivars	8 g/L
Enzymes	Aromazyme, Lallemand	4 g in 50 mL distilled water
Yeast nutrient	Wyeast Beer Nutrient Blend	approx. 7.5 g
Kettle fining agent	Protofloc tablets, Murphy & Son Ltd.	approx. 1.5–2 tablets

Note: Amounts refer to total quantities used during brewing unless otherwise stated. Aroma hop dosage refers to the dry-hop rate applied in the experimental treatments, which was selected based on dry-hopping rates reported by Lafontaine & Shellhammer (2018)

Spraymalt Extra Light was selected as the extract source to obtain a pale base beer with low color contribution and improved reproducibility compared with a full mash. BRY-97 was selected as a neutral American ale yeast to minimize yeast-derived flavor interference while providing a fermentation profile suitable for hop-forward beer styles. Its reported β-glucosidase activity was also considered relevant in relation to potential hop aroma biotransformation. The hop materials used in this study consisted of Swedish heritage cultivars obtained from private cultivators.

The cultivars are part of the Swedish national gene bank ("Grönt kulturarv") and were identified based on their registered names and accession codes, where the accession codes refer to the official classification within the Swedish Program for Diversity of Cultivated Plants (POM). East Kent Goldings was included as a commercial reference cultivar to provide comparison with the Swedish heritage hops, while the non-dry-hopped control was included to distinguish hop-derived effects from the base beer matrix.

Table 3. Experimental hop and enzyme treatment design.

Treatment code	**Treatment**	**Hop cultivar**	**Accession / origin**	**Dry-hop mass**	**Approx. dry-hop rate**	**Enzyme added**	**Replicates**
A	Control	None	—	0 g	0 g/L	No	A1, A2
B	Reference	East Kent Goldings	UK reference	55 g	7.9 g/L	Yes	B1, B2
C	Swedish heritage hop	Korsta	SWE25	50 g	8.3 g/L	Yes	C1, C2
D	Swedish heritage hop	Hulla Norrgård	SWE04	50 g	8.3 g/L	Yes	D1, D2
E	Swedish heritage hop	Spångby	SWE08	50 g	8.3 g/L	Yes	E1, E2
F	Swedish heritage hop	Grimsarbo	SWE53	50 g	8.3 g/L	Yes	F1, F2

Note: The control received neither dry hops nor enzyme. All dry-hopped treatments received Aromazyme.

The wort was divided into twelve fermentation vessels representing six treatments in biological duplicate. The control vessels received neither dry hops nor enzyme. All dry-hopped treatments received whole cone hops at approximately 8 g/L, corresponding to the upper range commonly reported for hop-forward beer styles in the range investigated by Lafontaine & Shellhammer (2018), who identified 400–800 g/hL as an effective range for static dry hopping. Aromazyme at a dosage corresponding to 5 g/hL. East Kent Goldings was included as a commercial reference treatment, while Korsta, Hulla Norrgård, Spångby and Grimsarbo represented the Swedish heritage hop treatments.

3.3 Brewing and fermentation

The beer was brewed on 9 March 2026 using an all-in-one electric brewing system (B80pro, Brewtools, Norway) with a total vessel capacity of 90 L. The system was equipped with integrated heating elements, internal recirculation and digital temperature control.

Approximately 80 L of wort was prepared by dissolving 11.6 kg spray-dried malt extract (Muntons Extra Light) in water heated to 68 °C under continuous stirring. The wort volume was adjusted prior to boiling.

The wort was boiled for 60 min. Bittering hops, consisting of 77 g Magnum at 11% α-acids, were added at the start of the boil using a hop spider. Additional external electric heating elements were used to maintain a stable rolling boil. Foam and hot break material were manually removed during boiling.

Yeast nutrient (Wyeast Beer Nutrient Blend) was added according to the manufacturer’s recommendation of 11 g/hL, corresponding to approximately 7.5 g in 80 L wort. A kettle fining agent (Protofloc tablets, Murphy & Son Ltd., UK) was added at a rate of approximately 1.5–2 tablets per 80 L wort. Both additions were made 15 min before the end of the boil.

After boiling, the wort was cooled to 18–19 °C using an immersion cooling coil and transferred into twelve fermentation vessels, each containing approximately 6–7 L. Fermentation vessels were cleaned with an alkaline detergent (PBW) and sanitized with Star San prior to use. Airlocks were sanitized and filled with a Star San–water solution.

Dry yeast (*Saccharomyces cerevisiae*, BRY-97 American West Coast Ale Yeast, Lallemand) was rehydrated in sterile water at approximately 30–35 °C for 30 min. The yeast slurry was added to the wort in the brewing vessel, after which the wort was recirculated for several minutes to promote even yeast distribution prior to transfer. Each fermentation vessel received approximately 5 g yeast. Fermentation was conducted at 18–19 °C.

Each fermentation vessel was labelled according to treatment and biological replicate (A1–F2) to ensure traceability throughout the experiment.

3.4 Experimental design

The wort was divided into twelve fermentation vessels representing six treatments conducted in biological duplicate (n = 2). The treatments consisted of different hop cultivars and one non-dry-hopped control. Two vessels (A1 and A2) served as negative controls and did not receive hop or enzyme additions.

3.5 Dry hopping and enzyme treatment

Dry hopping was performed during active fermentation, 4 days after yeast inoculation. Prior to dry-hop addition, fermentation vessels were gently swirled to homogenize the beer. Specific gravity and pH were measured for all vessels, and baseline samples were collected from six representative fermenters (A1, B1, C1, D1, E1 and F1).

Whole hop cones were added at a rate of approximately 8 g/L, corresponding to 50 g in 6 L vessels and 55–56 g in 7 L vessels. This dosage was selected to fall within the upper range of dry-hop rates commonly reported for hop-forward beers. The hops were manually broken apart and placed into cotton hop bags.

Hop bags and stainless-steel nuts used as weights were pre-boiled prior to use. As a final sanitation step, hop bags, stainless-steel weights and butcher’s twine were treated with Star San. The hop bags were tied with sanitized twine, leaving sufficient length for retrieval after the contact period. All handling was performed using sanitized gloves. After addition to the fermentation vessels, the hop bags were manually submerged and gently agitated to ensure full wetting and submersion.

A commercial enzyme preparation (Aromazyme, Lallemand) was added to all dry-hopped vessels. The control vessels (A1 and A2) received neither dry hops nor enzyme. The enzyme was prepared as a stock solution by dissolving 4 g Aromazyme in 50 mL distilled water. It was added at a dosage corresponding to 5 g/hL (0.05 g/L), resulting in approximately 3.8 mL for 6 L vessels and 4.4 mL for 7 L vessels. The enzyme solution was measured and transferred using a sterile 5 mL plastic syringe.

Following addition of hops and enzyme, the fermentation vessels were gently swirled to promote distribution. Fermentation and dry-hop contact were continued at 18–19 °C for 4 days after which the hop bags were removed.

3.6 Bottling and conditioning

Bottling was performed on day 15 after completion of fermentation. A priming solution was prepared by dissolving dextrose in water based on an estimated total beer volume of approximately 64 L, targeting a carbonation level of approximately 2.3 volumes of CO_2 at 20 °C. The solution was boiled for several minutes to ensure complete dissolution and reduce the risk of contamination and was subsequently cooled prior to use.

The priming solution was thoroughly homogenized, and 5 mL was added to each bottle individually using a sterile syringe prior to filling. Bottles were filled leaving approximately 2–3 cm headspace, sealed, and conditioned at 18–20 °C for 14 days prior to further analysis. Minor deviations from the theoretical carbonation level may have occurred due to uncertainty in total beer volume estimation and potential continued fermentation after dry hopping.
Alcohol by volume (ABV) was estimated using the empirical formula shown in Figure 2 (Partichelli et al. 2025), where OG is the original gravity and FG is the final gravity.

$$ABV = (OG - FG) \times 131.25$$

Figure 2. Empirical equation used to estimate alcohol by volume (ABV) from original gravity (OG) and final gravity (FG). Adapted from Partichelli et al. (2025).

3.7 Sampling and sample handling

Sampling was conducted at three points: immediately prior to dry hopping (day 4), after dry-hop removal (day 8), and prior to bottling (day 15).

Prior to dry hopping, specific gravity and pH were measured for all fermentation vessels. Baseline green beer samples were collected from six representative fermenters (A1, B1, C1, D1, E1 and F1). Approximately 12–15 mL of beer was transferred into sterile 15 mL tubes, sealed and placed on ice immediately after collection.

After four days of dry-hop contact (day 8), the hop bags were removed and allowed to drain without squeezing. Sampling was then performed for all twelve fermentation vessels. Prior to sampling, the outlet tap was sanitized with Star San and an initial volume of approximately 10–20 mL was discarded. Approximately 15 mL of beer was collected into sterile tubes. Duplicate samples were collected from each vessel, consisting of one sample for analysis and one backup sample.

Prior to bottling on day 15, an additional sampling was performed from all fermentation vessels using the same procedure as described above.

All samples were placed on ice immediately after collection, transported under cold conditions and stored at −20 °C until analysis to minimize chemical changes and loss of volatile compounds.

3.8 Sensory evaluation

3.8.1 Objective

The sensory evaluation was conducted to characterize and compare the aroma profiles of dry-hopped beers produced with Swedish heritage hop varieties, using an international reference hop for contextual comparison.

3.8.2 Experimental design

The study include six treatments (four Swedish hop varieties, one international reference hop, and one non–dry-hopped control), each produced in duplicate fermentation vessels, resulting in a total of 12 samples.

Due to the number of samples, the sensory evaluation was conducted in three sessions to reduce sensory fatigue. Each session will include four samples.

Replicate samples were randomly distributed across the three sessions rather than grouped, to minimize potential bias and recognition effects between sessions.

All samples were labelled with randomized three-digit codes, and a separate coding key will be maintained by the researcher. The coding will ensure that assessors cannot identify or link replicate samples across sessions.

Samples were presented in randomized orders within each session, three different serving orders divided by four tables. Each assessor evaluated all treatments (within-subject design).

3.8.3 Panel

A panel of 16 assessors was recruited, primarily consisting of beer-interested individuals with varying levels of experience.

