Master's thesis

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Norwegian University of Science and Technology
Faculty of Natural Sciences
Department of Biotechnology and Food Science

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Comparison of volatile compounds in five hop (*Humulus lupulus*) varieties and the effect of solvent and temperature on retainment using Headspace Gas Chromatography-Mass Spectrometry

Master's thesis in Biotechnology (MBIOT5)
Supervisor: Eivind Almaas
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Abstract

Hops (*Humulus lupulus*) is a critical ingredient in beer. In order to add the typical bitter taste, as well as an attractive aroma, hops have been an essential ingredient in beer production for centuries. It is especially the hoppy aroma, which stems from the volatile compounds found in the hop essential oil, that has become of interest to researchers recently. Now, brewers often base their hop selection on past experience and trial and error. This is very subjective, and it is therefore of interest for researchers to develop an unambiguous and objective method for the identification of the many hop varieties out there and their contribution. In order to increase the understanding of the volatile compounds and their behavior in different solvent states and temperatures, this study was conducted.

By the use of headspace gas chromatography-mass spectrometry, the essential oils of five hop varieties, "Saaz", "Hallertau Mittelfrüh", "Simcoe", "Citra" and "Centennial", were measured and compared. The samples were analyzed in conditions that resembled the brewing process, with an aim to unravel and highlight differences in how the volatile compounds of the hop essential oil were retained in different solvent states, as well as at different temperatures. From these findings, the plan was to highlight the importance of hop addition timing in the brewing process with regards to the retainment of these compounds, as well as to reveal analytical differences linked to the hop varieties.

The results from this study indicated that for better retainment of volatile compounds, hop additions should be done in wort rather than in beer, and rather at higher temperatures than at lower temperatures. Variations between the hop varieties were shown to be larger at lower temperatures. The results also indicated that when using hop varieties with a higher total oil content, a larger percentage of volatile compounds would evaporate than from hop varieties with a lower total oil content. Hence, their flavor and aroma contribution may not necessarily be more significant. β-myrcene was found to be the major volatile compound.

Due to the complexity of hop volatile compounds and the simplified setup of this experiment, it should be noted that no general conclusions could be drawn from the results in this study. The study is therefore meant only to highlight this complexity of the hops with regards to differences in how their volatile compounds are retained in wort and beer, and also to show how temperature plays an important role in this complexity.

Sammendrag

Humle (*Humulus lupulus*) er en viktig ingrediens i øl. For å få den typiske bitre smaken, samt en attraktiv aroma, har humle vært en del av produksjonen av øl i århundrer. Det er spesielt humlearomaen, som stammer fra flyktige forbindelser i humlens essensielle oljer, som har vært interessant for forskere den siste tiden. Nå til dags baserer ofte bryggere humlevalget sitt på tidligere erfaringer, samt prøving og feiling. Dette er svært subjektivt, og det er derfor av interesse for forskere å utvikle entydige og objektive måter å identifisere de mange ulike humlevariantene og deres bidrag på.

Ved å bruke headspace gass kromatografi-massespektrometri ble de essensielle oljene til fem humlevarianter, "Saaz", "Hallertau Mittelfrüh", "Simcoe", "Citra" og "Centennial", målt og sammenlignet. Prøvene ble analysert under forhold som forestilte de som er i bryggeprosessen, med sikte på å oppklare og belyse forskjeller i hvordan de flyktige forbindelsene fra humlens essensielle oljer ble bevart i ulike løsemidler, samt ved ulike temperaturer. Fra disse funnene var planen å belyse viktigheten av timing av humletilsetting i bryggeprosessen med hensyn til hvor godt disse flyktige forbindelsene blir bevart, samt for å avsløre analytiske forskjeller forbundet med humlevariantene.

Resultatene fra studien indikerer at for å bevare de flyktige forbindelsene bedre, bør humletilsettingen gjøres i vørter i stedet for i øl, og heller ved høye temperaturer enn ved lave temperaturer. Variasjoner mellom humlevariantene ble vist å være større ved lavere temperaturer. Resultatene indikerte også at når man bruker humlevarianter med høyere innhold av essensielle oljer, vil en større mengde av disse fordampe for disse variantene enn de vil for varianter med lavere innhold. Det er derfor mulig at selv om de har høyere oljeinnhold, er ikke nødvendigvis deres bidrag til smak og aroma større. β-myrcene ble vist å være den viktigste flyktige forbindelsen.

Grunnet kompleksiteten til de flyktige komponentene i humle og det forenklede oppsettet av dette eksperimentet, bør det merkes at ingen generelle konklusjoner kunne trekkes av resultatene i denne studien. Studien er derfor kun ment for å belyse kompleksiteten av humle med hensyn til forskjeller i hvordan deres flyktige forbindelser er bevart i vørter og øl, samt å også vise hvordan temperatur spiller en viktig rolle i denne kompleksiteten.

Preface

This master's thesis was written at the Norwegian University of Science and Technology (NTNU) as a part of the beer metabolomics project, and it marks the end of a 5-year long journey I would not be without. Not only have I gotten the opportunity to acquire knowledge from great teachers and fellow students through my studies, but I have also met people that I will forever be grateful for, and some I will stay in touch with for the rest of my life. Studying for exams and working on group projects together may not be seen as big hardships, but it sure brings people together.

Something else that sure is good at bringing people together is beer. I am not sure if I would have been as close as I am to those friends as I am today if we didn't celebrate our finished exams and projects with beer. Actually, by working on this thesis, the way I view beer has completely changed - I now almost see beer as a work of art. Knowing more about the efforts that lie behind getting a beer to taste a certain way, has made drinking beer an experience I appreciate a lot more than before.

In addition to being grateful for the existence of beer, I would also like to extend my gratitude to my main supervisor, Eivind Almaas, for letting me join the Almaas Lab group and for his great guidance whenever it was needed, and most importantly, to my co-supervisor Christian Schulz. Not only has he been there to guide me through the work behind this thesis, but he has also been a great motivator through it all. I couldn't have asked for a more patient co-supervisor, and I am very appreciative of his ability to trust the process and for being like a friend that I could text any time I had a question. I am also very grateful for Kåre Andre Kristiansen and for his help with teaching me how to work with the GC-MS.

Lastly, I wish to thank my family and friends for their never-ending support and for always being proud of me. It has been a significant source of motivation through this last year.

Cheers!

Maren Sigbjørnsen Jondal
Trondheim, May 15th 2021

Table of contents

List of figures

List of tables

Abbreviations

ABV	Alcohol by volume
GC	Gas chromatography
GC-O	Gas chromatography-olfactometry
HS	Headspace
IBU	International bittering unit
IPA	India pale ale
MS	Mass spectrometry
NMR	Nuclear magnetic resonance
PCA	Principal component analysis
RT	Retention time
SIM	Selected ion monitoring

1 Introduction

Of all the drinks in the world, beer has one of the oldest recorded recipes. It is in fact believed by some anthropologists that man moved away from the hunter-gatherer culture to an agriculture-based existence mainly to be able to grow large amounts of grain to be used in brewing, indicating the importance of beer already back then. For centuries, the basic formula for beer has remained the same, and the first chemically confirmed barley beer, which is similar to the beer that we know today, has been confirmed to date back to as early as the 5th millennium BC in Iran (1).

Through the times, beer has played many different roles - from being an offering to the gods, to being prescribed to treat various illnesses (2). Even though it would be great if the latter was still common in the modern society, the way we drink beer is constantly evolving. Now, beer is to be enjoyed and discussed, and many of the beer lovers that would usually go to the bar at the corner to grab a commercial beer, will now take a trip to the local brewery instead to drink craft beer. Craft beer is beer that is brewed in a craft brewery, also known as a microbrewery, and a craft brewery is loosely defined as a brewery that produces small amounts of beer and that is independently owned. Craft breweries are generally marketed and perceived as having an emphasis on new flavors, enthusiasm and varied brewing techniques, and in 2019, the global craft beer market size stood at $89.25 billion. This may already seem like a lot, but it is forecasted to reach a market size of $190.66 billion by 2027 (3). The U.S. market accounts for a lot of this growth (Figure 1.1).

Figure 1.1: U.S. Craft beer market revenue by distribution (USD billion). "On-trade" refers to business with hotels, bars and restaurants, whereas "off-trade" means sales to food retailers like supermarkets. Figure adapted from (4).

One of the reasons for the rising popularity of craft beer is that brewers can experiment with a huge diversity of beer styles and flavor profiles, and that is exactly what the consumers want now. It should also be noted that it is not only the craft beer market that is seeing a growth – home brewing is also becoming increasingly more popular, and the home beer brewing machine market is also expected to keep witnessing massive growth in the upcoming years (5). As the interest in beer is gradually increasing, it is also increasingly

interesting for brewers to gain more objective insight into why the finished beer products turn out the way they do, and if there are any alterations that can be done to potentially save money and/or make the beer turn out even better.

Then, if the recipe for beer has pretty much stayed the same for centuries, how is there any variation? The four main ingredients in beer are water, malt, yeast and hops (see section 2.2 for more details). In short, malt is the backbone of the beer, and in addition to giving the beer its sweetness, it provides carbohydrates for the yeast to use in the fermentation process. Without the malt, there would simply be no alcohol and no CO_2. To counterbalance the sweetness of the malt, the beer needs to be bittered. For centuries, beer cultivation in Europe used a mixture of spices and herbs called "gruit" for this purpose, and it was not until the first millennium A.D. that hops were commonly found in beer brewing. Originally, hops were added to preserve the beer – it was not until later that they were added mostly due to their bittering effect (6).

What is gaining more and more attention these days, however, is the ability the hops have to add flavor and aroma to the beer. The key driving factor for the growth of the global beer market is the fact that among millennials, it has become a huge trend in beer that there is an expanding availability of a variety of flavors (5). It has been known for a long time that the addition of certain hop varieties will provide a more citrusy or fruity flavor and aroma to the end product, whereas the addition of certain other hop varieties can provide a more grassy or herbal flavor and aroma (6). Figure 1.2 illustrates some flavors and aromas hops can contribute to the beer. The traditional way to evaluate the quality of hops has been to use an experienced brewer to organoleptically assess the hops. This is done by them simply crushing a few hops between their fingers and then smelling the aroma that is released. This is effective per se, but it is certainly not objective (7). Even though it is possible to brew good beer just knowing this information about the hop aroma properties, the increasing demand for flavorful beer also leads to an increasing demand for quantitative information that can be used to make correct decisions on how to best utilize the hops.

It is the essential oil, biosynthesized in the lupulin glands of the hops, which is of prime interest when studying the aroma and flavor properties. The volatile components make up between 0.2 and 3% of the total mass of the hops, with more than 400 hop aroma components having currently been identified (8). Researchers have been trying for more than 50 years to identify all the volatile compounds in the hop essential oil, but there are still more to be identified. It is actually thought that the essential oil of hops could potentially contain up to as many as 1000 compounds (9, 10). However, it is not until recent years that researchers have really started to work more on the optimization of the identification methods. They have also started to look even closer at how the brewing process may influence the contribution of the volatile compounds (11).

