Dynamics of Isomerization of Hop Alpha-Acids and Transition of Hop Essential Oil Components in Beer

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Abstract

Hops’ unique composition of essential oil components and bitter resins are crucial for beer aroma, which is important to consumers’ acceptance of beer. In this experiment the same wort was divided into four portions and each was hopped differently. To determine the dynamics of isomerization rates the concentrations of alpha- and iso-alpha-acids were continuously measured. Measurements of hop essential oil components were performed during each process to understand the dynamics of the transition into beer. The maximum isomerization yield of alpha-acids (18.1%) was achieved after 100 min. Longer boiling increased the reduction of iso-alpha-acids, as well as essential oil components. Dry hopping also affected not only on beer aroma but also on beer bitterness.

Keywords: Beer; hop; aroma; isomerization; hop essential oil

1. Introduction

In the brewing process hop is a quantitatively minor ingredient, but of paramount importance to the brewing industry. With their complex chemistry, hops have been the subject of investigation for decades.¹ During the brewing process hops are added into the boiling wort to provide a bitter taste and aroma to the final product.² One of the main reasons for boiling the wort is the isomerization reaction of the hop alpha-acids into their isomerized forms. The duration of kettle hopping depends on the time required for the isomerization to take place.³ Adding hops into the boiling wort is desired with intent to achieve the desired bitterness, however it causes a reduction in the yield of essential oils in the beer due to evaporation. Consequently, these components are not present in beer in the same ratios as they are in hops. Some components are very volatile and some have low solubility in water.⁴

During other stages of the brewing process, essential oil components decrease because of adsorption of yeast cells on the trub in addition to evaporation. The CO₂ produced during alcoholic fermentation also affects the decrease of aroma compound yields in beer.⁵ ⁶ To avoid the loss of aroma constituents and to achieve better yields dry hopping technique is in use, where hops are added at a later stages of brewing process, during fermentation or maturation.

The volatile aroma compounds of the hops are crucial for brewers, since they give beer its unique aroma. The identification of hop essential oil composition lasts for decades and the study of Roberts et.al concluded on the presence of over 1000 compounds in an oil fraction.⁹ Dried hops contain approximately 0.5–4.0% of essential oils. According to the chemical composition, compounds can be divided into three groups depending on their chemical structure: the hydrocarbon fraction which form approximately 75% of the total oil, the oxygen containing compounds forming approximately 25% of the total oil and the sulphur-containing compounds present in much lower quantities.²,¹⁰ Hydrocarbon fraction, consists of monoterpenes, sesquiterpenes and aliphatic hydrocarbons. Due to evaporation and low solubility in water, concentrations of these group of aroma compounds are very low in finished beer. Presence of alcohol in beer can increase solubility of monoterpenes. Oxygen containing compounds consist mostly of monoterpenic, sesquiterpenic and aliphatic alcohols and these components can be formed by the oxidation or reduction during beer production. In general, these compounds are more soluble in water compared to the hydrocarbons because of the higher polarity of the molecule.⁶,⁷ Generally, myrcene, which is the most abundant component in hop essential oil, is undesirable in beer, while linalool and geraniol – both from an oxygen fraction
present in lower concentrations than myrcene, present a pleasant aroma. The odour threshold is more important than the concentration level of a component in beer.\textsuperscript{11}

The analytical approach to determine the volatile flavour-active compounds in beer is based on different solvent extraction techniques and separation by gas chromatography with flame ionization detection (GC-FID) or gas chromatography-mass spectrometry (GC-MS).\textsuperscript{12} A newer approach to determine the volatile aroma compound is based on a solvent-free extraction technique, head-space solid phase micro extraction (HS-SPME),\textsuperscript{13,8} head-space approach to determine the volatile aroma compound is chromatography-mass spectrometry (GC-MS).\textsuperscript{12} A newer vent extraction techniques and separation by gas chroma-

Much research has been done to investigate the complexity of the final flavour compounds in beer aroma, but in our study the dynamics of the transfer of hop essential oil components as well as the dynamics of the isomerization of alpha-acids were studied directly during process. The aim of this study was to explore the levels of hop essential oils and alpha-acids during short- and long-time kettle hopping. By preparing the first two beer types (A–B), we investigated the behaviour of hop essential oil components (HEOC) during short-time (A) and long-time (B) wort boiling. In preparing the second two types of beer, we investigated dry hopping techniques of beer aroma compounds originated from hops. We tried to determine the difference if hop is added at the beginning of fermentation (C), or at the beginning of maturation (D).