Prior to evaluation, a short calibration session with two commercial beers, one New England DIPA and one West Coast IPA, was conducted to align the interpretation of attributes and the use of the intensity scale among assessors.

3.8.4 Methodological approach

Sensory evaluation was conducted using a descriptive approach, as sensory analysis is a scientific discipline used to measure, analyze and interpret human responses to stimuli (Gómez-López 2020).

A structured attribute-based evaluation similar to rate-all-that-apply (RATA) was applied, allowing assessors to both identify and rate the intensity of perceived attributes, which has been shown to provide reproducible data with semi-trained panels (Giacalone & Hedelund 2016).

3.8.5 Aroma attributes

The selected aroma attributes were based on commonly used descriptors in beer sensory analysis and were adapted to reflect the expected aroma profiles of dry-hopped beer. Attributes such as citrus, floral, fruity, herbal and green were included to capture hop-derived terpenoid compounds, which are known to contribute significantly to beer aroma and are influenced by both hop composition and brewing process conditions (Sharp et al. 2017b; a).

The following attributes was evaluated:

- Floral
- Citrusy
- Fruity
- Woody
- Green/grassy
- Herbal
- Spicy
- Garlic/onion (Sulfur-like)
- Sharp/edgy (aroma)
- Overall hop aroma intensity

All attributes were evaluated based on aroma perception (orthonasal and retronasal), and not on bitterness or mouthfeel.

3.8.6 Intensity scale

A structured 0–9 category scale was used:

- 0 = not perceptible
- 1–3 = low intensity
- 4–6 = medium intensity
- 7–9 = high intensity

The use of the scale was explained during the calibration session to ensure consistent interpretation among assessors.

3.8.7 Sample preparation and serving

Samples were served in identical glasses at a consistent temperature. Approximately 50 mL of beer was served per sample.

Assessors were provided with water and neutral crackers for palate cleansing between samples.

Participants were seated with sufficient spacing and instructed to evaluate samples individually without discussion.

3.8.8 Evaluation procedure and data treatment

For each sample and attribute, intensity scores were recorded using a structured 0–9 scale. Assessors first evaluated each sample orthonasally, followed by a small sip to allow retronasal perception. Samples were expectorated after tasting. Assessors were instructed to focus on aroma perception rather than bitterness or mouthfeel. Scores could be adjusted after tasting if necessary.

Missing responses (blank entries) were treated as missing values and were not replaced. Responses of 0 were retained as valid observations, representing “no perception”. For each treatment and attribute, mean intensity values and standard deviations were calculated.

Statistical analysis was performed using Minitab Statistical Software. A one-way analysis of variance (ANOVA) was applied to evaluate differences in mean intensity between treatments. Biological replicates were pooled prior to analysis. Unequal sample sizes resulting from missing values were accepted, and missing data were excluded from calculations. Post hoc comparisons were conducted using Tukey’s method at a significance level of $\alpha = 0.05$. The non–dry-hopped control was included in the analysis as a reference sample.

3.9 Hop material handling

For each hop cultivar, approximately two-thirds of the available material was used for dry hopping. The hops were supplied in 50 g packages, which were weighed prior to use. The remaining unopened portion of each hop variety (~50 g per cultivar) was retained for raw material analysis and stored at −20 °C.

3.10 GC-FID analysis of hop terpenes

3.10.1 Target Compounds

The extraction and chromatographic analysis procedures were adapted from previously published methods for the analysis of hop-derived volatile compounds in beer and hop material, with modifications to sample preparation, extraction conditions and chromatographic detection to accommodate the experimental design and available instrumentation (Oliveira et al. 2006; Mikyška et al. 2024)

A selection of hop-derived terpene compounds was chosen for analysis based on their documented occurrence in hop essential oils and their relevance for beer aroma and terpene biotransformation during brewing and fermentation. The

selected compounds included both hydrocarbon terpenes, primarily associated with hop varietal characteristics, and oxygenated monoterpenes known to contribute directly to beer aroma. Target compounds and their analytical relevance are presented in Table 4.

Table 4. Target terpene compounds selected for the planned GC–FID analysis.

Compound	**Chemical Class**	**Most Relevant Matrix**	**Analytical Role**	**Contributes to Beer Aroma?**	**Typical Aroma Description**
β-Myrcene	Monoterpene (hydrocarbon)	Raw hop	Varietal fingerprint; dominant hop oil component	Limited (often reduced after fermentation)	Green, resinous, citrus, pine
Limonene	Monoterpene (hydrocarbon)	Hop and beer	Aroma-active terpene; citrus marker	Yes	Citrus, orange peel, lemon
trans-Caryophyllene	Sesquiterpene (hydrocarbon)	Raw hop	Varietal marker; hop oil constituent	Limited as parent compound	Spicy, woody, herbal
β-Farnesene	Sesquiterpene (hydrocarbon)	Raw hop	Varietal marker; cultivar discrimination	Limited as parent compound	Woody, green apple, sweet, lavender
Linalool	Monoterpene alcohol	Beer	Aroma-active; stable oxygenated terpene	Yes	Floral, lavender, citrus
Geraniol	Monoterpene alcohol	Beer	Aroma-active; precursor to biotransformation products	Yes	Rose, citrus, fruity
Nerol	Monoterpene alcohol	Beer	Aroma-active terpene alcohol; geraniol isomer	Yes	Floral, rose, citrus

Note: Hydrocarbon terpenes were included primarily as varietal markers in raw hop material, whereas oxygenated monoterpenes were targeted due to their relevance for beer aroma, retention during brewing and potential biotransformation during fermentation (Takoi et al. 2010; Praet et al. 2016; Sharp et al. 2017b; Haslbeck & Benjamin 2022)

3.10.2 Beer sample extraction

Beer samples were kept cold prior to extraction and were only minimally degassed to avoid excessive loss of volatile compounds.
Approximately 10 mL of beer was first transferred to a measuring cylinder and then added to a glass extraction tube containing solid sodium chloride (NaCl). Addition of NaCl appeared to promote partial CO_2 release and salting-out effects within the sample matrix.
Approximately 2–3 mL dichloromethane (DCM) was then added to the extraction tube. The tube was sealed and placed on a slow rotational shaker/tube rotator for approximately 10–15 min to facilitate extraction of volatile compounds into the organic phase.
The same extraction procedure was used for:

- one representative green beer sample collected before dry hopping (GB),
- post dry-hop samples collected immediately after hop removal (A1 DH–F1 DH),
- and finished beer samples collected at bottling (A1 Beer–F1 Beer).

Only one green beer sample was analyzed since all fermenters originated from the same base wort prior to dry hopping and treatment separation.
After extraction, the samples were allowed to separate overnight to improve phase clarification. Following separation, three distinct layers became visible:

- an upper aqueous beer phase,
- an intermediate translucent gel-like/emulsion layer,
- and a lower organic DCM phase.

The upper beer phase was carefully removed manually using micropipettes and Pasteur pipettes in order to improve access to the organic phase. Some material from the intermediate layer may occasionally have been collected during transfer of the DCM phase.
The lower organic phase was subsequently collected carefully using glass Pasteur pipettes. The collected extracts were filtered through 0.2 µm PTFE syringe filters, which effectively removed remaining particulate and gel-like material, before being transferred into clean glass vials.
Prepared extracts were stored refrigerated until analysis.

3.10.3 Hop sample extraction

Hop extractions are prepared on the same day as the beer extractions.

For each hop sample, approximately 0.5 g of hop material is weighed into a glass extraction tube. Approximately 10 mL DCM is then added to the tube.

The hop material is manually disrupted and roughly ground directly in DCM using a glass rod to improve solvent contact, while avoiding excessive fine particle formation.

The samples are extracted for approximately 30–40 min. During extraction, the tubes are occasionally rotated and gently shaken by hand to facilitate extraction of hop-derived volatile compounds into the solvent phase.

Unlike beer samples, the hop extracts do not form separate liquid phases. Instead, the extracts consist of DCM containing dissolved hop-derived volatile compounds together with suspended hop particles.

After extraction, the DCM extracts are carefully transferred into new glass tubes in order to remove as much hop material and particulate matter as possible. The transferred extracts are then allowed to stand overnight to improve sedimentation and clarification.

The following day, the clearer DCM extracts are filtered through 0.2 µm PTFE syringe filters and transferred into clean glass vials.

Prepared extracts are stored refrigerated until analysis.

3.10.4 Standards GC-FID analysis

Analytical standards of β-myrcene (95%), limonene (97%), linalool (97%), geraniol (98%), β-citronellol (95%), trans-caryophyllene (80%) and nerol (97%) were purchased from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany). The selected compounds represent major hydrocarbon and oxygenated terpene constituents of hop essential oils and were chosen based on their relevance for hop aroma, beer aroma and terpene biotransformation during brewing and fermentation.

A terpene reference mixture was prepared using the analytical standards and analyzed prior to sample analysis. The reference mixture was used for compound identification through comparison of retention times between standards and sample chromatograms.

The GC oven program was adapted from (Oliveira et al. 2006) with minor modifications.

GC-FID analysis was performed using an Agilent 8890 gas chromatograph equipped with a flame ionization detector (FID) and a 30 m HP capillary column. Helium was used as the carrier gas. The oven temperature was initially held at 60°C for 5 min, increased at a rate of 3°C min^{-1} to 240°C, and held at the final temperature for 5 min. .

Terpene standards and beer extracts were analyzed using an injection volume of 5 µL, whereas hop extracts were analyzed using an injection volume of 2 µL due to the substantially higher concentration of extracted volatile compounds.

The analysis included hop extracts, green beer, post dry-hop beer samples and finished beer samples. Compound identification was performed by comparing retention times obtained from the terpene standard chromatogram with retention times observed in sample chromatograms.

Chromatographic data were processed using Agilent OpenLab software. Peak integration was performed manually when necessary to improve peak assignment and consistency between samples. Results were evaluated using relative peak areas and compound occurrence rather than absolute concentrations. Representative chromatograms of the terpene standard mixture, hop extracts and beer samples are presented in Appendix 2.

3.10.5 Method Limitations

The original analytical plan involved GC-MS analysis to enable compound confirmation and quantitative determination of hop-derived terpene compounds. However, due to delayed delivery of analytical standards and limited instrument availability, GC-FID was used instead.