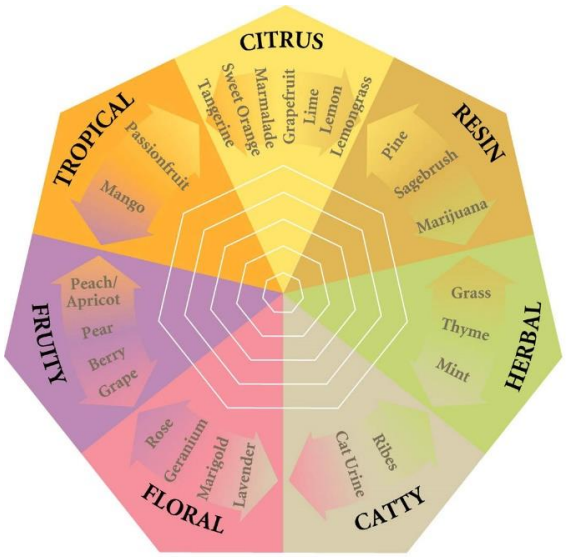


Figure 1.2: Hop flavor chart illustrating some flavors and aromas hops can contribute to beer. Figure from (12).

What is also important to consider is that aromas themselves are very complex. Subtle differences in the concentrations of components in complex mixtures can result in varying fragrance perceptions – and just a change in the ratio of two compounds can shift the aroma and have an impact on the overall character of the beer (13). In addition, not all of the compounds identified in hop oil are necessarily the same as those in hopped beer, as the boiling and fermentation processes affect the characteristics of the hops. This is also due to the fact that hop compound losses can occur by adsorption to the solid material or yeast cells, and also by oxidative transformations (14).

Another important aspect that needs to be taken into consideration is sensory threshold. Even though there are several hundred compounds present in the hop essential oil, only a certain number of these will be present at a concentration high enough to be above their human detection threshold and to actually contribute to the odor of the oil. Some compounds, on the other hand, have very low odor thresholds, such as sulfur compounds, and they can cause an off-flavor even when present at trace levels (9).

It must also be taken into account that the volatile compounds are exactly that – volatile. Hop additions can be done at many different time points throughout the brewing process; hence, the hops will be exposed to different temperatures for different lengths of time. Furthermore, different compounds will evaporate at different temperatures, and it is therefore interesting to further investigate which compounds are retained, and which evaporate. Some hop additions are done in wort, while some are done in the beer during fermentation or post-fermentation (15). Consequently, it is of interest to see if there is any variation when hops are placed in different solvent states, and how different temperatures affect the amount of evaporated volatiles. To analyze this, the analytical method gas chromatography-mass spectrometry can be used (8) .

By now, it should be apparent that even though beer brewing is full of traditions and has been done for centuries, there is a lot more to the beer brewing process than most people acknowledge. To say that beer is a complex topic is actually under-selling things by a large margin. Even just the hops themselves are vastly complex, as has been attempted shown in this introduction, and one can only think of all the possible different topics just within the subject of hops that can be studied. Due to the complexity of the hop volatile compounds, it should be noted that no definite conclusions could be drawn from the results in this study. This study is only one of many along the road to a better understanding of the volatile compounds of hops, and it is meant to give a good initial impression of possibilities for further studies, as well as function as a springboard for similar studies. Eventually, these studies can help brewers brew even better beer, and even save time and money.

From the results obtained from the headspace gas chromatography-mass spectrometry analyses in this study, there was a range of possibilities in how to interpret and analyze the data. To narrow it down so it was kept within the scope of this thesis, the following aims and sub-aims were decided on:

The aim of this thesis is to gain further insight into how different temperatures affect how well hop volatile compounds are retained in wort, as well as to explore differences in retainment of compounds between hop samples in wort and beer. This thesis also aims to highlight the importance of hop addition timing in the brewing process with regards to retainment of volatile compounds. Comparing 5 different hop varieties at 3 different temperatures in 4 different solvent states will also help to reveal analytical differences that are undoubtedly linked to the hop varieties.

2 Theory

In this chapter, research and theory relevant for this thesis will be presented. First, a general introduction on hops will be given, followed by a more in-depth look at the essential oil of hops and its composition. The next section covers the brewing process, where each step of the process is covered and emphasis is put on when hops can be added and how the hops play a role. Then, an introduction to the method used in this study, headspace gas chromatography-mass spectrometry is given. Finally, principal component analysis, which is used for most of the data analysis in this thesis, is introduced.

2.1 Hops (*Humulus lupulus*)

Hops are the cone-shaped flower of the female hop plant, *Humulus lupulus,* illustrated to the left in Figure 2.1. They have been used as an essential ingredient in beer production for centuries, and they contribute to the taste, aroma and foam stability of beer in a unique way, thus making the influences of hop-derived compounds interesting to research (16).

Hops are available to brewers in a couple of different forms, each with their advantages and disadvantages. One of these forms is pellets, illustrated to the right in Figure 2.1. Hop pellets account for the majority of hops used in the craft brewing industry, and in order to make them, the hop cones are dried, shredded, compressed and extruded into pellets (6). Their compressed state and lighter weight makes them easy to store and less susceptible to spoilage, which is important as the flavor will degrade as they age (7).

Figure 2.1: Hop cones (left) and hop pellets (right). Figure adapted from (17, 18).

There are over 100 hop varieties around the world, and the hop varieties can be divided into two broad categories: bittering hops and aroma hops. The bittering hops contain higher levels of alpha acids, making them more economical for bittering beer, as a small amount goes a long way. Aroma hops, on the other hand, tend to have more essential oils. It is these essential oils that contain the highly volatile compounds that contribute most of what people perceive as “hoppiness” to the beer (6). Section 2.1.1 covers more details on how the alpha acids and essential oils actually contribute to the bittering and aroma.

2.1.1 Alpha acids, beta acids and essential oils

Alpha acids, beta acids and essential oils are all found in the pinhead-sized lupulin glands of the hop cone. Lupulin is a sticky substance that is secreted when boiled, and it is found surrounding the center of the cone underneath leaf-like structures, as seen in the cross-section of a hop cone in Figure 2.2. Each kind of hop is distinctive in its bittering, aroma and flavor profile, and these differences are due to variations in the alpha acid and beta acid content, as well as differences in the essential oil content and composition. The alpha acids account for the largest percentage share of dry weight of the three, followed by the beta acids and then the essential oils. They will be discussed in this order.

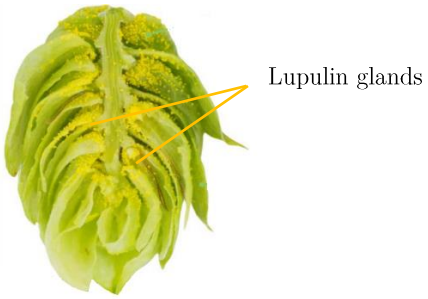


Figure 2.2: Cross-section of a hop cone. The lupulin glands, which contain the alpha acids, beta acids and essential oils, are found surrounding the center of the cone, underneath leaf like structures. Figure adapted from (19).

The alpha acids are commonly in a range of 2-18% of the dry weight of the hop, and they are the main bittering agent. The alpha acid level of hops is measured in a laboratory, and brewers can use different equations to figure out the amount of hops needed to get a certain amount of bitterness in the beer, commonly quantified as international bittering units (IBU) (20).

What we generally call “alpha acids” is actually a bunch of different alpha acids that are similar in structure, and they do not bring any bitterness to the beer as they are. Some of these analogues include humulone, cohumulone and adhumulone, and in order for them to bring bitterness to the beer, they have to be boiled and isomerized into iso-alpha acids, which are more soluble. Alpha acids are non-volatile, and in their non-isomerized form, they are stubbornly insoluble in aqueous solutions such as beer. This is why they have to be boiled, and the longer the hops are boiled while brewing, the more alpha acids are transformed into iso-alpha acids. The composition of the alpha acids also plays a role, and there is some debate among experts as to which of the humulones gives the cleanest bitterness. Still, there seems to be agreement that high levels of cohumulone are an indicator for a potentially harsh bitterness, which is why cohumulone levels often are listed next to the alpha acid percentage (20).

The second type of acid that is found in the lupulin glands are beta acids, and they commonly account for 3-10% of the hop's weight. You could say that if hops were a band, the alpha acids would be the lead singer, whereas the beta acids would be the fourth guitarist - Most people are not really sure of what he does, but his contribution is still important to the song. The beta acids do not really contribute much to the beer as a whole, but they do help in two areas: First, some of the bittering of the beer stems from the beta acids. Although the bitterness is a lot harsher than it is from the alpha acids, not much comes off in the beer due to the insolubility of beta acids. In contrast to the alpha acids, the beta acids do not isomerize, but rather oxidize in the beer. So, while alpha acids dissolve into solution almost immediately after being added to the boil, beta acids break down over time. Therefore, their effect is best seen over time in beer storage and lagering. Beta acids have also been found to have great antiseptic qualities and to counteract and delay the inevitable effects of bacterial spoilage, thereby giving beer a longer shelf life (21).

Essential oils, on the other hand, do not contribute to bitterness, but instead to flavor and aroma. Hops can influence beer aroma in terms of mainly floral, herbal, woody, spicy and fruity characters, which can be attributed to the different compositions of the volatile compounds in the essential oils. The total oil content amounts to about 0.1-2.0% of the dry weight, and the amount of and composition of the oil is dependent on the hop variety (8). The composition of the oil is very complex, and it potentially contains up to 1000 compounds. In addition to there being differences in the composition due to hop variety, other factors also play a role in further increasing the chemical complexity of the composition. These factors include processing conditions, intrinsic and extrinsic factors during growth, and oxidation and hydrolysis reactions during storage (22).

One might then think that the higher the total oil content, the more the hops will contribute to a "hoppy" flavor and aroma in the end product. Research has shown that this is not true at all, and that some of the hop varieties with the highest total oil contents do in fact contribute very little to overall flavor and aroma (23). This is due to the composition of the oil, which is thoroughly discussed in the next section, 2.1.2: Hop essential oil composition and volatile compounds.

Brewers often use several different hop varieties in a single beer to achieve the desired balance of bitterness and aroma, and this is usually just based on alpha acid percentage, past experience, and trial and error (9). Trying to balance alpha acids, beta acids, and oils all along with aroma, taste, and overall impression of a beer is not easy, and it makes choosing the right hop for your beer very tricky. This is exactly why it is of interest for researchers to develop an unambiguous method for the identification of hop varieties. An important part of this research is obtaining a better understanding of the hop essential oil composition and the volatile compounds (10).

2.1.2 Hop essential oil composition and volatile compounds

The essential oil fraction of hops has been attributed as the primary source of hop-derived aroma in beer. As mentioned earlier, the total oil content amounts to about 0.1-2.0% of the hops' dry weight, and the amount and composition of the oil is mainly dependent on the hop variety. More than 400 hop aroma components have currently been identified, and of the many classes of compounds found in the essential oil, the majority of them belong to the class of terpenes or terpenoids. Terpenoids are terpenes that have been modified with functional groups, often oxygen-containing (22). There are many ways to classify the compounds into categories, and some choose to divide the components into two broader main classes: the hydrocarbons and the oxygen-containing compounds (8). Though, nowadays it is conventionally described as three classes: hydrocarbons, oxygen-containing compounds and sulfur-containing compounds (24).