2. Experimental
2.1. Experimental Beer Brewing
For 90 L of wort 100 L of water and 20 kg of Pilsner malt (Castle Malt, Belgium) were used. The mashing process started at 52 °C for 30 min, then the temperature was increased to 63 °C for 30 min, the temperature was increased once more to 72 °C for 30 min and finally the temperature was increased to 76 °C and held for 10 min. After mashing and lautering the wort was divided into four parts, 1 × 60 L and 2 × 15 L. For beers A (short time boiling) and B (long time boiling) the whole amount of hop was added at the beginning of the wort boiling and left it in for 60 min. After that, the wort was divided in 2 equal parts (30 L). The second part of the wort continued with boiling for another 60 min (120 min in total). In each 30 L 250 g of hop cones were added. For beers C and D wort was boiling without hop in the same way as in the first two trials. In one 15 L fermentation tank 125 g of hop was added and left it until the end of six days of fermentation (beer C). In the second 15 L, 125 g of hop was added after the fermentation process, at the beginning of a two week maturation (beer D). Yeast strain used in fermentation process was Saflager W34/70 (Fermentis) 1 g per L. The duration of the fermentation was 5 days at 12 °C, while maturation take place at 2 °C for 2 weeks. After adding hops into boiling wort, samples were collected every 10 min. During fermentation, samples were collected on each day of fermentation and during maturation, samples were collected every week of maturation.

2.2. Beer Analyses
Basic beer characteristics were measured. Determination of extract, alcohol and degree of fermentation were carried out according to the MEBAK II, method 2.10.4.17 Determination of pH value was carried out according to the Analytica EBC, method 9.35.\textsuperscript{18} Bitterness of beer was measured according to the Analytica EBC, method 9.8.\textsuperscript{197} Measurement of CO$_2$ was carried out according to the Analytica EBC, method 9.28.\textsuperscript{30} and the determination of beer colour was carried out according to the Analytica EBC, method 9.6.\textsuperscript{21}

2.3. Analytical Determination of Hop Metabolites
Determination of the content of hop essential oil was carried out according to Analytica EBC, method 7.10,\textsuperscript{22} using standard steam distillation. The determination of hop essential oil components was carried out according to the Analytica EBC, method 7.12\textsuperscript{23} using GC-FID. The determination of alpha-acids was carried out according to the Analytica EBC, method 7.4.\textsuperscript{24}

2.4. Determination of Hop Essential Oil Components (HEOC) in Beer
For the hop essential oil component analysis in beer, an Agilent 6890 GC, equipped with FID detector (Agilent Technologies, USA) was used. The determination of essential oils was performed on a 60 m × 0.32 mm × 0.25 μm DB-Wax capillary column (Agilent Technologies, USA) according to the MEBAK, method 2.23.6\textsuperscript{25} with some modifications, where the aroma compounds are driven out of the sample by steam distillation. The extraction of aroma compounds was performed by shaking 80 mL of distillate in 100 mL glass centrifuge tubes with 1 mL dichloromethane (Sigma-Aldrich, USA). Helium 5.0 was used as a carrier gas with a constant flow of 2.8 mL min$^{-1}$. The temperatures of the injector and detector were set to 250 °C. The temperature program started at 60 °C for 4 min, then increased by 5 °C min$^{-1}$ to 220 °C and was held at 220 °C for 30 min, then increased by 20 °C min$^{-1}$ to 240 °C and held for 5 min at 240 °C. The sample injection volume was 4 μL. Methyl heptanolate (Sigma-Aldrich, USA) was used as an internal standard. Identifications and quantifications of all investigated compounds presented in Table 1 were performed using standards purchased through Sigma-Aldrich, USA. For all measured parameters, the repeatability, accuracy and linearity for analytes were determined. RSDs for repeatability were from 1.3 to 1.9%. All measurements were done in duplicate.
2. 5. Alpha-Acids and Iso-Alpha-Acids Determination

To determine alpha-acids and iso-alpha-acids in wort and beer, samples were taken and frozen at –20 °C until analysis. Prior to analysis, samples were warmed to room temperature and centrifuged at 3000 rpm for 15 min in Heraeus Biofuge Primo (Switzerland) centrifuge. The clear upper phase was filtered through 0.45 µm PET filters into 2 mL glass vials. Injection of 2 µL was made with an auto sampler of an Agilent 1200 HPLC chromatograph, using HPLC grade methanol (Merck, Germany), distilled water and 85% ortho-phosphoric acid (Sigma Aldrich, USA) in a ratio of 77.5: 21: 0.9 as a mobile phase. Separation was performed on a 150 mm long Nucleodur C18 Column (Macherey Nagel, Germany). Iso-alpha-acids were recorded at wavelength 270 nm, while alpha-acids were recorded at 314 nm.