As flame ionization detection does not provide structural information, compound identification was based on comparison with authentic analytical standards and retention time matching. Consequently, compound assignments should be regarded as tentative until confirmed by GC-MS analysis.

Furthermore, no calibration curves or internal standards were employed, preventing absolute quantification of terpene concentrations. Since different injection volumes were used for hop extracts (2 µL) and beer extracts (5 µL), chromatographic peak areas cannot be directly compared between matrices. The results were therefore interpreted semi-quantitatively, focusing on compound occurrence and relative abundance patterns throughout the brewing process.

3.11 HPLC Analysis of α- and β-Acids

3.11.1 Reference Standard and Calibration

The extraction and HPLC procedure was adapted from Jaskula et al. (2007) and Baker et al. (2008), with minor modifications.
An International Calibration Extract (ICE-4) was used as an external reference standard for quantification of α- and β-acids. The extract contained 42.58% total α-acids and 26.54% total β-acids.

The composition of individual bitter acid fractions was as follows:

- Cohumulone: 10.98%
- Humulone + adhumulone: 31.60%
- Colupulone: 13.02%
- Lupulone + adlupulone: 13.52%

A stock solution was prepared by dissolving 117 mg of ICE-4 in 25 mL of mobile phase. Serial dilutions (STD1–STD5) were prepared to generate a five-point calibration range for each analyte group.

Calibration curves were constructed individually for cohumulone, humulone + adhumulone, colupulone, and lupulone + adlupulone. Peak area responses were plotted against known concentrations, and linear regression was applied to obtain calibration equations.

Quantification of analytes in samples was performed using the respective calibration functions according to:

$$x = (\text{area} + \text{intercept}) / \text{slope} \times \text{dilution factor}$$

where x represents the concentration of the compound in the injected sample.

Total α-acids and β-acids were calculated by summing the concentrations of their respective components:

- Total α-acids = cohumulone + (humulone + adhumulone)
- Total β-acids = colupulone + (lupulone + adlupulone)

This approach accounts for differences in detector response between individual compounds and improves quantification accuracy compared to the use of a single total calibration curve.

3.11.2 Mobile phase and chromatographic conditions

The mobile phase consisted of 85% (v/v) HPLC-grade methanol and 15% (v/v) distilled, deionized water acidified with 0.025% (v/v) formic acid, based on established methods for hop acid analysis (Baker et al., 2008).

Separation was performed using a reversed-phase C18 column with a flow rate of approximately 1.0 mL/min. Detection was conducted using UV absorbance at 326 nm, corresponding to the absorbance maximum of α- and β-acids (Baker et al., 2008).

3.11.3 Hop Sample Preparation

Hop samples (1.000 g) were extracted in 100 mL of extraction solvent (mobile phase). Each hop variety was prepared in triplicate.

Extraction was performed using an ultrasonic bath for 30 min at approximately 25°C, following established laboratory procedures. The extracts were vacuum filtered and subsequently filtered through a 0.20 µm PTFE syringe filter prior to transfer into HPLC vials.

3.11.4 Beverage sample preparation

Beer samples were degassed prior to analysis to remove dissolved carbon dioxide. Degassing was performed manually to avoid excessive agitation and foaming, as sonication was found to be too vigorous for the beer matrix.

Samples were acidified prior to extraction to facilitate recovery of hop-derived bitter compounds.

Solid phase extraction (SPE) was used to concentrate α- and β-acids prior to HPLC analysis, following established methodologies (Jaskula et al., 2007).

SPE cartridges were conditioned with methanol and equilibrated with deionized water before sample loading. Samples were passed through the cartridge at a controlled flow rate, followed by washing and elution with organic solvent.

The resulting extracts were filtered through a 0.20 µm PTFE syringe filter and transferred into HPLC vials. Further details of the SPE procedure are provided in Appendix A.

3.11.5 HPLC analysis

HPLC analysis was performed using isocratic elution with the mobile phase described above.

Samples included calibration standards, hop extracts, and extracted beer samples. Each sample was analyzed in replicate where possible.

Peak identification was based on retention times obtained from the ICE-4 standard, where cohumulone elutes first, followed by humulone + adhumulone, colupulone, and lupulone + adlupulone (Baker et al., 2008).

High-performance liquid chromatography is widely used for selective and quantitative determination of hop-derived bitter acids in beer, offering improved specificity compared to spectrophotometric methods (Jaskula et al., 2007). Representative chromatograms of the ICE-4 standard and the calibration curves used for quantification are provided in Appendix 1.

4. Results

4.1 Fermentation performance and final beer parameters

OG was measured on brew day (day 0) before the wort was divided between fermentation vessels. SG before dry hopping was measured on day 4, SG after dry-hop removal on day 8, and FG at bottling on day 15 of the fermentation process.

Table 5. Fermentation performance and final beer parameters by treatment.

Treatment	OG	SG before dry hopping	SG after dry hopping	FG	pH before dry hopping	pH after dry hopping	Final pH
Control	1.063	1.034 (1.032–1.035)	1.017 (1.017–1.017)	1.012	4.63 (4.61–4.65)	4.44 (4.35–4.52)	4.51 (4.50–4.51)
East Kent Goldings	1.063	1.035 (1.034–1.035)	1.017 (1.015–1.018)	1.012	4.61 (4.57–4.64)	4.77 (4.76–4.77)	4.85 (4.84–4.85)
Korsta	1.063	1.034 (1.034–1.034)	1.015 (1.015–1.015)	1.012	4.58 (4.57–4.58)	4.81 (4.79–4.83)	4.93 (4.92–4.94)
Hulla Norrgård	1.063	1.035 (1.035–1.035)	1.015 (1.014–1.015)	1.012	4.63 (4.62–4.64)	4.77 (4.75–4.78)	4.89 (4.88–4.89)
Spångby	1.063	1.035 (1.034–1.035)	1.015 (1.014–1.015)	1.012	4.65 (4.63–4.66)	4.65 (4.63–4.66)	4.72 (4.71–4.73)
Grimsarbo	1.063	1.035 (1.035–1.035)	1.016 (1.015–1.016)	1.012	4.64 (4.63–4.64)	4.63 (4.61–4.64)	4.73 (4.72–4.73)
Overall mean	**1.063**	**1.035**	**1.016**	**1.012**	**4.62**	**4.67**	**4.77**
Overall SD	**0.000**	**0.001**	**0.001**	**0.000**	**0.03**	**0.14**	**0.15**

Note: Treatment values are presented as means with replicate ranges in parentheses (n = 2). Overall mean and standard deviation were calculated across all fermentation vessels (n = 12).

Since all 12 fermentation vessels had the same base wort, the OG is assumed to be consistent after the separation into individual vessels. The overall mean for the SG before dry hopping, measured 4 days after fermentation, was 1.035, indicating active breakdown of available sugars. This was consistent in the measurements after dry hopping, with a mean SG of 1.016, and in the final gravity (FG) of 1.012, approximately 14 days after the initial fermentation start. Alcohol by volume (ABV) was calculated with the ABV formula to an ABV of 6.7%.

The pH measurements before dry hopping and after dry hopping were within the typical range for beer pH, around 4.6 to 4.7, primarily due to the production of organic acids by the yeast. The initial pH of wort typically starts around 5.4 and gradually decreases as fermentation progresses, reaching a final pH closer to 4.6–4.7 at bottling. What was observed, however, was a slight pH increase following the dry hopping treatment, likely linked to the extraction of hop-derived compounds during dry hopping. This pH increase was observed throughout the fermentation vessels, with the exception of the control, which maintained a consistent pH drop.

4.2 HPLC: Alpha and Beta acids

Table 6. HPLC-derived composition of α- and β-acids in hop samples. Data are presented as mean ± standard deviation (n = 3). Concentrations are expressed in mg/g, while the relative contribution of each fraction (cohumulone, humulone + adhumulone, colupulone, and lupulone + adlupulone) is given as percentage of total α- or β-acids.

Variety	Coh (mg/g)	Hum+ Adh (mg/g)	Total α (mg/g)	Coh (%)	Hum+ Adh (%)	Col (mg/g)	Lup+ Adlup (mg/g)	Total β (mg/g)	Col (%)	Lup (%)	α/β
MAG	29.55 ± 1.54	88.22 ± 4.76	117.77 ± 6.30	25.09 ± 0.03	74.91 ± 0.11	29.42 ± 1.50	26.93 ± 1.27	56.34 ± 2.77	52.20 ± 0.11	47.80 ± 0.11	2.09 ± 0.01
EKG	17.53 ± 1.03	50.19 ± 3.30	67.73 ± 4.33	25.90 ± 0.12	74.10 ± 0.40	20.94 ± 1.60	13.62 ± 1.27	34.56 ± 2.87	60.61 ± 0.40	39.39 ± 0.40	1.96 ± 0.08
KOR	24.76 ± 1.37	80.87 ± 6.02	105.63 ± 7.39	23.46 ± 0.35	76.54 ± 0.21	61.54 ± 2.04	61.44 ± 2.10	122.99 ± 4.11	50.04 ± 0.21	49.96 ± 0.21	0.86 ± 0.06
HUL	18.13 ± 1.43	54.74 ± 4.72	72.87 ± 6.15	24.88 ± 0.16	75.12 ± 0.16	33.07 ± 3.13	28.42 ± 2.40	61.49 ± 4.89	53.77 ± 2.13	46.23 ± 2.13	1.18 ± 0.06
SPA	18.94 ± 3.74	53.47 ± 10.87	72.41 ± 14.61	26.17 ± 0.19	73.83 ± 0.19	25.38 ± 5.08	16.05 ± 3.09	41.43 ± 8.17	61.23 ± 0.21	38.77 ± 0.21	1.75 ± 0.02
GRI	6.79 ± 0.20	22.21 ± 0.26	29.00 ± 0.42	23.40 ± 0.43	76.60 ± 0.43	16.31 ± 0.62	14.33 ± 0.90	30.64 ± 1.48	53.24 ± 0.81	46.76 ± 0.81	0.95 ± 0.05

HPLC analysis of the hop samples revealed substantial variation in α- and β-acid composition between varieties. Magnum and Korsta exhibited the highest bittering potential, with total α-acid contents exceeding 100 mg/g (117.8 ± 6.3 mg/g and 105.6 ± 7.4 mg/g, respectively). In both varieties, the α-acid fraction was dominated by humulone and adhumulone (88.22 ± 4.76 mg/g for Magnum and 80.87 ± 6.02 mg/g for Korsta). Spångby and Hulla Norrgård showed intermediate α-acid levels, with total α-acid concentrations of 72.41 ± 14.61 mg/g and 72.87 ± 6.15 mg/g, respectively, while Grimsarbo exhibited the lowest bittering potential at 29.00 ± 0.42 mg/g. The α/β ratio varied between the hop varieties. Magnum, East Kent Goldings, and Spångby displayed typical profiles with α-acid dominance (α/β ≈ 2), whereas Grimsarbo showed nearly equal concentrations of α- and β-acids (0.95 ± 0.05). A similar trend was observed for Hulla Norrgård (1.18 ± 0.06). In contrast, Korsta exhibited an unusual profile with β-acids exceeding α-acids, resulting in an α/β ratio below unity (0.86 ± 0.06).