The majority of the aromatic compounds are derived from a few key parent terpenes, and it is thought that they are biosynthesized by the plant as a defense against insects. The oxygen-containing terpenes, the terpenoids, on the other hand, function as photosynthetic pigments, plant hormones and membrane constituents, among others. Terpenes contain carbon atoms in multiples of five as they are composed of isoprene units (C_5H_8), ranging from 10 to 40 carbon atoms. Depending on the number of repetitions of the isoprene units, the hydrocarbons can be subdivided into groups, with monoterpenes and sesquiterpenes being most common in hops (8, 9). Monoterpenes (C_{10}) are composed of two isoprene units and include compounds such as α-pinene, β-pinene, β–myrcene, and limonene, while the monoterpenoids include compounds such as geraniol, nerol, linalool and geranyl acetate. Sesquiterpenes (C_{15}) are composed of three isoprene units, and the sesquiterpenes and sesquiterpenoids include caryoplhyllene, β–farnesene, humulene, farnesol and humulene epoxides among others. If the terpene or terpenoid has a backbone larger than C_{15}, it is generally either not found in the hop oil at all, or it is considered to not be volatile enough to contribute to the aroma due to its higher molecular weight. Other classes of compounds found include aldehydes, ketones, methyl esters and sulfur compounds (22).

Generally, some of the classes contribute to the following aromas: Herbal, woody and spicy flavors are often attributed to sesquiterpenes, green flavors to aldehydes, citrus flavors to esters and linalool, and fruity and floral flavors to citronellol, geraniol, linalool, ketones, epoxides and esters (14). Which compounds actually stay in the finished beer are affected by many parameters, but polarity is especially important to consider. The oxygen-containing compounds are hydrophilic, i.e. polar, and they will therefore be more easily retained in wort and beer than the hydrocarbons, which are hydrophobic, i.e. non-polar. The non-polar compounds will solubilize better in a high-alcohol environment though, so if you make the exactly same beer with the only difference being the alcohol content, the flavor contributed from the hops will actually be substantially different. The non-polar

compounds also take longer time to solubilize in beer, so the time you leave the hops in also needs to be considered depending on what one is after (23). Some common compounds found in hops can be seen in Table 2.1.

Table 2.1: Some common volatile compounds found in hop essential oil and their characteristics. Their respective odor type and flavor type is marked in bold. Characteristics for all compounds were retrieved from The Good Scents Company (http://www.thegoodscentscompany.com/).

Compound	Characteristics
β-caryophyllene	Woody-**spicy,** dry and tenacious odor. **Spicy**, pepper, woody and herbal flavor
D-limonene	**Citrusy**, lively odor with nuances of tangerine and lemon oil and a sweet, terpy and **citrusy** flavor
Farnesene	**Woody**, green vegetative odor with a hint of a floral nuance. Fresh **green** vegetative taste, with celery and hay nuances and somewhat fatty and tropical fruity afternotes (α-Farnesene)
Geraniol	**Floral,** sweet, rose, fruity and citronella-like odor with a citrus nuance. **Floral** flavor used in many flavors such as cherry, peach, raspberry, grapefruit
β-myrcene	**Spicy**, green, resinous and piney odor, with a **woody**, citrusy and fruity flavor
α-pinene	**Herbal**, woody, piney and turpentine-like, with a slight cooling camphoraceous nuance and a fresh herbal lift. Intense **woody**, piney and terpy flavor
β-pinene	**Herbal,** cooling, woody, piney and turpentine-like odor with a fresh minty, eucalyptus and camphoraceous note and a spicy peppery and nutmeg nuance. Fresh, **piney** and woody flavor with a spicy nuance
α-humulene	**Woody**, oceanic-watery and spicy-clove odor
Linalool	Citrus, orange, **floral**, waxy odor with a **citrus**, orange, lemon and woody flavor.
Isobutyl isobutyrate	Penetrating **fruity** odor (ethereal, tropical fruit, pineapple, banana) with a ripe **fruit** flavor
Hexanal	Fresh **green**, vegetative, fruity, clean odor with a woody nuance. **Green,** woody, apple, grassy flavor with a fresh, lingering aftertaste
α-terpinene	Citrusy, **woody**, terpenic with camphoraceous and thymol notes. It has spicy and juicy citrus nuances and a **terpenic,** woody, citrusy, lime with spice flavor
Isoamyl isobutyrate	**Fruity**, waxy, apricot, pineapple and banana odor. Sweet, **fruity**, green flavor with berry nuance
Nerol	**Floral**, sweet, citrus, fresh, lemon/lime and waxy with a spicy depth odor. Lemon, bitter green and fruity flavor with a terpenic nuance
2-methylbutyl isobutyrate	**Fruity,** ethereal, tropic, banana odor

Some of the volatile compounds are actually called "survivables" due to their ability to "survive" in the beer. They often show up in beer analyses, and they consist of seven compounds, including linalool, 2-methylbutyl isobutyrate and geraniol. It is also important to take into account how well compounds survive in the beer throughout the brewing process - If you use a hop variety low in survivables, or that has mostly compounds that do not really survive heat, fermentation or CO_2 that well, it will make a better post-run hop than a whirlpool hop (see section 2.2: Beer brewing and the role of hops) (23).

Still, although there may be several hundred compounds present in the hop essential oil, only a certain number of these will be present at concentrations above their detection threshold and actually contribute to the aroma of the beer. These compounds are known as character-impact odorants, and they are responsible for, or at least significantly contribute to, a sample's distinctive odor profile (9). β-myrcene and linalool are considered the most aroma-active volatiles in all analyzed hop varieties, but myrcene usually does not make a contribution to the hop aroma in beer. This is due to the fact that the concentration is often far below the sensory threshold level, as it is a hydrophobic hydrocarbon and most of it evaporates during wort boiling. Oxygenated compounds on the other hand, such as linalool, are hydrophilic and they can therefore be more easily retained in wort and beer compared to the hydrophobic hydrocarbons (8). A nice saying by Patrick Jensen that makes this easy to remember is that (23):

"If it ends with –ene, it does not make the scene"

Another good example of the importance of the sensory threshold is when it comes to sulfur-containing compounds. They are often associated with unpleasant aromas, and their thresholds are often low, meaning that they can cause an off-flavor even when present at trace levels (9). So, dominant constituents in hops may not be the most flavor-impacting compounds if they have high sensory thresholds, and vice versa, as seen for sulfur-containing compounds. Therefore, in order to be able to say something about the aroma contribution of a compound, one cannot just look at the amount of it - one also has to take into account sensory thresholds. This is why an increasing number of flavor scientists are now paying more attention to the aroma-active compounds in hops, instead of only looking at the overall chemical composition (24).

As if sensory thresholds were not enough to consider, another aspect that has to be taken into account is synergisms and antagonisms. Synergism refers to when the combined effect of the interaction between two or more "things" are greater than if they were on their own, and it has been shown that aroma compounds with similar attributes often have additive interactions that can lower the sensonsoric thresholds of the individual compounds (25). This means that changing the ratios of the same hops, even just the same two compounds, can really shift the aroma of the beer. Antagonism, on the other hand, is when the combined effect is lower than if the compounds were on their own. With so many various compounds in the hop essential oil, researchers are actively looking into what these synergisms and

antagonisms might be so that synergy can be maximized and antagonism minimized (23). Studies have also shown that factors such as cultivation, locality, seasonality, harvesting, processing and storage practices contribute to the differences observed in hop essential oil composition, further increasing the complexity of it (22).

Lastly, to add even more to the complexity, it must be mentioned again that the compounds identified in hop oil are not all the same as those observed in hopped beer. The boiling and the fermentation process affect the hop characteristics, and during the brewing process, hop compound losses can occur due to a range of different reasons (14). First of all, hop compound losses can occur due to adsorption of compounds on residual hop matter, on the walls of the fermenter, as well as on yeast cells. Based on charge, some compounds will more easily stick to things, so this must also be kept in mind. Second, you can have a problem with conversion of the volatile compounds due to heat and oxidization, as well as by action during the fermentation by yeast. Other factors that may affect the observed compounds include change in pH, alcohol level, CO_2 level, dropping of oxygen levels and so on (23). This is another reason to why it is important to consider at what time of the brewing process you choose to add your hops, which is the topic of the next section.

2.2 Beer brewing and the role of hops

Brewers use hops primarily to get bitterness, flavor and aroma, and hops can be added at several different time points throughout the brewing process to enhance one or the other. Most of the hops are added in the boil kettle, but they can also be added at various stages both prior to and after the boil as well. First, a brief introduction to beer and beer brewing will be given, before a further look is taken at what time points the hops can be added and why you would want to add them at these time points. To put it simply, beer is the fermented, alcoholic product of a careful combination of malt, water, yeast and hops (Figure 2.3).

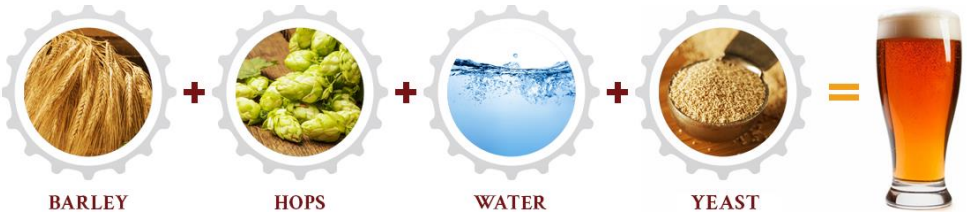


Figure 2.3: The four key ingredients in the basic formula of beer: barley, hops, water and yeast. Figure from (26).

Malt is cereal grain, often barley, that has been made to germinate by soaking it in water, and then halted from further germinating by drying it with hot air. By halting the

germination, the resources of the seed are made available to the brewer. These resources include the starch reserves of the seed and other smaller carbohydrates, lipids and amino acids, and in addition to giving the beer its sweetness, these resources can be modified and used by yeast in the fermentation process. Essentially, yeast is what gives beer its alcohol content and carbonation. This is done through fermentation, which is the process where fermentable sugars are chemically converted into approximately equal parts of ethanol and carbon dioxide (CO_2). ABV, or alcohol by volume, is the standard measurement used to assess the strength of a particular beer, with percentages getting higher the heavier style of the beer.

As for the brewing process and the hop additions, "kettle hops" is what the hops added to the kettle during the boil are called, and these will be discussed before the other hop additions. The brewing process is illustrated in Figure 2.4. Prior to the boiling, we first have the mashing and the lautering to obtain the sweet liquid we know as wort. During the mashing, malt is combined with warm water to convert the starch into sugars that can be fermented by yeast later on in the process. After this, the grains and liquid are transferred over to the lautering wessel to separate the sweet wort from the grains (15).

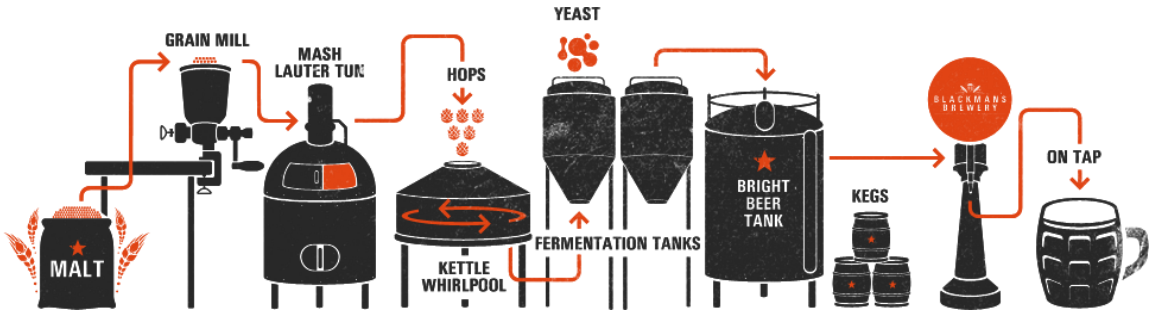


Figure 2.4: Illustration of the main processes in beer brewing: malt milling, mashing, lautering, boiling, whirlpool, fermentation, filtration and dispensing (27). Figure from (27).