3. Results and Discussion

In each type of beer brewed, the same amount (g L⁻¹) of the traditional Slovenian variety, Dana, with the alpha-acids content 9.0% and 1.9 mL of hop essential oil on 100 g of dried hop was used. Basic beer specifications of all investigated beer types are presented in Table 2. Data consists of extract, alcohol, degree of fermentation, pH, bitterness, content of CO₂ and content of iso-alpha-acids.

3. 1. Isomerization of Alpha-Acids

The content of iso-alpha-acids is the sum of all three peaks of isomerized analogues of alpha-acids: iso-cohumulone (t<sub>R</sub> = 4.19 min), iso-adhumulone (t<sub>R</sub> = 5.42 min) and iso-humulone (t<sub>R</sub> = 4.97 min). In brewing science, the iso-alpha-acids are expressed as the sum of all three components, because for brewers, the sum value is relevant. The content of alpha-acids is the sum of cohumulone (t<sub>R</sub> = 8.39 min) and n-adhumulone (t<sub>R</sub> = 10.70 min). In Figure 1 where the dynamics of isomerization of alpha-acids for beer type B are presented, one can see a high isomerization rate in the first 30 min.

The highest utilization of 18.1% was achieved after 100 min of boiling. Isomerization yield was calculated as the ratio of actual mass concentration of iso-alpha-acids in beer, divided by the known content of alpha-acids in hop that was added into wort. Each sample taken later had smaller concentrations of iso-alpha-acids and smaller concentrations of alpha-acids. There was also a small part (1.6% of total alpha-acids added in wort) of alpha-acids that remained un-isomerized. This is a sign that longer boiling causes further degradation of iso-alpha acids. During fermentation we can see the concentration of iso-alpha-acids decreasing but a small increase in concentration was noticed on the last, fifth day of fermentation. Concentration during the maturation process increased to the final isomerization yield of 14.6%. The rise of IAA is occurred during maturation, where all bioprocess are finished, all the components reacts between each other's and beer gets its flavour and aroma. The pattern was the same (not shown) in beer type A, with one difference. The maximum isomerization yield was achieved at the end of boiling for 60 min, but after this time there is a part (4.6% of total alpha-acids added in wort) of alpha-acids that remain un-isomerized. Again, the concentrations decreased during fermentation and slightly increased during the maturation process.

In beer C, the concentration of iso-alpha-acids decreased every day until the final isomerization yield was below 1% (Figure 2).

Table 1: Composition of Dana essential oil

| Essential oil component | % in hop oil |
|-------------------------|-------------|
| Methyl hexanoate        | 0.4         |
| α-Pinene                | 0.2         |
| Myrcene                 | 37.5        |
| Linalool                | 1.1         |
| Bornol                  | 0.1         |
| α-Terpineole            | 0.4         |
| Methyl nonanoate        | 0.2         |
| Nerol                   | 0.3         |
| β-Citronellol           | 0.3         |
| Geraniol                | 0.5         |
| Methyl caprate          | 1.0         |
| Neryl acetate           | 0.2         |
| Geranyl acetate         | 0.4         |
| β-Cariophyllene         | 9.4         |
| α-Humulene              | 21.1        |
| β-Farnesene             | 5.1         |
| Tridecanone             | 0.8         |
| Geranyl iso-butyrante    | 1.0         |
| Cariophyllene oxide     | 0.9         |
| Farnezol                | 0.2         |
| Limonene                | 0.3         |

Table 2. Basic beer characteristics for each beer type.

| Beer type | Extract (%) | Alcohol (vol. %) | Degree of fermentation (%) | pH  | Bitterness (IBU) | CO₂ (g L⁻¹) | Iso-alpha-acids (mg L⁻¹) |
|-----------|-------------|------------------|----------------------------|-----|------------------|-------------|--------------------------|
| A         | 11.8        | 5.1              | 66.2                       | 4.7 | 112              | 3.0         | 98                       |
| B         | 12.6        | 6.1              | 67.1                       | 4.8 | 120              | 3.1         | 110                      |
| C         | 11.3        | 5.2              | 68.8                       | 4.7 | 14               | 4.7         | 6                        |
| D         | 11.4        | 5.2              | 69.1                       | 4.7 | 8                | 4.8         | 6                        |
Figure 1: Dynamic of isomerization of alpha-acids in beer B. Sampling was made every 10 min during boiling, every day of fermentation and every week of maturation.