Table 7. α- and β-acid concentrations in beer samples determined by HPLC. Data are shown as individual replicates and mean ± standard deviation (n = 2) in mg/L. Values below the detection limit are reported as <LOD.

Sample	Variety	Coh (mg/L)	Hum+Adh (mg/L)	Total α (mg/L)	Total β (mg/L)
A1	Control	11.12	23.29	34.41	<LOD
A2	Control	9.94	22.41	32.35	<LOD
A (mean ±SD)	Control	10.53 ± 0.83	22.85 ± 0.62	33.38 ± 1.45	<LOD
B1	EKG	11.97	<LOD	11.97	
B2	EKG	26.18	34.71	60.89	<LOD
B (mean ± SD)	EKG	19.07 ± 10.05	34.71 (n=1)	36.43 ± 34.59	<LOD
C1	KOR	44.65	69.16	113.81	<LOD
C2	KOR	39.25	68.23	107.48	9.69
C (mean ± SD)	KOR	41.95 ± 3.82	68.70 ± 0.66	110.65 ± 4.48	9.69 (n=1)
D1	HUL	31.82	50.81	82.63	<LOD
D2	HUL	21.1	36.56	57.65	<LOD
D (mean ± SD)	HUL	26.46 ± 7.58	43.68 ± 10.08	70.14 ± 17.66	<LOD
E1	SPA	23	35.48	58.48	<LOD
E2	SPA	21.72	35.01	56.73	<LOD
E (mean ± SD)	SPA	22.36 ± 0.90	35.25 ± 0.33	57.60 ± 1.23	<LOD
F1	GRI	18.55	34.13	52.69	<LOD
F2	GRI	18.47	32.23	50.71	<LOD
F (mean ± SD)	GRI	18.51 ± 0.06	33.18 ± 1.34	51.70 ± 1.40	<LOD

Continuing with the HPLC analysis of the beer samples, β-acids were below the limit of detection in all samples except C2 (Korsta), where total β-acids were detected at 9.69 mg/L. This may be related to the high β-acid content observed in the Korsta hop material; however, since β-acids were not detected in the biological replicate C1, this result should be interpreted with caution.

For α-acid residues, most dry-hopped beer samples showed higher concentrations than the non-dry-hopped control, which had a total α-acid concentration of 33.38 ± 1.45 mg/L. Korsta showed the highest mean total α-acid concentration in beer, with 110.65 ± 4.48 mg/L, followed by Hulla Norrgård at 70.14 ± 17.66 mg/L. The comparatively high standard deviation for Hulla Norrgård indicates greater variation between biological replicates. Spångby and Grimsarbo showed lower but still elevated total α-acid concentrations compared with the control, with 57.60 ± 1.23 mg/L and 51.70 ± 1.40 mg/L, respectively.

East Kent Goldings showed inconsistent replicate behavior. In B1, only the cohumulone fraction was detected at 11.97 mg/L, whereas B2 contained both cohumulone and humulone + adhumulone, resulting in a total α-acid concentration of 60.89 mg/L. Because of this discrepancy, the East Kent Golding beer results should be interpreted cautiously and may indicate analytical or sample-specific variation.

Previous studies done on dry hopped New England IPA showed that residual α-acids and iso-α- acids can be found in substantial concentration at the same time. The studies data resulted in an average of 31 mg/l residual α-acids and 20 mg/L iso-α-acids in the beers. Pointing at the fate of the alpha acids is multiple, such as degradation, precipitation and adsorption leading to the unrecovered fraction (Maye, J. P., & Smith, R. 2018)

To further contextualize the HPLC results, apparent α-acid recovery estimates were calculated by comparing the theoretical α-acid contribution from hop additions with the residual α-acid concentrations detected in the finished beer.

Table 8. Apparent residual α-acid retention after kettle hopping and apparent α-acid recovery following dry hopping.

Hop	Process	α-acids in hops (%)	Theoretical α added (mg/L)	Beer α-acids (mg/L)	Apparent recovery (%)
Magnum	Kettle	11.77	113.28	33.38	29.47
EKG	Dry hop	6.77	531.93	36.43	0.57
Korsta	Dry hop	10.56	880	110.65	8.78
Hulla Norrgård	Dry hop	7.29	607.5	70.14	6.05
Spångby	Dry hop	7.24	603.33	57.6	4.01
Grimsarbo	Dry hop	2.9	241.67	51.7	7.58

Note: Dry-hop recovery was estimated after subtraction of the residual α-acid concentration detected in the control beer (33.38 mg/L). Values represent apparent α-acid recovery and not direct measurements of iso-α-acids or true isomerization efficiency.

Literature suggests that non-isomerized α-acids can be extracted during dry hopping, although their solubility in beer is limited. The dry-hopped beers in the present study generally showed elevated residual α-acid concentrations compared with the non-dry-hopped control, indicating transfer of hop-derived α-acids into the beer during dry hopping. Previous studies have reported dry-hop α-acid extraction levels around 4–5%, which is comparable to several of the apparent recovery values observed in the present study. Previous studies reported α-acid extraction levels typically below 10% of theoretical α-acid additions and also increase in residual α-acids concentrations after dry hop in comparison with control beer without any dry hop addition (Cocuzza et al. 2019; Kemp et al. 2021)

The residual α-acid concentration detected in the control beer corresponded to an apparent α-acid retention of approximately 29% of the theoretical α-acid addition from the Magnum bittering hops. However, this value does not represent true kettle utilization, as the HPLC method quantified residual α-acids rather than iso-α-acids directly. The unrecovered fraction therefore likely reflects a combination of α-acid isomerization, adsorption to trub and yeast biomass, oxidative degradation, and other process-related losses.

In contrast, dry hopping occurs at fermentation temperatures where significant α-acid isomerization is not expected. Therefore, the apparent recovery values

observed in the dry-hopped beers primarily reflect the extraction and retention of non-isomerized α-acids rather than formation of iso-α-acids.

4.3 Descriptive sensorics analysis: hop aromas

Table 9. Mean sensory intensity values (± standard deviation) for all evaluated attributes across treatments. Data represent pooled results from 16 assessors across both biological replicates. High standard deviations indicate considerable variability among participants.

Treatment	Citrus	Fruity	Floral	Pine/ Resin	Herbal	Green/ Grassy	Onion/ Garlic	Woody	Sharp	Hop Intensity
Control	2.63 ± 1.96	2.39 ± 2.06	2.30 ± 2.02	3.26 ± 2.72	3.12 ± 3.11	2.23 ± 2.41	1.05 ± 1.33	2.48 ± 2.11	3.79 ± 2.77	3.89 ± 1.88
EKG	2.62 ± 2.14	3.41 ± 2.28	2.72 ± 2.25	2.23 ± 1.88	3.88 ± 2.71	2.50 ± 2.67	0.90 ± 1.24	1.54 ± 1.54	3.00 ± 2.51	4.75 ± 1.73
Grimsarbo	1.40 ± 1.32	2.31 ± 1.83	2.50 ± 2.08	2.21 ± 2.67	2.89 ± 2.74	1.33 ± 1.53	0.80 ± 1.51	1.78 ± 1.48	2.75 ± 2.49	3.58 ± 2.36
Hulla Norrgård	2.36 ± 2.31	2.55 ± 1.82	2.73 ± 2.03	3.08 ± 2.57	3.57 ± 2.53	1.96 ± 1.68	0.82 ± 1.10	2.46 ± 2.09	3.21 ± 2.80	4.04 ± 2.23
Korsta	2.00 ± 1.53	2.96 ± 2.10	2.54 ± 2.12	2.80 ± 2.31	2.64 ± 2.52	2.00 ± 1.89	0.57 ± 0.81	2.00 ± 2.00	3.74 ± 2.80	4.46 ± 1.82
Spångby	2.09 ± 1.83	2.57 ± 2.33	2.26 ± 1.75	2.71 ± 2.42	2.52 ± 2.36	2.13 ± 2.07	1.00 ± 1.83	2.26 ± 2.14	3.13 ± 2.76	3.84 ± 2.01

Sensory data were collected during a structured tasting of the 12 beer samples, representing the different fermentation vessels with a semi-trained/ consumer panel. Typical hop aroma attributes were chosen for the participants to score from 0-9. Even though the panel consisted of beer-interested participants and we had a calibration session with known commercial beers with these aroma attributes, the standard deviations are high among the panel participants. The only aroma attributes that is closer to a difference is the hop intensity score, where the deviation is more reasonable and the hop varieties with the highest score is EKG (4.75 ± 1.73) and Korsta (4.46 ± 1.82).

Table 10. Summary of one-way ANOVA results for all sensory attributes. No statistically significant differences were observed between treatments (α = 0.05). ns = not significant.

Attribute	**F-value**	**p-value**	**Significance**
Citrus	1.51	0.189	ns
Fruity	1.09	0.368	ns
Floral	0.29	0.918	ns
Pine/Resin	0.76	0.578	ns
Herbal	1.09	0.370	ns
Green/Grassy	0.75	0.589	ns
Onion/Garlic	0.34	0.888	ns
Woody	0.68	0.496	ns
Sharp	0.56	0.730	ns
Hop Intensity	1.13	0.347	ns

According to the one-way ANOVA analysis, none of the evaluated sensory attributes showed statistically significant differences between treatments ($p > 0.05$). The sensory dataset was characterized by substantial inter-individual variability among assessors, which contributed to large within-group variation across treatments.