After the lautering, the wort is transferred to the boil kettle and the boiling starts. The boiling process usually lasts for one hour, and during this time hops can be added at different intervals. Hops that are added early during the boil are often called "bittering hops", as the long boil time allows the alpha acids to isomerize. They are usually added at the beginning of the boil, or with at least 60 minutes of boiling time left. "Flavor hops" are added with about 20 to 40 minutes of the boil remaining, as too early addition will lead to a large amount of the volatile components being lost to evaporation. Lastly, "aroma hops" can be added in the last minutes of the boil, to further minimize the loss of volatile components to evaporation (6).

Hops added towards the end of the boil are often called whirlpool additions. They are added after flameout while the wort is still hot, typically around 75 °C to 90 °C, and the name comes from the fact that in commercial systems, this is done in "whirlpool" systems. The purpose is to help separate the wort from hops and grain materials. Homebrewers commonly refer to "whirlpool" hops as hops added to the wort after the boil but before the wort is chilled (28). When the boil is complete, a coiled heat exchanger is used to cool the wort down to pitching temperature, usually around 20 °C, and the wort is ready for fermentation. When fermentation ends, the wort has become what we call beer, and is ready for packaging (6).

It is also possible to add hops at other points in the brewing process, mainly with the goal to enhance the flavor and aromatic qualities of the beer. The most common non-boil use of hops is dry-hopping, which nowadays often refers to hop addition after the wort has been cooled for a week or two and leaving them in there, allowing the essential oils to dissolve. This does not add any bitterness since there is no boil to make the alpha acids isomerize. Other time points for hop-additions, which are somewhat controversial, include first wort hopping, which is a pre-boil addition of hops to the hot wort as it is running into the kettle after the lautering, and mash hopping, which is the addition of hops already during the mashing (6).

It should also be briefly mentioned that there are many styles of beer out there, but one style that has been on the rise lately is the India Pale Ale (IPA). The story goes that in the 18th century, IPA was just a British pale ale brewed with extra hops. These hops made the beer so bitter that it was stable enough to survive the long boat trip to India without spoiling. Due to the extra hops, IPAs are full of flavor compared to other common beer styles, and early craft brewers capitalized on this. Now, IPAs are synonymous with the craft beer movement and as craft beer has become more popular, so has IPAs (29).

There are several types of IPAs out there, but generally, they are known for being the most aggressively hopped beer style of them all. Hops can be added at any time point throughout the boil, but for IPAs, it is very common to finish off the big aromas by dry-hopping. By doing this, one can really put an exclamation point on the flavor one wants to achieve (30). Since IPAs have become so popular, it is especially of interest for brewers to know more about how hops and their volatile compounds behave in the beer style in a dry-hopping situation.

2.3 GC/MS and headspace sampling

Different methods are available to analyze the essential oils in hops and to quantify their flavor composition, and researchers are continuously working on optimizing these methods (10). A commonly used method for analyzing hop volatiles is headspace gas chromatography-mass spectrometry (HS GC-MS) (8).

First, a weighed amount of hops is placed in a glass vial together with the relevant solvents and standards. Then it is sealed, either with a screw top or a crimp top (Figure 2.5, left). The vial is then heated in an oven for a set fixed time at a set fixed temperature. A portion of the vapor is then extracted from the vial by the headspace sampling system and is introduced to the GC column (Figure 2.5, right). The headspace sampling can be done in two ways: static (loop) or dynamic (trap). Both ways will work, but dynamic is superior to static, as the amount of sample value introduced to the GC column is increased. This is favorable for hop volatile analysis, as low levels of some components can still be critical to the overall aroma of the sample. Static headspace sampling, on the other hand, only delivers a very small fraction of the headspace vapor into the GC column and is more suitable when you have high concentrations of compounds (7).

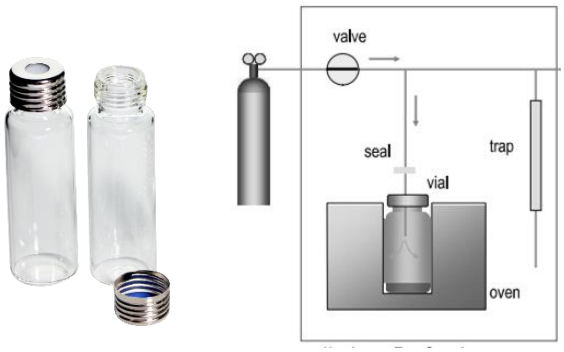


Figure 2.5: Empty glass vials with screw tops (left) and schematic diagram of the HS trap system (right). In the schematic diagram, it can be seen that the equilibrated vial is being pressurized with carrier gas. The pressurized gas will be released from the vial into the adsorbent trap, where the volatile compounds are collected before being thermally desorbed and introduced into the GC column (7). Figure adapted from (7, 31).

GC-MS is an analytical method that combines the features of the GC and the MS to identify different substances within a test sample. In the GC, you have a mobile phase and a stationary phase. The mobile phase, which is the carrier gas that typically is helium, carries the sample through the column. On the inside of this column is the stationary phase, and depending on their chemistry, the different molecules in the sample will have different affinity for the stationary phase. This leads them to travel through the column at different speeds, separating them. The molecules will then be eluted at different times, which is known as the retention time (RT) (Figure 2.6) (32).

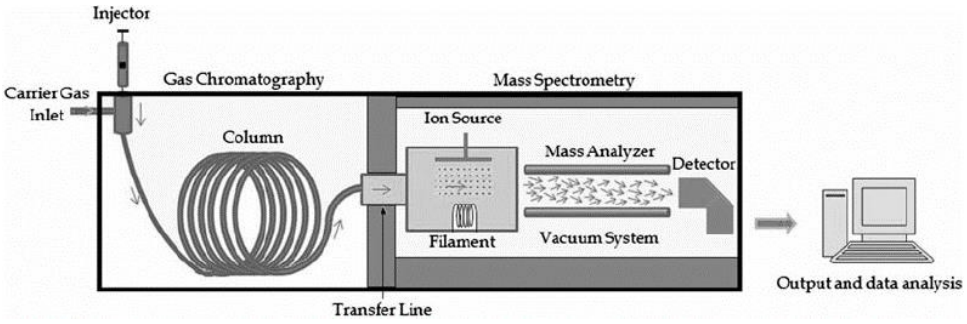


Figure 2.6: Schematic overview of the main components of gas chromatograph-mass spectrometer instruments. Adapted from (33).

As the molecules are then captured by the mass spectrometer at different times, they are ionized by an ion source. This may cause some of the sample's molecules to break into charged fragments or to simply become charged without fragmenting. These ions are then separated according to their mass-to-charge ratio by accelerating them and subjecting them to an electric or magnetic field. When this is done, ions of the same mass-to-charge ratio will undergo the same amount of deflection, which means a small change in direction. The final steps of the process is then ion detection and analysis. Fragmented ions appear as a function of their mass-to-charge ratios; meanwhile peak areas are proportional to the quantity of the corresponding compound. Therefore, when a complex sample is separated by GC-MS, many different peaks will be produced in the chromatogram and each peak generates a mass spectrum. By using extensive commercially available libraries of mass spectra, such as the NIST library, unknown compounds can be identified from these peaks (32).

An increasing number of scientists are paying more and more attention to the aroma-active compounds in the hops instead of only looking at the overall chemical composition, and they therefore use gas chromatography-olfactometry (GC-O) in addition in their research (7, 24). As mentioned in earlier sections, to determine the importance and influence of individual volatiles in hops, the relevant sensory thresholds of the compounds must also be considered. Some of the volatile compounds that are present at only trace amounts can really contribute to the aroma due to low thresholds, and vice versa. GC-O is a method where the GC-MS system is equipped with an olfactory detection port, which is a sniffer mask placed at the outlet of the GC. Here, a trained panelist can smell the gas and provide information about the presence of odor in it (34).

The trap system is characterized by a good repeatability without carryover effects, but some errors may still happen in the process. For example, the injector or column may be contaminated, causing some peaks of the chromatogram to change in size. Peaks may also change in size if there has been decomposition of the sample, due to temperature changes or leaks causing evaporation from the sample. There is a whole range of other common mistakes, which is why preventative maintenance is carried out regularly to avoid this (35).

2.4 Principal component analysis (PCA) and data preprocessing

The purpose of this section is to provide an uncomplicated explanation of principal component analysis (PCA), without diving too deep into the mathematics behind it. PCA is an unsupervised method of multivariate analysis, and in general, almost any data matrix can be simplified by PCA. The analysis is fast and resourceful, and it is often used for the analysis of metabolomics, as it can provide visual information about observed tendencies, arrangements or outliers in the data set (36).

When using PCA, it is important to make sure that the data matrix used is properly transformed and scaled first, which is covered on the next page. The word "scaled" is often used interchangeably with "normalize" or "standardize", and it will also be so here. Without scaling, features that show a large variance often tend to dominate the result, and in many cases, this is not desired. This is one of the main weaknesses with PCA - it tends to be highly affected by outliers in the data. PCA is a least squares method, and outliers will therefore severely influence the model. Hence, outliers have to be found and corrected or eliminated before the final PC model is developed. Though, it is important to note that before removal, one should be sure that it can be justified that it in fact is an outlier, and not an influential point. A reduction of the data set should only be made if there is a cost, experimental or computational, connected with keeping the variables in the model (36). Figure 2.7 shows an example of a score plot (left) and a biplot (right) for the same dataset (37).

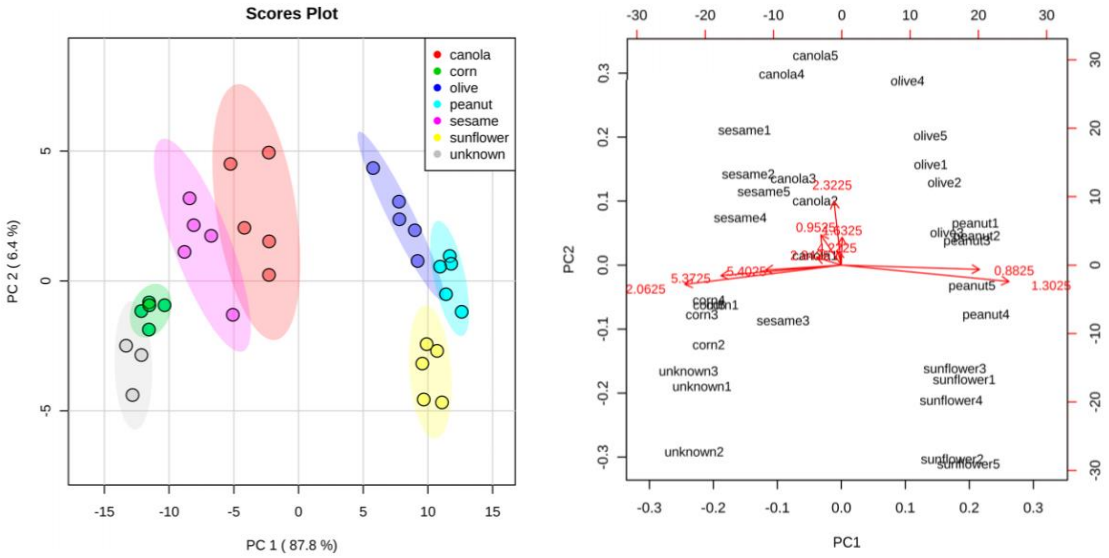


Figure 2.7: Example of a score plot for PC1 and PC2 using peak intensities as input (left). The color-shaded area in each cluster is the 95% confidence region. To the right is a biplot for PC1 and PC2 from the same data input. Figure adapted from (37).