Figure 2: Dynamic of isomerization of alpha-acids in beer C.
The alpha-acid content in the beer was approximately 2 mg L\(^{-1}\) from the beginning of the process to the end of the fifth day. The cause of low isomerization yield was the low temperature of the process. The cause is also limited solubility of alpha-acids in the wort and further losses of iso-alpha-acids post wort boiling; the absorption of trub and because of losses during clarification.\(^{26-28}\) Knowledge of the utilization value of iso-alpha-acids for each brewery is of crucial importance for brewers to achieve a desired level of bitterness in beer. In Table 3, where the dynamics of isomerization in beer D is presented, one can see that the concentrations of alpha-acids are slightly higher than

| Time of sampling | Alpha-acids (mg L\(^{-1}\)) | Iso-alpha-acids (mg L\(^{-1}\)) |
|------------------|-----------------------------|-----------------------------|
| After 1 week of maturation | 7.2 | 5.8 |
| After 2 weeks of maturation | 8.6 | 6.0 |

Table 3. Dynamics of isomerization in beer D

![Figure 3a and 3b. Transition of hop essential oil components during wort boiling.](image)
the concentrations of iso-alpha-acids. Both concentrations are very low, as the isomerization process depends on the temperature of the medium.

3. Determination of the Transition of HEOC in Beer

The most abundant components of Dana essential oils were focused on. These components with their relative abundance in hop are presented in Table 1. Concentrations of investigated HEOC at the beginning of the hopping process and at the end of the experiment are presented in Table 3. Concentrations of HEOC during wort boiling in beer types A and B (first 60 min for beers A + B and next 60 min for beer B) decreased at different rates (Figures 3a and 3b).

Some were decreased to the values < LOD. The exception is geraniol, where a small increase in concentration is noticed. It is important to know that these are not the final concentrations in beer. The literature states that later in the fermentation or maturation process concentrations of some HEOC can increase, because of thermal re-

![Figure 4a and 4b. Transition of hop essential oil components during fermentation.](image-url)
actions or by biotransformation processes such as oxidation, reductions, isomerization or hydrolysis of volatiles that occur in beer brewing.\textsuperscript{30–35} Myrcene and α-humulene, which belong to the terpene hydrocarbon fraction of essential oils, are hydrophobic and would not pass from the hops through to the finished beer during the brewing process. On the other hand, hydrophilic terpene alcohols, such as linalool, are easier to retain in the beer.\textsuperscript{30,33} Due to evaporation during wort boiling, myrcene, although it is the most common component in essential oils, does not contribute to the beer aroma because the concentrations are normally far below the odour threshold level.\textsuperscript{32} In beer C we observed the transition of HEOC during fermentation (Figure 4a and 4b).

In general, concentrations of HEOC begin high, and then some fluctuation in concentrations is observed, until the last day of fermentation when the HEOC are again in the same range as at the beginning. One of the main reasons for such fluctuations in concentrations during dry hopping, which seems to be simple, but from a physicochemical point of view dry hopping is very complex and complicated, is not just extraction time and amount of hops but also the presence of yeast cells and carbon dioxide which can wash out the essential components from beer.\textsuperscript{6,7}

It is interesting, that concentrations of limonene, α-terpineole, neryl acetate and geranyl-iso-butyrate remain almost the same as they were after the first day of dry hopping. Concentration of linalool increases around 50 times. The concentrations of HEOC in beer C are generally higher than in both “kettle hopped” beers A and B. In beer D, where hop was added only at the beginning of the maturation process, two types of HEOC dynamics are observed. In Table 4, some concentrations decrease with time while some of the concentrations increase.