Among the evaluated attributes, citrus showed the strongest tendency toward treatment-related variation, with the lowest p-value ($p = 0.189$) and the highest F-value ($F = 1.51$) observed in the study. Although not statistically significant, this suggests a weak tendency toward differences in perceived citrus character between some hop varieties. Apart from citrus, no other attribute showed a meaningful tendency toward treatment-related variation based on the ANOVA results. While attributes such as fruitiness and hop intensity exhibited slightly lower p-values than the remaining attributes, these effects were weaker statistically and should not be interpreted as clear trends.

Despite measurable differences in α- and β-acid composition and the subsequent transfer of hop-derived compounds into the beer, these chemical differences were not reflected in statistically significant sensory differences between treatments.

4.4 GC-FID Analysis of Hop-Derived Terpenes

4.4.1 Standard Chromatogram and Compound Identification

Table 11. Retention times of terpene reference standards analyzed by GC-FID. The standard mixture was used for compound identification in hop extracts, green beer, post dry-hop beer and finished beer samples through comparison of retention times.

Compound	Retention time (min)
β-Myrcene	13.1
Limonene	14.8
γ-Terpinene	16.2
Linalool	18.2
Geraniol	25.4
Trans-Caryophyllene	32.3

4.4.2 Terpene Detection in Hop Extracts

Table 12. Detection of selected terpene compounds in hop extracts.

Compound	MAG	EKG	KOR	HUL	SPÅ	GRI
β-Myrcene	+	+	+	+	+	+
Limonene	-	-	-	-	-	-
γ-Terpinene	-	-	-	-	+	-
Linalool	-	-	+	+	+	+
Geraniol	-	-	-	-	-	-
trans-Caryophyllene	-	+	+	-	-	-

β-Myrcene was detected in all analyzed hop varieties and represented the most consistently observed terpene compound. Additional hydrocarbons was detected less frequently such as trans-Caryophyllene found in Korsta and East Kent Goldings and γ-Terpinene only detected in Spångby. Among the oxygenated terpenes only Linalool was detected, present in all four varieties of the Swedish heritage hops. Neither Geraniol nor limonene was present in the raw hop material.

4.4.3 Terpene Transfer During Dry Hopping

Table 13. Detection of selected terpene compounds in green beer and post dry-hop beer samples.

Compound	Green beer	A1 DH (control)	B1 DH (EKG)	C1 DH (KOR)	D1 DH (HUL)	E1 DH (SPÅ)	F1 DH (GRI)
β-Myrcene	-	-	-	+	+	-	-
Limonene	-	-	+	+	+	+	-
γ-Terpinene	-	-	-	-	-	-	-
Linalool	-	-	+	+	+	+	+
Geraniol	+	+	+	+	+	+	+
trans-Caryophyllene	-	-	-	-	-	-	-

The green beer sample collected prior to dry hopping showed only geraniol among the target terpene compounds. Geraniol was subsequently detected in all post dry-hop samples, suggesting persistence throughout the brewing process. Following dry hopping, several hop-derived terpenes became detectable in the beer samples. Linalool was the most frequently detected terpene compound and was present in all dry-hopped treatments (B1–F1), followed by limonene, which was detected in four of the five dry-hopped treatments (B1–E1). β-Myrcene was detected only in treatments C1 and D1. These observations indicate successful transfer of volatile hop-derived compounds from the hop material into the beer matrix during dry hopping. In contrast, trans-caryophyllene and γ-terpinene were not detected in any post dry-hop beer sample. Overall, the results indicate a shift from hydrocarbon terpenes towards oxygenated monoterpenes following dry hopping.

4.4.4 Terpene Composition of Finished Beer

Table 14. Detection of selected terpene compounds in finished beer samples.

Compound	A1 (Control)	B1 (EKG)	C1 (KOR)	D1 (HUL)	E1 (SPÅ)	F1 (GRI)
β-Myrcene	-	-	-	+	+	-
Limonene	-	+	+	-	+	-
γ-Terpinene	-	-	-	-	-	-
Linalool	-	+	+	+	+	+
Geraniol	+	+	-	+	+	+
trans-Caryophyllene	-	-	-	-	-	-

The shift towards oxygenated terpenes became more evident in the finished beer samples. Geraniol was detected in all beers except C1, while linalool was detected in all beers except the control (A1), making them the most frequently detected terpene compounds. Limonene was detected in B1, C1 and E1, but was not detected in D1 despite being present in the corresponding post dry-hop sample. β-Myrcene was detected in D1 and E1, whereas it was only detected in C1 and D1 immediately following dry hopping.

Overall, the GC-FID analysis demonstrated a shift in terpene composition throughout the brewing process. Hydrocarbon terpenes, particularly β-myrcene, were more frequently detected in the hop extracts, whereas oxygenated monoterpenes such as linalool and geraniol were more consistently detected in the finished beers. Limonene was detected at intermediate frequencies in both post dry-hop and finished beer samples. Collectively, these observations indicate substantial changes in terpene composition between the raw hop material, post dry-hop beer and finished beer.

5. Discussion

5.1 Raw material, process and extraction

The brewing process parameters, particularly pH measurements, demonstrated that dry hopping affected the beer during fermentation. An increase in pH was observed in all dry-hopped fermentation vessels, resulting in final pH values ranging from 4.72 to 4.93 compared with 4.51 in the control beer. The average pH increase of approximately 0.31 units was most pronounced in the Korsta and Hulla Norrgård treatments.

The observed pH increase is consistent with previous studies investigating the effects of dry hopping. Cocuzza et al. (2019) reported pH increases of up to 0.4 units following dry hopping with hop pellets, while Maye et al. 2016) reported a linear increase of approximately 0.14 pH units per pound of hop pellets added during dry hopping. Based on the dry-hop dosage used in the present study (~8 g/L), the relationship described by (Maye et al (2016) would predict an increase of approximately 0.29 pH units, which is in close agreement with the values observed here.

Although the pH increases corresponded well with previous studies, the use of whole hop cones instead of pellets may have influenced the extraction efficiency of hop-derived compounds. Pelletization disrupts the hop structure and ruptures lupulin glands, generally resulting in greater extraction of bitter acids and aroma compounds compared with whole hop cones (Wolfe 2012). Consequently, the extraction of hop constituents affecting beer pH may have been somewhat limited in the present study relative to studies employing hop pellets.

5.2 Consequences of α and β acids ratios

Korsta exhibited the highest β-acid content among the investigated hop varieties, resulting in the lowest α/β-acid ratio. This distinguishes Korsta chemically from the other hop varieties and may influence its brewing characteristics. Although β-acids possess limited solubility in beer and previous studies have shown that a large proportion remains in the spent hop material after dry hopping, their oxidation products may still be relevant (Maye, J.P., Smith, R. and Leker, J.S. 2016).

β-Acids can oxidize to form hulupones, compounds that have been reported to exhibit approximately 84% of the bitterness intensity of iso-α-acids, while humulinones, formed from α-acids, exhibit approximately 66% of the bitterness intensity of iso-α-acids (Algazzali & Shellhammer 2016). However, hulupones are generally found at substantially lower concentrations than humulinones in dry-hopped beers and are therefore considered to contribute less to overall bitterness ((Maye, J.P., Smith, R. and Leker, J.S. 2016; Klimczak & Cioch-Skoneczny 2023)

Consequently, the elevated β-acid content observed in Korsta may represent a distinctive chemical characteristic of the variety. However, because hulupones were not quantified in the present study and no significant bitterness-related sensory differences were observed between treatments, the practical impact of the elevated β-acid content on beer flavor and bitterness remains uncertain. Nevertheless, the findings highlight the chemical diversity present among Swedish heritage hop varieties and suggest that β-acid composition may be a relevant parameter for future investigations.

5.3 Terpene changes during process

In the raw hop material, a diversity of terpene compounds was detected that was either substantially reduced or not detected in the post dry-hop samples or finished beers. One example was β-myrcene, a hydrocarbon monoterpene characterized by high volatility and low water solubility (Dieckmann & Palamand 1974; Haefliger & Jeckelmann 2013). Previous studies have shown that β-myrcene is particularly susceptible to losses during fermentation through CO_2 stripping and adsorption onto yeast cells (Haslbeck et al. 2018). Similarly, trans-caryophyllene, detected in East Kent Goldings and Korsta, and γ-terpinene, detected only in Spångby, were only observed in the raw hop material. These compounds are volatile hydrocarbons and are often reported only at trace levels in finished beer (Salanță et al. 2012; Praet et al. 2014).

The observed reduction of these compounds is consistent with previous reports describing β-myrcene, β-caryophyllene and α-humulene as dominant constituents of hop essential oils, representing approximately 2.22–45.30%, 9.97–24.62% and 20.20–67.64% of total hop volatiles, respectively (Duarte et al. 2020). Despite their abundance in raw hops, these hydrocarbon terpenes generally exhibit poor retention during brewing due to their hydrophobic nature and volatility.

In contrast, linalool was detected in Korsta, Hulla Norrgård, Spångby and Grimsarbo hop extracts and remained detectable throughout the brewing process. The persistence of linalool in both post dry-hop and finished beer samples is consistent with its physicochemical properties. Linalool is an oxygenated monoterpene alcohol characterized by relatively high water solubility, lower volatility and a low sensory threshold, making it one of the most important hop-derived aroma compounds in beer (Praet et al. 2014; Sharp et al. 2017b).

Furthermore, linalool has been shown to exhibit limited adsorption onto yeast cells compared with hydrocarbon terpenes such as β-myrcene (Haslbeck et al. 2018), which may explain its persistence throughout the brewing process.

Geraniol was also detected in the green beer, post dry-hop beer and finished beer samples, exhibiting similar persistence to linalool throughout the brewing process. Its continued presence from dry-hopped beer to finished beer is consistent with previous studies identifying oxygenated monoterpene alcohols as important contributors to hop-derived aroma in beer (Takoi et al. 2010; Praet et al. 2014; Sharp et al. 2017b).