On the x-axis of both plots, we see PC1, which means principal component 1. The principal components are new variables that are constructed as linear combinations or mixtures of the initial variables, and this is done in such a way that the principal components (i.e. the new variables) are uncorrelated, and so that most of the information within the initial variables is compressed into the first components. The idea is that if you have 9-dimensional data, you get 9 principal components. PCA tries to put maximum possible information into the first component (PC1), then the maximum remaining information in the second component (PC2) and so on. By doing this, dimensionality is reduced without losing much information. What is important to note is that the principal components are less interpretable, and they do not really have any real meaning due to the fact that they are linear combinations of the initial variables (38).

In the example in Figure 2.7, the variation explained by PC1 accounts for 87.8%, while for PC2 it is 6.4% (left). With this information, one can further interpret the figure based on what the dataset contains. To the right, a biplot is included. In this example, they have looked at edible oil authentication, and the biplot here has linked the score plot of different oils and the loading plot of NMR peaks. Similarly, one can link the score plot of different hop samples with the loading plot of peak retention times. A biplot is used to identify which variables have the largest effect on each principal component, and they can range from -1 to 1. The vectors are pinned at the origin of the PCs (PC1 = 0 and PC2 = 0), and their projected values on each PC show how much weight they have on that PC. The closer the loading is to -1 or 1, the more the variable influences the component. If the loading is close to 0, it indicates that the variable has a weak influence on the component (39).

There are several tools out there that can help with these analyses, and one of them is MetaboAnalyst. MetaboAnalyst is a comprehensive online tool dedicated for metabolomics data analysis via a user-friendly and web-based interface, where for example PCA can be carried out (40). More information about MetaboAnalyst can be found in section 3.6. MetaboAnalyst can also carry out scaling of the data for the user, which was mentioned to be important earlier in this section. The purpose of scaling the data is because PCA seeks to maximize the variance of each component; hence, one needs to ensure that no extra weight is given to the “larger” variables, which can lead to biased outcomes. Still, larger variables may sometimes be more important, and it can be tricky to proceed when different variables can bring different amounts of quality information. Scaling can give equal importance to a variable that is a small source of noise, and to another variation that is a large source of wanted signal. In this case, noise would be boosted and signal shrunk. If all your variables are measured on the same scale and have the same unit, it is not necessarily any need to scale the variables. If you want to maximize variation, it is fair that variables that have more variation get to contribute more. On the other hand, if you

have different types of variables with different units, such as height and weight, it should definitely be scaled (41, 42).

Different preprocessing methods emphasize various aspects of the data, and each method has its advantages and disadvantages. The choice of preprocessing method depends on the biological question of interest, characteristics of the data and the chosen data analysis. Two common scaling methods used are pareto scaling and auto scaling (also called unit variance scaling). Both of these methods are aimed at adjusting the variance of the different metabolites. (43)

The simplest of these approaches is the auto scaling method, which uses the standard deviation of the data as a scaling factor. After autoscaling, all metabolites have a standard deviation of one, and therefore the data is analyzed on the basis of correlations instead of covariances. Auto scaling renders all features equally important. However, measurement errors will also be inflated (44). The other method, pareto scaling, uses the square root of the standard deviation of the data. It is similar to auto scaling, but its normalizing effect is less intense, making the normalized data stay closer to its original values. Comparing it to auto scaling, this method is able to more significantly reduce the weights of large fold changes in metabolite signals, and large fold changes are decreased more than small fold changes (43). In addition, the data does not become dimensionless, as after autoscaling. It should be noted, though, that when using pareto scaling, the dominant weight of extremely large fold changes may still be unchanged and they may still show a dominating effect (45).

As mentioned, biological question of interest, characteristics of the data and the chosen data analysis has to be taken into account when deciding on how to scale your data. Data pretreatment methods can correct for aspects that hinder the biological interpretation of metabolomics data sets by emphasizing the biological information in the data set and thus improving their biological interpretability. Choosing a suitable scaling method is not always simple, but when analyzing metabolomics data, pareto scaling is often chosen for its ability to keep the data structure partially intact, while the relative importance of smaller quantities is increased. The only thing important to note when using this scaling method is that, as mentioned above, the dominant weight of large fold changes may still show a dominating effect, and this should be taken into account. If it is not sutable for your question of interest or for the analyses you are planning to do with the data, another scaling method should be used (43).

Finally, it should again be emphasized that the resulting principal components are only meant to guide your continued investigation or chemical experimentation, and not to function as an end in themselves.

3 Methods

3.1 Hop selection

Five different hop varieties were chosen for this study based on their flavor and aroma profiles. Two of the varieties, Centennial and Citra, are known to have a citrusy profile (46, 47), whereas two of the other varieties, Saaz and Hallertau Mittelfrüh, are known to have a more spicy and herbal profile (48, 49). The fifth hop variety, Simcoe, is characterized to be both fruity and earthy and is placed somewhere in between the two pairs (50). The hop pellets were obtained from the Brewshop webshop (https://brewshop.no/), and they were packaged in airtight bags, stored at -20°C prior to analysis. Table 3.1 shows an overview of the five hop varieties and their characteristics.

Table 3.1: Overview of the five hop varieties and their respective alpha acid percentage, total oil content and characteristics. Alpha acid percentage was stated on the individual hop pellet packages, whereas average total oils content and hop variety characteristics was retrieved from beermaverick.com (46-50)

Hop variety	Alpha acid (%)	Avg. total oils (ml/100g)	Characteristics
Citra	12.6	2.3	Dual-purpose hop that can be used in all hop additions throughout the brewing process. Has a strong, yet smooth floral and citrus aroma and flavor.
Centennial	8.0	2.0	Dual-purpose hop that can be used in all hop additions throughout the brewing process. Characterized by aromatic pine, citrus, and floral notes.
Saaz	2.9	0.7	Aroma hop typically used in only late boil additions, including dry hopping. Mild with pleasant earthy, herbal and floral overtones.
Hallertau M.	3.8	1.0	Aroma hop typically used in only late boil additions, including dry hopping. Mild, yet spicy, with floral and citrus tones.
Simcoe	14.1	2.0	Dual-purpose hop. Alongside its fruity and slightly earthy aromas, specific descriptors include grapefruit, passion fruit, pine and berry characteristics

3.2 Wort and beer preparation

For the use as solvent states for the samples, wort and beer was made. The wort was prepared by mixing 65.1 grams of the dry malt extract "Briess CBW Pilsen light spraymalt" with 0.5 liters of distilled water, aiming for a 1.050 gravity (i.e. ~ 5% ABV). The wort was then autoclaved before use.

The beer was prepared by following an IPA recipe, "IPA Schnipa", that can be found in Appendix B.

3.3 Sample preparation

1.0 g of ground hop pellets were weighed into headspace glass vials (20 ml). Samples were either left dry, or 10 ml of wort, beer or distilled water was added. 30 µL 100x diluted 4-methyl-2-pentanol ($C_6H_{14}O$) was added as an internal standard to all samples, including the blanks. A total of 47 samples were made. Sample overview can be found in Appendix A, and a flowchart illustration of sample preparation in Figure 4.1. For the Saaz variety, triplicates were made for each sample.

3.4 Headspace analysis

For the headspace (HS) analyses, a Teledyne Tekmar HT3™ Static and Dynamic Headspace System (Teledyne Tekmar, Mason, OH) was used. HS-trap sampling parameters were based on work done by Aberl and Coelhan, and a selection of them are displayed in Table 3.2 (8). The rest of the parameters can be found in Appendix C.

Table 3.2: Selected HS-trap sampling conditions. For the vial equilibration, the temperature was either 30°C, 60°C or 80°C depending on the sample. Every other parameter remained the same.

Headspace system	Teledyne Tekmar HT3™ (dynamic)
Vial equilibration	30°C/60°C/80°C for 20 minutes
Trap column	Supelco™ Purge/Trap K Vocarb® 3000
Transfer line	100°C, column connected directly to HS trap
Carrier gas	Helium
Dry purge	2 minutes
Sweep	50 ml/min for 5 minutes
Split	5:1 with split flow 9 ml/min

3.5 GC-MS analysis

Analysis of the injected headspace sample was performed by the Agilent Technologies 7000 Triple Quadrupole Mass Spectrometer system, often referred to as the 7000 Triple Quad GC/MS (Agilent Technologies, Santa Clara, CA). The 7000 Triple Quad GC/MS system consists of a 7890A gas chromatograph (GC) and a 7000 triple quadrupole mass spectrometer (MS). An Agilent J&W DB-624 UI capillary column (30 m x 250 µm x 1.4 µm) was used for chromatographic separation. A selection of the parameters are found in Table 3.3. All parameters are found in Appendix C.

Table 3.3: Selected GC conditions

Gas chromatograph/Mass spectrometer	Agilent 7890A (GC) and 7000 triple quadrupole mass spectrometer (MS)
Column	Agilent J&W DB-624 UI (30 m x 250 µm x 1.4 µm)
Flow	1.8 ml/min
Pressure	14.7 psi
Oven	35°C (6 min), 8.8°C/min to 100°C, 13.3°C/min to 220°C and 22.1°C/min to 250°C (3.4 min)

The MS transfer line temperature was set to 250°C and the ion source temperature to 230°C. The MS was operated in selected ion monitoring (SIM) mode using electron impact ionization (70 eV). Identification of separated compounds was made based on the comparison of the obtained mass spectra with the ones from the mass spectra library NIST05a. Selected parameters are displayed in Table 3.4. All MS parameters are found in Appendix D.

Table 3.4: Selected MS conditions

Scan range	m/z 30 to 400
Scan time	70 minutes
Mode	Selected ion monitoring (SIM)
Source temp	230 °C
Inlet line temp	250 °C

3.6 Data processing and scaling

3.6.1 Data processing and peak area measuring

The data from the HS GC-MS analysis were processed using the Agilent MassHunter Qualitative Analysis B.05.00 software. 97 compounds were chosen to investigate further based on their occurrence in previous studies. Compounds were identified based on the comparison of the obtained mass spectra with the ones from the mass spectra library NIST05a. To measure the peak area for each of the 97 compounds in each of the 35 samples (only 1 sample was used from each triplicate, see section 4.1.1: "Triplicates" for details), the "integrate chromatogram" function of the software was used. Peak area values, tallying up to 3395 values, were manually added to a Microsoft Office Excel spreadsheet (Appendix E). The 97 peak area values from each sample were then normalized to the respective sample's peak area value of the internal standard, and blanks were subtracted.