Some concentrations are higher than in beer C. One of the reasons is a low temperature during the maturation process and live environment in the fermentation process. Beside extraction of HEOC in wort or beer, chemical reactions between some components can lead to an increase of concentrations. Linalool can come from nerol or geraniol, and β-citronellol can come from geraniol. Linalool could be also cyclized to α-terpineol.\textsuperscript{31,33} Although linalool is not abundant in hops, its contribution to beer aroma is high because of its very low odour threshold value of 2.2 µg L\textsuperscript{−1}.\textsuperscript{34} Henke et al. also reported that concentrations of geraniol increase during fermentation, which is in line with our results.\textsuperscript{35}

### 4. Conclusions

From this experiment, it can be concluded that the optimal time of hopping to achieve high bitterness is no longer, than 100 min. Dry hopping also affects beer bitterness, but the isomerization rates are very low, and consequently higher concentrations of un-isomerized alphas-acids were detected. During brewing, severe losses, of hop essential oils occur by evaporation in the hot part

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**Table 4.** Concentrations of hop essential oil components in beer types A-D (LOD = 0.1 µg L\textsuperscript{−1})

|       | Start [µg L\textsuperscript{−1}] | Final [µg L\textsuperscript{−1}] |       | Start [µg L\textsuperscript{−1}] | Final [µg L\textsuperscript{−1}] |       | Start [µg L\textsuperscript{−1}] | Final [µg L\textsuperscript{−1}] |       | Start [µg L\textsuperscript{−1}] | Final [µg L\textsuperscript{−1}] |
|-------|-------------------------------|-------------------------------|-------|-------------------------------|-------------------------------|-------|-------------------------------|-------------------------------|-------|-------------------------------|-------------------------------|
| Methyl hexanoate | 0.9 ± 0.1 | 0.4 ± 0.1 | α-Pinene | 0.2 ± 0.1 | 0.2 ± 0.1 | Myrcene | 4.8 ± 0.1 | 1.9 ± 0.1 | Linalool | 14.6 ± 0.2 | 0.9 ± 0.1 | Borneol | 1.0 ± 0.1 | 0.3 ± 0.1 | α-Terpineole | 2.5 ± 0.1 | 0.9 ± 0.1 | Methyl nonanoate | 1.5 ± 0.1 | 0.9 ± 0.2 | Neryl | 0.7 ± 0.1 | 0.4 ± 0.1 | β-Citronellol | 43.9 ± 0.7 | 17.3 ± 0.4 | Geraniol | 12.7 ± 0.2 | 24.3 ± 1.0 | Methyl Caprate | 1.8 ± 0.1 | 0.7 ± 0.1 | Neryl acetate | 0.2 ± 0.1 | 0.1 ± 0.1 | Geranyl acetate | 0.5 ± 0.1 | 0.2 ± 0.1 | β-Caridoiphyllene | 24.4 ± 0.3 | 9.8 ± 0.2 | α-Humulene | 3.1 ± 0.1 | 0.4 ± 0.1 | β-Farnesene | 11.6 ± 0.2 | 0.2 ± 0.01 | Tridecanone | 1.8 ± 0.1 | 0.4 ± 0.1 | Geranyl isobutyrate | 9.5 ± 0.2 | 7.7 ± 0.2 | Carioiphylene oxid | 3.2 ± 0.1 | 1.4 ± 0.1 | Farnezol | 0.5 ± 0.1 | 0.6 ± 0.1 | Limonene | 0.4 ± 0.1 | 0.3 ± 0.1 |
of the process. Losses in the cold part of beer production are mainly consequence of washing out aroma compounds by carbon dioxide and adsorption of the aroma compounds on the yeast cells. Nevertheless, oxidation and reaction between aroma compounds can lead to rise of concentration of these compounds. Dry hopping does not result in increased aroma intensity in all cases.

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**Abbreviations**

HEOC – hop essential oil components, $t_R$ – retention time

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**Povzetek**

Edinstvena sestava eteričnega olja hmelja in grenkih smol je ključnega pomena za aromo, kar je pomembno za sprejemanje piva s strani potrošnikov. V tem poskusu je bilo isto pivo razdeljeno na štiri dele, vsak je bil hmeljen drugače. Za določitev dinamike hitrosti izomerizacije so bile izvedene kontinuirane meritve koncentracij alfa-izooalfa-kislin. Prav tako so bile med postopkom merjene tudi koncentracije določenih komponent eteričnega olja, da bi razumeli dinamiko njihovega prehoda v pivo. Največji izkoristek izomerizacije alfa-kislin (18,1 %) je bil dosežen po 100 min varjenja pivine. Daljše vretje povečuje zmanjšanje koncentracij izo-alfa-kislin, kot tudi komponent eteričnega olja. Hladno hmeljenje vpliva ne le na aromo piva, ampak tudi na gremkobo.

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