The behavior of oxygenated monoterpenes during fermentation is complex, as compounds such as linalool, geraniol, nerol and β-citronellol may be directly extracted from hop material, released from glycosidically bound precursors, adsorbed onto yeast cells, or transformed through yeast metabolism (Sharp et al. 2017b; Takoi et al. 2017).

Previous studies have shown that geraniol can decrease during fermentation while β-citronellol increases, suggesting yeast-mediated biotransformation, whereas linalool generally appears more stable (Takoi et al. 2010; 2017).

Although standards for both β-citronellol and nerol were included in the present study, reliable assessment of these compounds was not possible due to co-elution during GC-FID analysis. Consequently, specific biotransformation pathways could not be confirmed.

Limonene was also detected sporadically in beer samples. Limonene is a hop-derived monoterpene associated with citrus-like aroma characteristics and has been reported among the terpene fraction of hop essential oils (Salanță et al. 2012; Roberts et al. 2024). The inconsistent detection observed in the present study may reflect low concentrations close to the analytical detection limit as well as losses during brewing and fermentation.

Overall, the results indicate that the fate of hop-derived terpenes during brewing is strongly dependent on their physicochemical properties. Hydrocarbon terpenes such as β-myrcene, trans-caryophyllene and γ-terpinene generally decreased or disappeared during fermentation and maturation, whereas oxygenated monoterpene alcohols such as linalool and geraniol persisted into the finished beer. These findings are consistent with previous studies and suggest that oxygenated terpenes may contribute more substantially to the final aroma profile than the more abundant hydrocarbon terpenes present in the raw hop material. However, due to the use of GC-FID rather than GC-MS, compound identification should be interpreted cautiously, particularly for co-eluting compounds and low-abundance peaks.

5.4 Sensory evaluation and relationship to analytical results

The sensory evaluation did not reveal statistically significant differences between the dry-hopped beers and the control for the investigated aroma attributes. This indicates that, under the brewing conditions used in the present study, the chemical differences observed between hop treatments were not translated into clearly distinguishable sensory differences. However, the absence of statistical significance should not be interpreted as evidence that the beers were identical. Rather, the sensory outcome may reflect the limited panel size, assessor variability, moderate dry-hop intensity, use of whole hop cones, and the complexity of the beer matrix (Takoi et al. 2010; 2017).

Previous studies investigating hop-derived aroma compounds in beer have reported similar observations, demonstrating that the relationship between hop chemistry and sensory perception is complex. Compound concentration, aroma interactions, matrix effects and assessor variability all influence sensory perception, and measurable differences in hop chemistry do not necessarily result in statistically significant sensory separation between beers (Praet et al. 2014; Sharp et al. 2017a; Dietz et al. 2021a). Furthermore, aroma intensity may have been limited by the dry-hop rate used in combination with whole hop cones, as hop utilization and extraction efficiency differ between whole cones and pelletized hop products (Maye et al. 2016; Cocuzza et al. 2019).

The sensory results are also consistent with the analytical findings. Several hydrocarbon terpenes detected in the raw hop material were reduced or not detected in the finished beer, while the compounds that persisted, such as linalool and geraniol, were present across several treatments. This may have reduced the sensory contrast between beers. Similar observations have been reported in previous studies, where differences in hop composition were measurable analytically but did not necessarily translate into clearly distinguishable sensory profiles (Dietz et al. 2021b; Mikyška et al. 2024).

5.5 Implications for Swedish hop cultivation and future research

The present study demonstrated measurable differences in α-acid content, β-acid content and terpene composition among the investigated Swedish heritage hop varieties compared with the international reference cultivar East Kent Goldings. These findings support previous work suggesting that Swedish-grown hops possess distinct chemical characteristics and contribute to the growing body of knowledge regarding Swedish heritage hop varieties. The observed variation in bitter acid composition and terpene occurrence is consistent with previous reports describing substantial genetic diversity among Swedish remnant hop populations (Karlsson Strese et al. 2014).

Several of the varieties identified as chemically distinctive in the present study, particularly Korsta and Hulla Norrgård, have previously been highlighted for their favorable agronomic performance, α-acid content and essential oil composition under Swedish cultivation conditions (Mackegård 2021). The agreement between these findings suggests that certain Swedish heritage hop varieties may possess characteristics of particular interest for brewing applications.

Several studies have investigated the effects of cultivating the same hop cultivar in different geographical locations and under varying environmental conditions. These studies have demonstrated that cultivation environment can influence essential oil composition, volatile aroma compounds and the sensory characteristics of the finished beer. Furthermore, variation between harvest years has been shown to affect hop chemical composition, supporting the application of both terroir and vintage concepts when describing hop quality and aroma potential (Van Holle et al. 2021; Hagemann et al. 2024; Herkenhoff et al. 2024).

This may also be relevant for Swedish heritage hop varieties. Future studies incorporating multiple cultivation sites and harvest years would enable evaluation of how environmental conditions influence essential oil composition and aroma-active compounds while helping to distinguish cultivar-specific characteristics from terroir and vintage effects.

The present study represents one of the first investigations combining brewing trials, bitter acid analysis, sensory evaluation and terpene profiling of Swedish heritage hops. While sensory differences between beers were limited, the analytical results demonstrated cultivar-dependent variation in both bitter acid composition and terpene occurrence throughout the brewing process. These findings provide a foundation for future studies investigating the brewing potential and aroma characteristics of Swedish heritage hops.

Future studies should include multiple cultivation sites, harvest years, larger sample sizes, pelletized hop material, higher dry-hop rates and quantitative GC-MS analysis to better evaluate the relationship between hop chemistry, aroma transfer and sensory perception. Future work should also investigate the influence of beer style and recipe design on the expression of hop-derived aroma compounds. Different beer matrices, hopping regimes and fermentation conditions may influence the extraction, retention and sensory perception of hop-derived terpenes. Beer styles utilizing higher dry-hop rates, pelletized hop products or formulations specifically designed to emphasize hop aroma may provide greater sensory differentiation between Swedish heritage hop varieties.

Such work could contribute to a deeper understanding of Swedish hop terroir, vintage effects and cultivar-specific brewing characteristics while supporting the development of regionally distinctive hop products for the brewing industry.

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Popular science summary

Hops are one of the key ingredients used in beer brewing and are responsible for much of the bitterness and aroma found in modern beers. In recent years, there has been increasing interest in locally produced ingredients and unique hop varieties that can contribute distinctive flavors to beer. Sweden has several historical hop varieties preserved through the Swedish national gene bank program, but relatively little research has been conducted on their brewing potential.

The aim of this study was to investigate whether selected Swedish heritage hop varieties differ in chemical composition and how these differences influence beer. Four Swedish heritage hop varieties (Korsta, Hulla Norrgård, Spångby and Grimsarbo) were compared with the commercial variety East Kent Goldings. The hops were used for dry hopping, a brewing technique where hops are added during fermentation to increase aroma rather than bitterness.

Beer was brewed on a pilot scale and analyzed using several methods. The content of bitter compounds known as α- and β-acids was measured using high-performance liquid chromatography (HPLC), while hop aroma compounds called terpenes were analyzed using gas chromatography (GC-FID). In addition, a sensory evaluation was carried out using a semi-trained tasting panel.

The results showed clear chemical differences between the hop varieties. Korsta contained the highest concentration of β-acids and had the lowest α/β-acid ratio among the hops investigated. Differences were also observed in terpene composition. Several terpene compounds present in the raw hop material decreased during brewing and fermentation, while compounds such as linalool and geraniol remained detectable in the finished beer. These compounds are known to contribute floral and citrus-like aroma characteristics and are considered important contributors to hop aroma in beer.

Despite the observed chemical differences, the sensory evaluation did not reveal statistically significant differences between the beers. This suggests that differences in hop chemistry do not always translate into clearly detectable flavor differences under practical brewing conditions. Factors such as extraction efficiency, fermentation processes and interactions between aroma compounds may influence how hop characteristics are expressed in the final beer.

Overall, the study demonstrates that Swedish heritage hops possess unique chemical characteristics and show potential for brewing applications. The results

contribute to a growing understanding of Swedish hop resources and provide a foundation for future research into hop terroir, aroma development and the use of locally adapted hop varieties in brewing.

Acknowledgements

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I would like to thank Lennart Johansson and the Uppsala Homebrewing Community for their assistance during the brewing trials, and Lena Vinterbäck for her help with the planning and execution of the sensory evaluation.

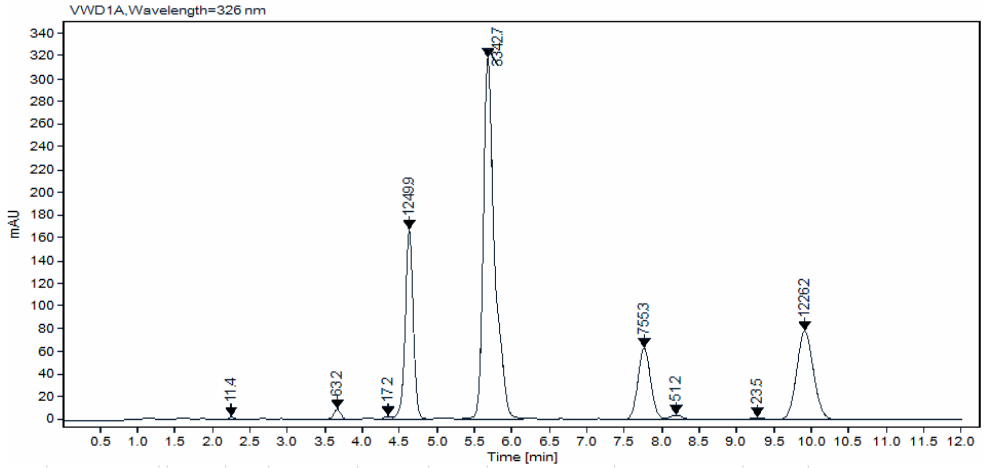
Furthermore, I would like to express my gratitude to Hans-Björn Eriksson and Örjan Berglund for generously providing Swedish heritage hop material from their private cultivations, making this study possible.

Finally, I would like to thank all sensory panel participants, as well as my family, friends and fellow students, for their encouragement and support throughout my studies.