For further preprocessing, modeling and statistical analysis of the normalized peak area data, MetaboAnalyst 5.0 was used. MetaboAnalyst was developed by the Wishart Research Group of the University of Alberta, and is a comprehensive online tool dedicated for metabolomics data analysis via a user-friendly and web-based interface (40). More details about the tool, as well as user manuals and the tool itself, can be found on the webpage: https://www.metaboanalyst.ca/home.xhtml. The next section covers details how the data was preprocessed prior to data analysis.

3.6.2 Data scaling

As mentioned in section 2.4: "Principal component analysis (PCA) and data preprocessing," scaling of the data prior to PCA is important. PCA is a variance maximizing exercise, so one needs to ensure that no extra weight is given to the "larger" variables, since that potentially can lead to biased outcomes. However, larger variables can bring quality information, and should therefore for some purposes be kept.

For the purpose of this study, it was decided to use pareto scaling on the data. This was due to the fact that it keeps the data structure partially intact, while the relative importance of smaller quantities is increased. It is known that some volatile compounds are present at much higher concentrations than other volatile compounds in hops, so the weight of the changes in these may be large and still show a dominating effect. Though, for the purpose of this study, these variables can work well for the purpose of highlighting differences.

Still, it was also of interest to see that the trends were still the same when the data was auto scaled, to make sure scaling was not the cause of the observations. Auto scaling renders all features equally important, and larger variables will not be as dominant as with pareto scaling. Therefore, 2D score plots, tables of loadings and biplots from the PCA of data from the same samples used in section 4.3 and 4.4, with the data auto scaled instead of pareto scaled, are also included in Appendix G: Results with auto scaling". These will be brought up again and discussed in chapter 5.

MetaboAnalyst was used for both the preprocessing, modeling and statistical analyses of the obtained data. When it comes to the preprocessing, relevant data sets were uploaded as peak intensity tables with samples in columns (unpaired), and no data filtering was performed as the data set contained less than 5000 features. "None" was then chosen for the sample normalization and data transformation, and "pareto scaling" was chosen as the data scaling method. For the additional results in appendix G using auto scaling, the only difference was that "auto scaling" was chosen as the data scaling method instead.

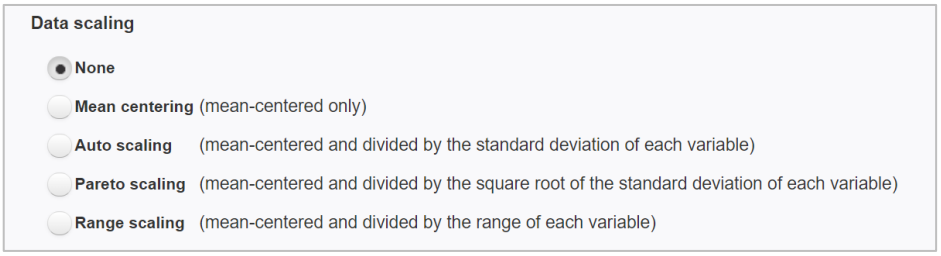


Figure 3.1: Different scaling method options in MetaboAnalyst, along with a statistical description of what the scaling methods do. Figure is adapted from a screenshot of the MetaboAnalyst website, https://www.metaboanalyst.ca/home.xhtml, during data preprocessing.

4 Results and analysis

In this chapter, results obtained from the headspace gas chromatography-mass spectrometry (HS GC-MS) analyses are presented and analyzed. The results are discussed and put into context in chapter 5. As mentioned in the introduction, this study had the following aims: Firstly, it was of interest to gain further insight into how different temperatures affect how well hop volatile compounds are retained in wort, as well as to explore differences in retainment of compounds between wort and beer samples. The study also aimed to highlight the importance of hop addition timing in the brewing process. Lastly, the study sought to reveal analytical differences linked to the hop varieties. This chapter was therefore split into three main sections that each cover one of these aims:

4.2 Differences and similarities in hop oil composition between the five hop varieties
4.3 The effect of temperature on retainment of hop volatile compounds in wort
4.4 Differences in retainment of hop volatile compounds in wort versus beer

Prior to these sections there is a short section covering the identified compounds and the results from the blanks and the triplicates, section **4.1.** Then, the differences and similarities between the five hop varieties are covered first, before differences due to solvent state and temperatures are taken into account. To discuss differences between the five hops varieties, 12 selected compounds known to be important to hop aroma and flavor were chosen to be focused on. The next section covers the effect of temperature on retainment of hop volatile compounds in wort, with a following section on differences in retained hop volatile compounds in wort versus beer. These two last sections are used to highlight the importance of hop addition timing in the brewing process.

A total of 47 samples were prepared and analyzed by HS GC-MS, and a flowchart illustration of how the individual samples were prepared and their self-explanatory sample names can be seen in Figure 4.1. Samples from each of the five hop varieties were tested in four solvent states: dry, distilled water (wet), wort and beer. Dry and wet samples were only tested at 80°C, beer samples only at 30°C and wort samples at 30°C, 60°C and 80°C. The beer samples were tested only at 30°C as that was the closest possible resemblance to room temperature, which is the temperature the beer often is kept at when dry-hopping. The beer prepared for the beer samples was an IPA, due to the current popularity of the beer style, and since it's a commonly dry-hopped beer style. The wort samples, on the other hand, were tested for all three temperatures to resemble the common temperatures in the brewing process when hop additions are done in the wort. Dry and wet samples were tested only at 80°C with the goal to get out as many volatile compounds as possible.

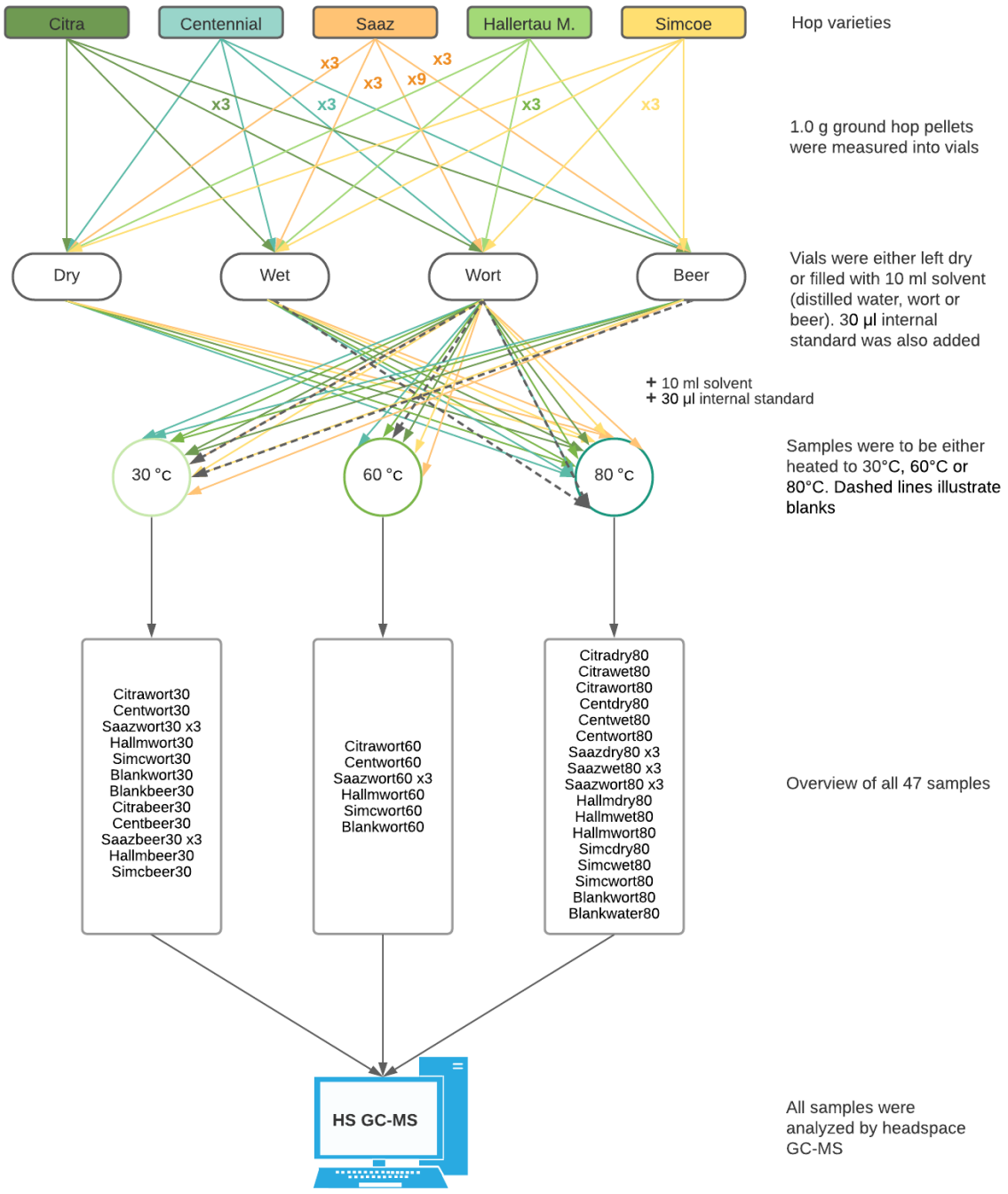


Figure 4.1: Flowchart illustration of how 1.0 gram samples from each of the hop varieties were assigned to the four different solvent states (dry, wet, wort, beer). For the Saaz variety, triplicates were made (x3). The samples were then assigned different temperatures, and blanks were made. Internal standard (100x diluted 4-methyl-2-pentanol) was added to all samples. All 47 samples were then analyzed by headspace GC-MS.

It should be clear from the flowchart illustrated in Figure 4.1 that the amount of data obtained from these samples is large, and that they can be analyzed and compared in numerous ways. For the scope of this thesis, only a couple of aims were chosen to focus on and some suitable analyses were conducted with these in mind. It should be noted that there are still many opportunities with the data and several ways to analyze them. This will be discussed more in the Conclusion and outlook chapter at the end of the thesis.

The experimental data used for the analyses in this chapter are found in Appendix E: Peak area values. Throughout this chapter, it should be kept in mind that the bigger the measured peak area, the more has evaporated, meaning less is retained in the wort or beer.

4.1 Identified compounds, triplicates and blanks

This section will give an overview of what makes up the foundation for the upcoming sections, including information about the triplicates and blank samples, as well as information about the identified compounds.

4.1.1 Triplicates

For the first sample run, triplicates (three identical replicates) were made for each of the Saaz samples. This was done to ensure that the variation potentially observed between the hop varieties was not due to differences in sample preparation or due to the analysis method, but due to actual differences in the hops. Since each sample run costs a certain amount of money, and given the aims of this study, it was prioritized to have a larger number of single samples.

By starting the experiment by running triplicates for all solvent states (except from dry) and for all relevant temperatures, the goal was that the differences observed within the triplicates were insignificant, and that it could be justified to carry on with single samples. Figure 4.2 shows sections of total ion chromatograms for two Saaz triplicates in two different conditions compared to the chromatograms of Centennial samples in the same respective conditions. They are included to show the significant differences observed between the hop varieties for the same conditions compared to the barely visible differences within the triplicates. To ensure that the variation observed within the triplicates was insignificant for the purpose of this study, a few peak areas were measured, normalized to the internal standard and then compared. Deviation was seen to be low and likely due to biological variation, so it was then concluded to carry on with single samples for the rest of the samples (data not included).

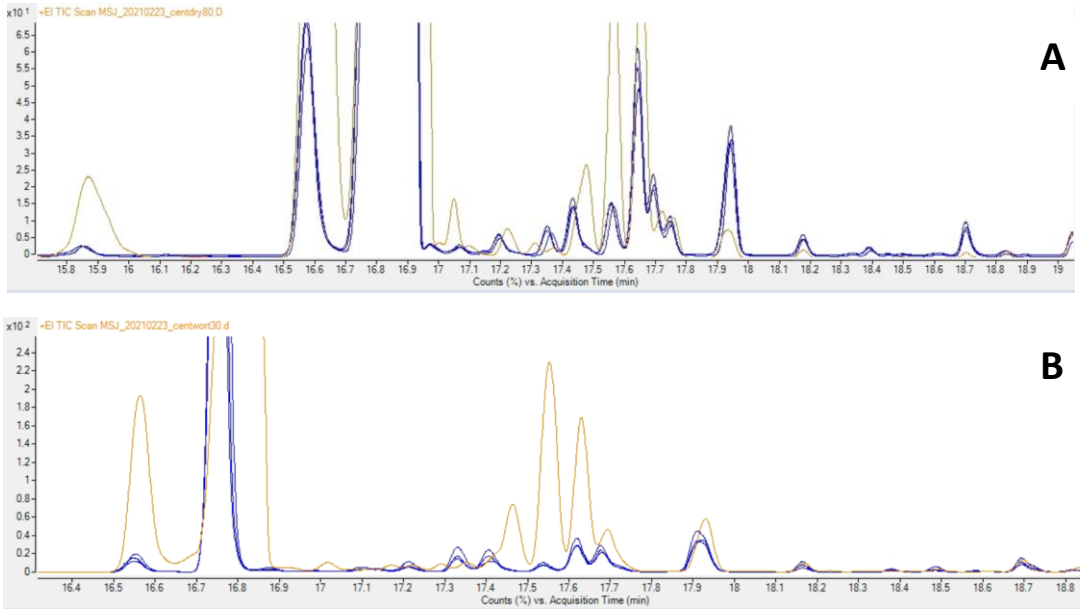


Figure 4.2: Sections of HS-trap GC-MS total ion chromatograms for dry Saaz sample triplicates (all three in blue) compared to the dry Centennial sample (yellow), all analyzed at 80°C (A), and for Saaz in wort triplicates (all in blue) compared to Centennial in wort (yellow), all analyzed at 30°C (B). The x-axis represents time and the y-axis represents signal intensity.

4.1.2 Blanks

Blanks, meaning vials containing everything but grounded hops, were also made to ensure that volatile compounds stemming from the solvent states themselves or potential contaminations would not interfere with the results from the sample analyses and further affect the interpretation of them. One blank was made for distilled water at 80°C, one for beer at 30°C and three for wort, analyzed at 30°C, 60°C and 80°C, respectively (see Figure 4.1).

The blank made for the "wet" samples with distilled water, blankwater80, showed no volatile compounds. The beer and wort blanks, blankbeer30, blankwort30, blankwort60 and blankwort80, on the other hand, all contained a range of volatile compounds. This is not surprising, as the beer was brewed using a range of hops throughout the brewing process, and it also makes sense that the wort itself will contain some volatile compounds that are not from hops.

For the wort blanks, the general trend was that not much evaporated at 30°C (i.e. small or non-existing peaks), whereas a larger amount of evaporated volatile compounds was observed at 80°C (i.e. larger peaks). Peak areas for blankwort60 tended to fall somewhere in between. However, there were some exceptions: For isoamyl isobutyrate (retention time 17.55) in wort for example, which has a fruity flavor and odor type, some evaporated at 30°C, but nothing evaporated at 60°C and at 80°C. Other compounds identified from the

wort samples include benzeneacetaldehyde (phenylacetaldehyde), which has a honey, floral and chocolate flavor and aroma, and linalool, which contributes a citrusy and floral flavor and aroma.

For this study, an IPA was used as the beer for the beer samples. As mentioned earlier in the theory, IPAs are heavily hopped, and it was therefore no surprise that the beer blank, blankbeer30, also contained some volatile compounds, with most of them most likely stemming from the hops used in the brewing process. Some compounds found mainly in the beer blank (and hence the beer samples) include ethyl acetate, a compound produced by yeast that is quantitatively the major ester found in both beer and wine, ethyl propionate, which has a sweet and fruity odor and flavor, and isoamyl acetate, a key ester present in all beers that provides a pronounced fruity-fresh or banana-like aroma at its threshold (51-53). The rest of the compounds found in the wort and beer blanks can be seen in Appendix E: Peak area values,

In the data used for the analyses, the peak areas of the blanks were subtracted from the respective peak areas of the respective normalized sample.

4.1.3 Identified compounds

A total of 97 chromatogram peaks were chosen to be investigated further for each sample. The identification of separated compounds was made based on the comparison of the obtained mass spectra with the ones from the mass spectra library, NIST05a. It was not possible to confidently identify which compound each of these 97 peaks corresponded to, but comparison of the obtained mass spectra gave sufficiently high scores when compared to the library to be relatively confident in the identification of around 80 compounds. A couple of smaller peaks were observed, but they were hard to identify. Thus, for the purpose of this study, the 97 peaks selected were sufficient. To ensure that the identification was most likely correct, relevant literature was searched for the identified compounds. It should be noted that none of the previous studies had used the same column in their analyses, nor had they used a similar setup; thus, it was not possible to directly compare retention times and simplify the identification process. Some identifications, especially those of compounds present in very small amounts, may not be correct, but for the purpose of this study, this is not crucial. This is due to the fact that this study focuses on highlighting differences, which is largely influenced by the data from the more common and major volatile compounds. If this study had put more emphasis on aroma and flavor contributions, the compounds that were present in small amounts would be of much more importance and they should have been confidently identified by the use of standards.

It should also be noted that although mass spectrometry is among the most sensitive methods used to identify molecules, it can be ill-suited for distinguishing structural isomers, which are chemically distinct entities that have the same mass. It is known that a few isomers exist in hop essential oil (24).

The identified compounds and the respective peak area values for each sample can be seen in Appendix E: Peak area values.

4.2 Differences and similarities in hop oil composition between the five hop varieties

One of the sub-aims of this study was to compare the five different hop varieties, Citra, Centennial, Saaz, Hallertau Mittelfrüh and Simcoe, and reveal differences between them. Since the volatile compounds of hops is the focus of this study and their contribution to flavor and aroma, this section focuses on differences in hop oil composition between these hop varieties with regards to some important compounds. The purpose of this section is to highlight these differences before the upcoming sections, as they will be brought up again throughout them.

To do this, 12 common compounds found in hop essential oil were chosen – half of them known to have a more citrusy and floral profile, and the other half known to be more on the spicy, woody and herbal side. These compounds were chosen with regards to the chosen hop varieties and to put emphasis on differences in their characteristics (see section 3.1: Hop selection). The compounds are listed in Table 4.1. In addition to the compounds and their characteristics, their polarity and class is also included. As mentioned earlier, polarity plays a role in how well the compounds are retained in wort and beer, and will be relevant for later discussion. Class is also relevant to include, as it is known that hop oil rich in monoterpenes often tend toward fruity or citrusy aroma, whereas hop oil rich in sesquiterpenes tend toward earthy, woody, herbal and spicy aromas (13).

Table 4.1: 12 selected common hop volatile compounds. The first 6 contribute a floral and citrusy aroma, whereas the 6 last contribute earthy, woody, herbal and spicy aromas. For each compound, their respective retention time (RT) for this study, class and polarity is also included. Information on class and polarity was retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov/), and characteristics were retrieved from the Good Scents Company (http://www.thegoodscentscompany.com/).

Compound	RT	Class	Polarity	Characteristics
Linalool	19.29	Monoterpene	Polar	Floral, citrusy
D-limonene	17.62	Monoterpene	Non-polar	Citrusy
Nerol (cis-geraniol)	21.47	Monoterpene	Polar	Sweet, floral, citrus
Isobutyl isobutyrate	14.55	Ester	Polar	Fruity
2-methylbutyl isobutyrate	15.17	Ester	Polar	Fruity
Isoamyl isobutyrate	17.55	Ester	Polar	Fruity
α-humulene	23.71	Sesquiterpene	Non-polar	Woody, spicy
β-caryophyllene	23.33	Sesquiterpene	Non-polar	Woody, spicy
α-pinene	17.91	Monoterpene	Non-polar	Pine, woody, herbal
β-myrcene	16.75	Monoterpene	Non-polar	Spicy, woody, fruity
β-pinene	16.56	Monoterpene	Non-polar	Pine, woody, minty
β-phellandrene	17.75	Monoterpene	Non-polar	Minty

To illustrate the differences in hop oil composition between the five hop varieties with regards to these 12 compounds, graphs were made with data from the dry 80°C samples in GraphPad Prism v9.1.0 (GraphPad Software Inc.) (Figure 4.3). The peak areas of β-myrcene were generally so high compared to those of the other compounds, that they were included in their own graph. Overall, it can be seen a clear variety in the hop oil composition with regards to these 12 compounds.