Appendix

Appendix 1: HPLC Calibration Curves

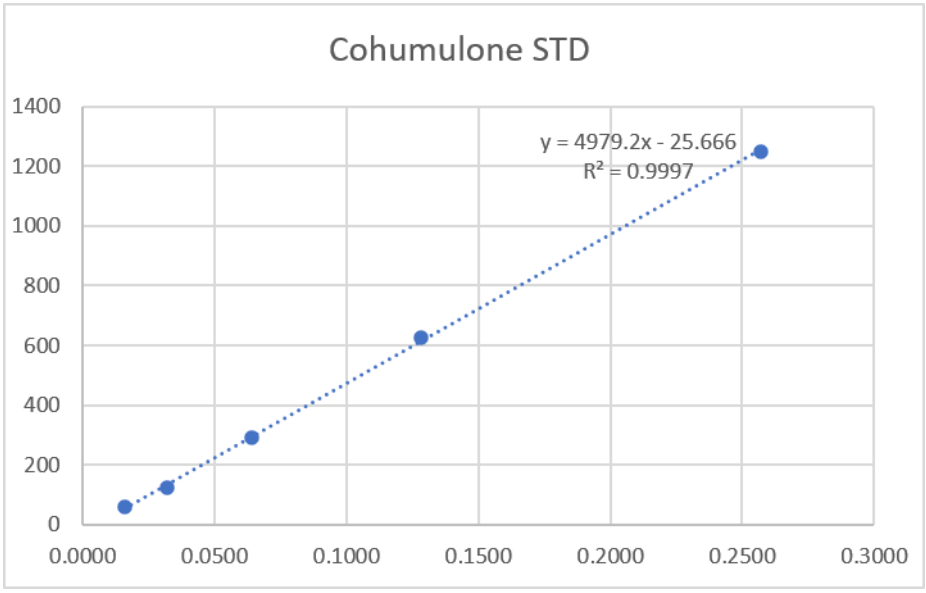
Figure 3. HPLC chromatogram of the ICE-4 reference standard.



Signal: VWD1A,Wavelength=326 nm

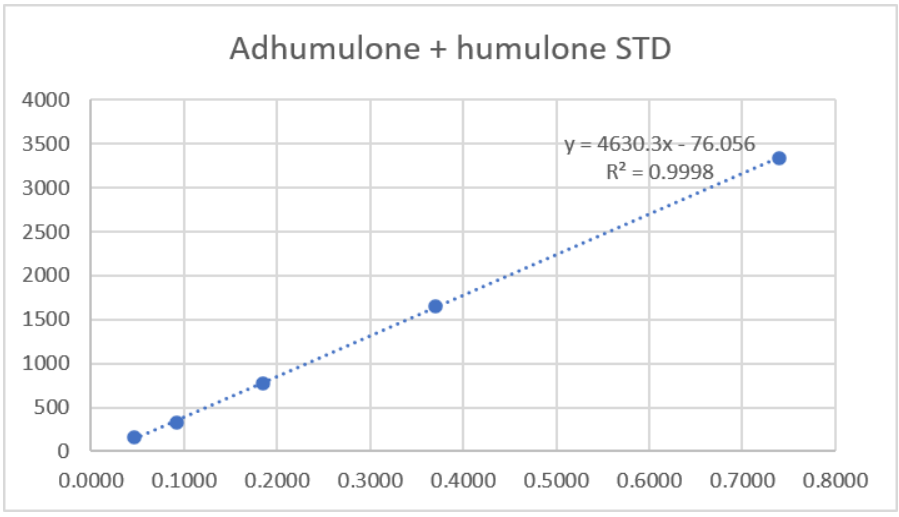
RT [min]	Type	Width [min]	Area	Height	Area%	Name
2.237	VV	0.2356	11.4461	2.2709	0.1698	
3.654	VB	0.4659	63.1885	8.9875	0.9374	
4.332	BV	0.1894	17.185	2.5388	0.2549	
4.616	VB	0.4921	1249.9018	166.6313	18.5429	**Cohumulone**
5.666	BV	0.8766	3342.6818	317.8685	49.5903	**Adhumulone+Humulone**
7.753	BV	0.5975	755.3211	63.0419	11.2055	**Colupulone**
8.179	VB	0.4443	51.1603	4.1556	0.759	
9.264	BV	0.5084	23.4791	1.7885	0.3483	
9.898	VB	1.0916	1226.2368	77.9696	18.1918	**Adlupulone+ Lupulone**
		Sum	**6740.6005**			

Figure 4. Calibration curve for cohumulone quantification using ICE-4 reference standard.



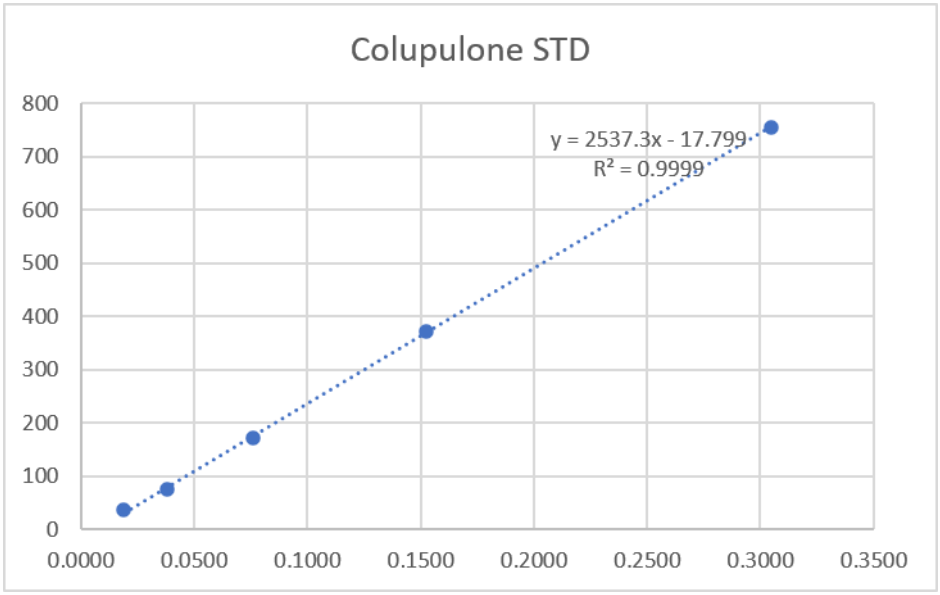
Note: The calibration curve was generated from five concentrations of the ICE-4 standard. Linear regression was used for quantification of hop bitter acid fractions.

Figure 5. Calibration curve for humulone + adhumulone quantification using ICE-4 reference standard.



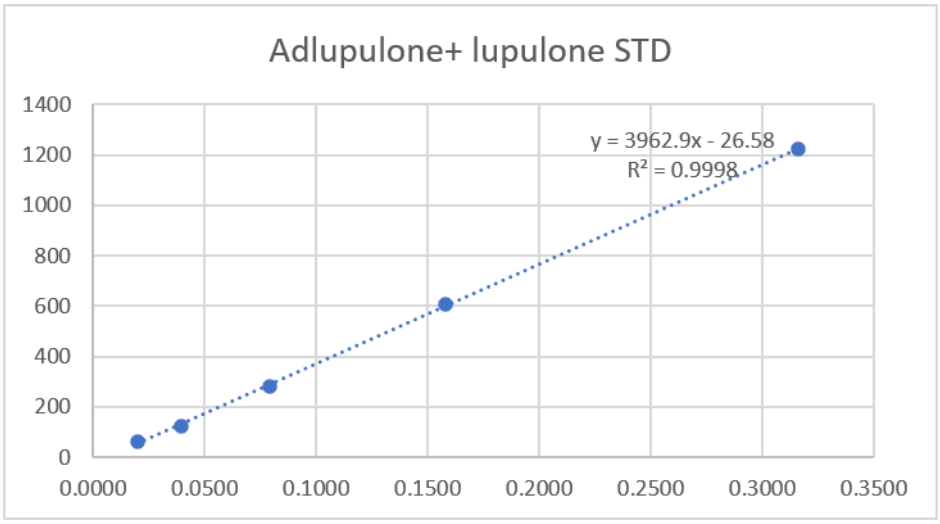
Note: The calibration curve was generated from five concentrations of the ICE-4 standard. Linear regression was used for quantification of hop bitter acid fractions.

Figure 6. Calibration curve for colupulone quantification using ICE-4 reference standard.



Note: The calibration curve was generated from five concentrations of the ICE-4 standard. Linear regression was used for quantification of hop bitter acid fractions.

Figure 7. Calibration curve for lupulone + adlupulone quantification using ICE-4 reference standard.



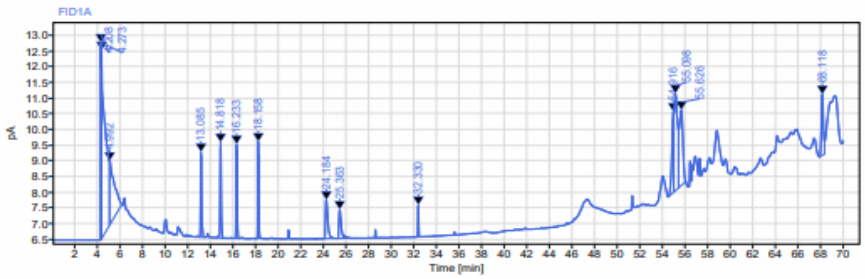
Note: The calibration curve was generated from five concentrations of the ICE-4 standard. Linear regression was used for quantification of hop bitter acid fractions.

Appendix 2

Figure 8. GC-FID chromatogram of terpene standard mixture.

Single Injection Report — Agilent

Data file:	2026-05-30 23-26-15+02-00-30.dx		
Sequence Name:	260529_C-R_Master	**Project Name:**	KE0076
Sample name:	8MIX	**Operator:**	admin (admin)
Instrument:	8890 GC	**Injection date:**	2026-05-30 23:29:36+02:00
Inj. volume:	5.000 µL	**Location:**	102
Acq. method:	Terpenes C-R 2.amx	**Type:**	Sample
Processing method:	*GC_LC Area Percent_C-R_terpeneMethod.pmx	**Sample amount:**	0.00
Manually modified:	Manual Integration		



Signal: FID1A

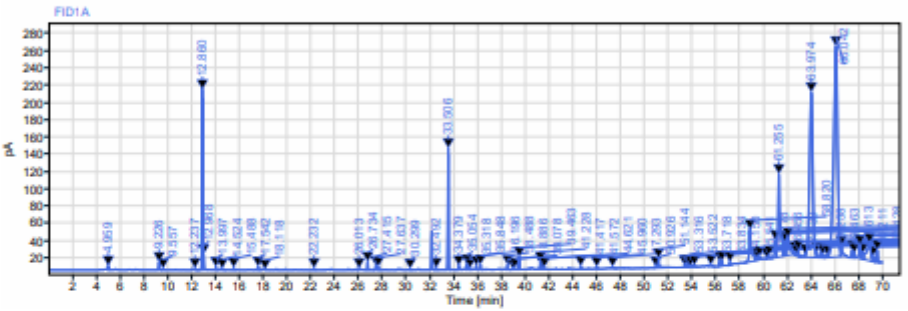
RT [min]	Type	Width [min]	Area	Height	Area%	Name
4.208	BV	0.05	16.40	6.30	3.30	
4.273	VV	0.74	149.70	6.01	30.12	
4.992	VB	1.08	58.37	2.05	11.74	
13.085	BB	0.39	16.67	2.72	3.35	beta-myrcene
14.818	VB	0.33	18.49	3.06	3.72	limonene
16.233	BB	0.35	16.79	3.01	3.38	gamma-terpinene
18.158	BB	0.32	15.28	3.09	3.07	linalool
24.184	MM m	1.83	17.00	1.24	3.42	nerol
25.363	MM m	1.65	13.09	0.92	2.63	geraniol
32.330	MM m	1.52	4.42	1.02	0.89	trans-caryophyllene
54.916	BV	0.47	28.19	2.58	5.67	
55.098	VV	0.43	64.95	3.10	13.07	
55.626	VB	0.62	54.61	2.41	10.99	
68.118	BV	0.44	23.05	1.95	4.64	
		Sum	**496.99**			

Figure 9. Representative GC-FID chromatogram of hop extract (Korsta).

Single Injection Report

Agilent

Data file:	2026-05-30 18-32-44+02-00-26.dx		
Sequence Name:	260529_C-R_Master	**Project Name:**	KE0076
Sample name:	KOR	**Operator:**	admin (admin)
Instrument:	8890 GC	**Injection date:**	2026-05-30 18:36:02+02:00
Inj. volume:	2.000 µL	**Location:**	132
Acq. method:	Terpenes C-R hops w. split 2.amx	**Type:**	Sample
Processing method:	*GC_LC Area Percent_C-R_terpeneMethod.pmx	**Sample amount:**	0.00
Manually modified:	Manual Integration		



Signal: FID1A

RT [min]	Type	Width [min]	Area	Height	Area%	Name
4.959	VB	0.28	18.61	5.20	0.12	
9.226	VB	0.38	45.73	9.69	0.29	
9.557	BV	0.28	9.40	1.99	0.06	
12.237	VB	0.36	11.94	2.62	0.08	
12.860	BV	0.33	836.69	209.32	5.34	
12.968	VV	0.38	78.74	18.51	0.50	beta-myrcene
13.997	BB	0.28	15.01	3.86	0.10	
14.524	VB	0.40	13.37	2.01	0.09	
15.488	BB	0.27	13.09	3.15	0.08	
17.542	VB	0.29	19.98	4.21	0.13	
18.118	MM m	0.16	2.95	0.86	0.02	linalool
22.232	VB	0.24	11.54	2.58	0.07	
26.013	BV	0.22	9.80	2.43	0.06	
26.734	BB	0.23	39.81	10.00	0.25	
27.415	BV	0.36	25.44	5.50	0.16	
27.637	VB	0.21	17.50	4.36	0.11	
30.299	BB	0.22	7.57	1.77	0.05	
32.492	BB	0.26	12.11	2.74	0.08	trans-caryophyllene
33.506	VB	0.40	633.10	141.19	4.04	
34.379	BV	0.29	24.79	5.39	0.16	

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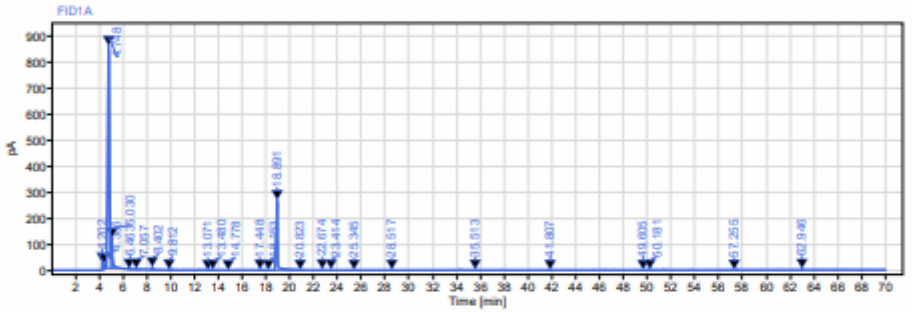
Figure 10. Representative GC-FID chromatogram of a post dry-hopped beer sample.

Single Injection Report

Agilent

Data file:	2026-05-30 09-58-56+02-00-19.dx		
Sequence Name:	260529_C-R_Master	**Project Name:**	KE0076
Sample name:	D1 DH	**Operator:**	admin (admin)
Instrument:	8890 GC	**Injection date:**	2026-05-30 10:02:31+02:00
Inj. volume:	5.000 µL	**Location:**	119
Acq. method:	Terpenes C-R 2.amx	**Type:**	Sample
Processing method:	*GC_LC Area Percent_C-R_terpeneMethod.pmx	**Sample amount:**	0.00
Manually modified:	Manual Integration		



Signal: FID1A

RT [min]	Type	Width [min]	Area	Height	Area%	Name
4.202	BV	0.14	124.33	30.23	1.02	
4.386	VB	0.21	127.06	22.89	1.04	
4.748	BV	0.43	8728.64	852.87	71.27	
5.030	VV	0.47	596.47	117.53	4.87	
6.463	BV	0.27	13.30	2.58	0.11	
7.057	BV	0.23	20.40	4.22	0.17	
8.402	BV	0.25	64.04	11.33	0.52	
9.812	BV	0.49	30.26	3.72	0.25	
13.071	VV	0.54	21.56	1.74	0.18	beta-myrcene
13.480	VB	0.36	16.48	2.85	0.13	
14.778	MM m	0.23	4.76	1.00	0.04	limonene
17.448	BV	0.37	22.74	3.96	0.19	
18.153	BV	0.33	15.85	2.02	0.13	linalool
18.891	BV	2.08	2301.35	269.61	18.79	
20.823	VV	0.46	15.40	2.29	0.13	
22.674	VB	0.27	19.03	4.40	0.16	
23.414	BV	0.22	18.40	3.93	0.15	
25.345	VB	0.42	10.93	2.14	0.09	geraniol
28.517	BB	0.21	10.94	2.86	0.09	
35.513	VB	0.32	12.70	3.28	0.10	

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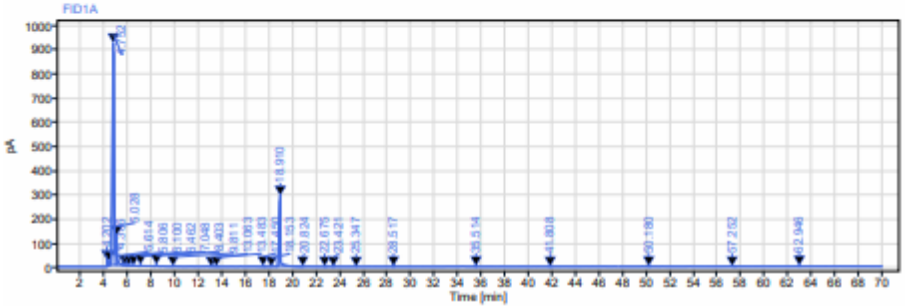
Figure 11. Representative GC-FID chromatogram of a finished beer sample.

Single Injection Report — Agilent

Data file:	2026-05-30 08-45-33+02-00-18.dx		
Sequence Name:	260529_C-R_Master	**Project Name:**	KE0076
Sample name:	D1	**Operator:**	admin (admin)
Instrument:	8890 GC	**Injection date:**	2026-05-30 08:48:53+02:00
Inj. volume:	5.000 µL	**Location:**	104
Acq. method:	Terpenes C-R 2.amx	**Type:**	Sample
Processing method:	*GC_LC Area Percent_C-R_terpeneMethod.pmx	**Sample amount:**	0.00
Manually modified:	Manual Integration		



Signal: FID1A

RT [min]	Type	Width [min]	Area	Height	Area%	Name
4.202	BV	0.14	121.13	28.97	0.93	
4.386	VB	0.21	106.88	20.61	0.82	
4.752	BV	0.43	9083.61	915.86	69.94	
5.028	VV	0.57	714.32	122.89	5.50	
5.614	VV	0.23	48.76	5.16	0.38	
5.806	VB	0.24	16.34	2.23	0.13	
6.100	BV	0.21	9.93	1.82	0.08	
6.462	BV	0.26	16.32	3.15	0.13	
7.048	BV	0.28	35.94	7.33	0.28	
8.403	BV	0.25	47.55	8.75	0.37	
9.811	VV	0.18	20.32	3.53	0.16	
13.063	MM m	0.20	1.60	0.36	0.01	beta-myrcene
13.483	VB	0.34	12.52	2.07	0.10	
17.450	BB	0.42	23.61	4.04	0.18	
18.153	MM m	0.24	6.45	1.11	0.05	linalool
18.910	BV	2.09	2600.03	293.70	20.02	
20.824	VB	0.20	10.48	2.51	0.08	
22.675	VB	0.27	10.62	2.35	0.08	
23.421	BV	0.21	10.41	2.17	0.08	
25.347	MM m	0.17	5.89	1.38	0.05	geraniol

C:\CDSProjects\KE0076\Report Templates\Short_Area_mod.rdl [Rev. 1.0] Printed: 2026-06-01 15:35:50+02:00 Page 25 of 60

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