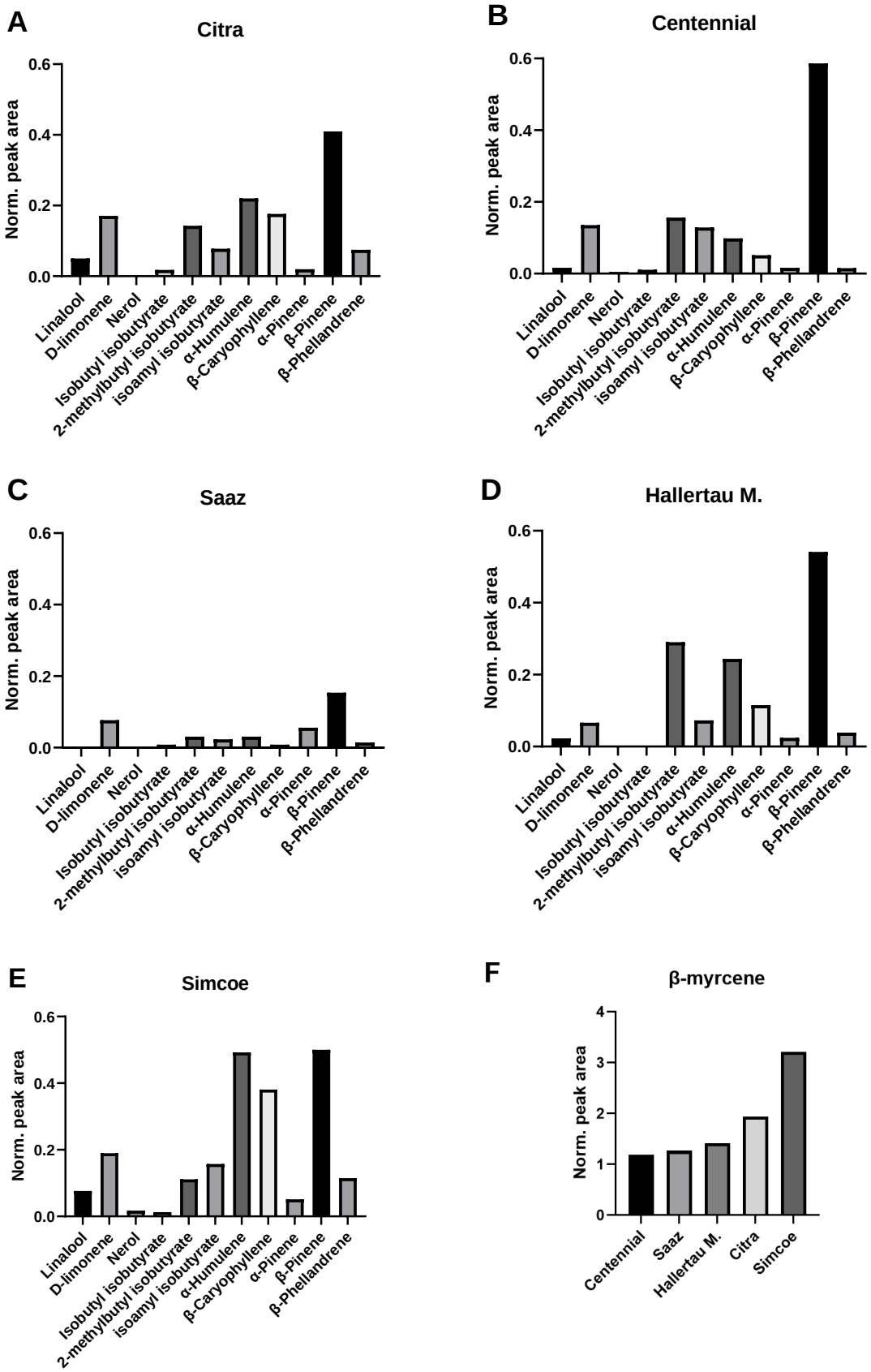


Figure 4.3: Bar graphs illustrating the normalized peak areas from dry samples (80°C) for 11 selected compounds for each of the five hop varieties used in this study (A-E). Graph F illustrates the peak area for β-myrcene for each hop variety at the same conditions, sorted in ascending order.

From Figure 4.3 it can clearly be seen that when it comes to these 12 selected compounds, Simcoe has the overall largest amount of essential oil, followed by Citra. This is consistent with what we saw in Table 3.1 in the hop selection part – Simcoe has an average total oil content of 2.0 ml/100 g, whereas Citra has an average of 2.3 ml/100 g, so it is expected to see a higher values for them. β-myrcene is known to be the major compound of the hop essential oil, and this is also observed here. Both Simcoe and Citra are shown to contain the largest amounts of also this compound.

From the same table mentioned above, it is known that Saaz has an average total oil content of 0.7 ml/100g, which is the smallest amount among the five selected hop varieties. From the figure, Saaz can be seen to have an overall small amount of most of the selected compounds, which is consistent with this information.

For Hallertau Mittelfrüh and Centennial, with average total oil contents of 1.0 and 2.0 ml/100 g, respectively, the differences are not too apparent and it seems like they almost have the same overall oil content with respect to these 12 compounds. The figure does not show the remaining 80+ compounds, so differences could have been more apparent if they were shown as well. Still, it should be noted again that there are annual variations in essential oil content in hops, and that the Centennial hops used may have had a lower essential oil content for this harvest, and the Hallertau Mittelfrüh hops the opposite.

4.3 The effect of temperature on retainment of hop volatile compounds in wort

From section 4.2 it should be apparent that the hop oil composition is not the same for the five hop varieties used in this study. Moving on, one of the main aims was to see how temperature affects how the volatile compounds are retained in wort. In order to look further into this, the wort samples for each hop variety were analyzed at 30°C, 60°C and 80°C. First, it was of interest to see if there actually was a difference in retainment of hop volatile compounds in wort at different temperatures. Then, it was of interest to see which compounds potentially contributed most to the observed differences.

Already just from looking at the total ion chromatograms when collecting the peak area data for the analyses, it was clear that there seemed to be some differences. For some compounds, a lot evaporated at 30°C, whereas for other compounds, more evaporated at 80°C (Figure 4.4). Figure 4.4 also shows a great example of an area where there was a peak separation issue, where the "integrate chromatogram" function was impossible to use and the cells for these values were therefore left blank (Appendix E: Peak area values. As can be seen in the figure, α-terpinene elutes for all three temperatures at around 17.44 minutes. Yet, as can be seen for the 30°C sample, a second peak comes right after that is not clearly separated from the prior peak. At around 17.47 minutes, it is propanoic acid, 2-methyl-, 3-methylbutyl ester that elutes right after α-terpinene. Peak areas of these two compounds are not measured at 30°C.

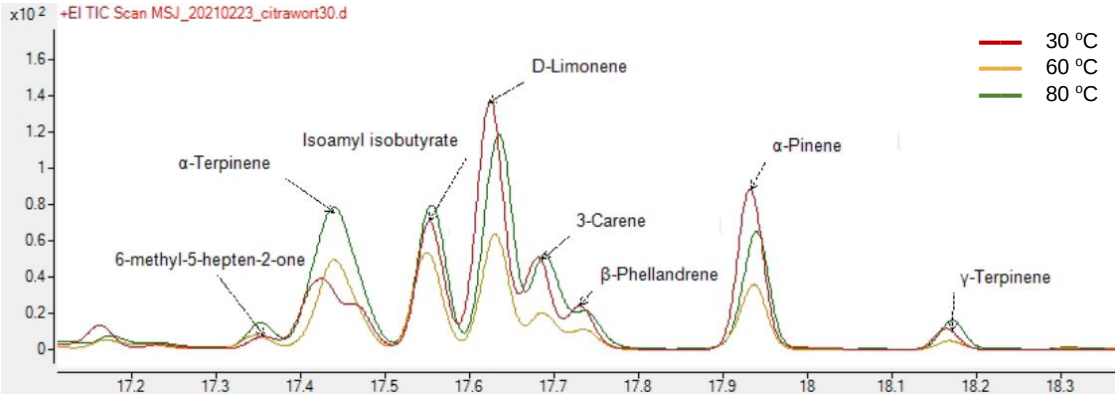


Figure 4.4: Section of HS-trap GC/MS total ion chromatograms from wort samples analyzed at 30°C, 60°C and 80°C for the Citra hop variety, with results from each temperature represented with its own color. Peaks of relevant volatile compounds are annotated with the name of its respective compound. The x-axis represents time and the y-axis represents signal intensity.

In order to analyze these differences further, principal component analysis (PCA) was used (Figure 4.5). More information and relevant code for the analyses can be found in Appendix F: *Data Analysis with MetaboAnalyst 5.0*. The PCA procedure is unsupervised and is both a data reduction procedure and a way to study interrelationships within a complex data set, such

as the data set used in this study. It can tell us which variables are the most important for clustering the data, and here it is first used to visualize the grouping of the samples due to the sample treatment, i.e. temperature. The temperatures will in this section be referred to as low (30°C), medium (60°C) and high (80°C) from here on. In terms of data reduction, PCA was used to find the factors (i.e. volatile compounds) which had the greatest influence on the differences observed between the sample treatments.

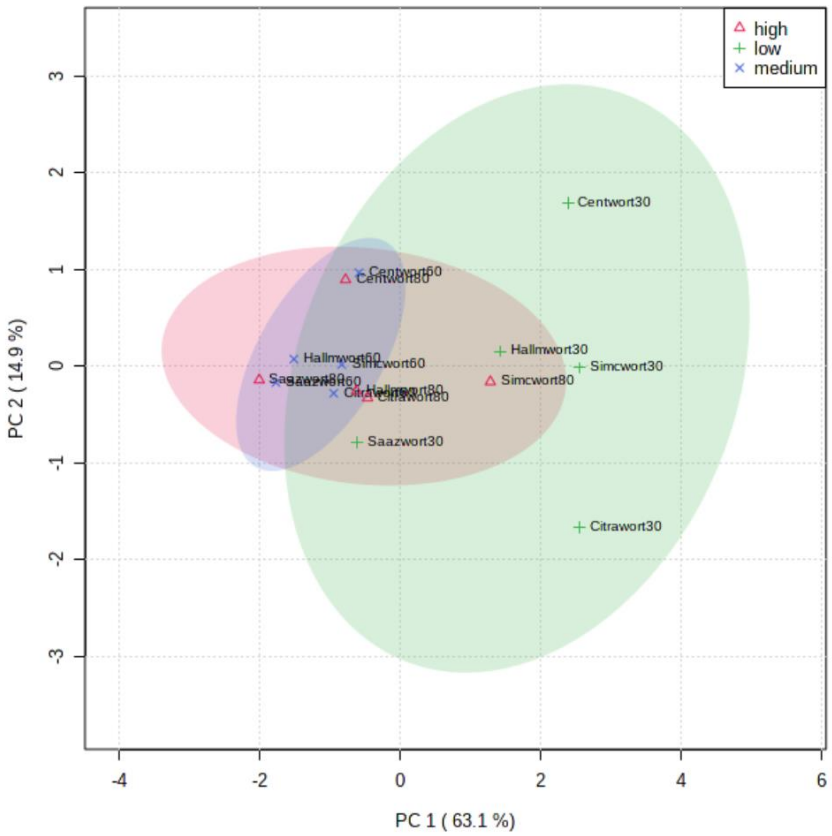


Figure 4.5: Principle component analysis (PC1 and PC2) 2D score plot from pareto scaled peak area data from all five hop varieties in wort at 30°C (low), 60°C (medium) and 80°C (high). PC1 and PC2 account for 78% of the total variation. Colored circles represent 95 % confidence intervals.

The purpose of Figure 4.5 is to visualize the differences between the hop varieties and sample treatments with respect to the first two principal components, principal component 1 (PC1) and principal component 2 (PC2). The variation explained by PC1 accounts for 63.1%, while the variation explained by PC2 accounts for 14.9%. The color shaded area in each cluster is the 95% confidence region. PC1 is anchored in the positive direction by low temperatures and in the negative direction medium and high temperatures, indicating that the variation associated with PC1 is most likely explained by differences due to temperature. In addition to stretching horizontally, the groups also stretch vertically. Thus, PC1 does not seem to explain the variation between the data points within the three groups. This variation can rather be explained by PC2, but one cannot know for sure what PC2 explains. Though, it is likely that the variation explained by PC2 can be related to hop variety, and also potentially to technical errors in the sample preparation method.

As can be seen from the figure, the groups are not clearly separated and there are some clear separation spaces. Still, there is some degree of clustering, with the medium temperature-samples clustering the most. It seems for most of the low temperature-samples that the horizontal variation between them, i.e. variation due to temperature, is not too big, especially for Centennial, Simcoe and Citra. Saaz, on the other hand, stands out. For the high temperature-samples it can also be observed some variation, mostly horizontal, and for the medium temperature-samples, the clustering is even tighter. Though, for all three temperatures, the Centennial samples are sticking out vertically, indicating that there is something else than temperature that makes these samples vary from the rest.

So, what we can tell from Figure 4.5 is essentially that differences between the hop varieties are more apparent at lower temperatures. At medium temperatures, on the other hand, the samples cluster tightly together, indicating that these differences are not that apparent. From this, it is reasonable to assume that when doing hop additions at lower temperatures, it is more important to make wise decisions when picking out the hops you want to use than it is with medium and high temperatures. Further, it is of interest to see which compounds cause the most of this variation. To do this, a biplot can be used (Figure 4.6).

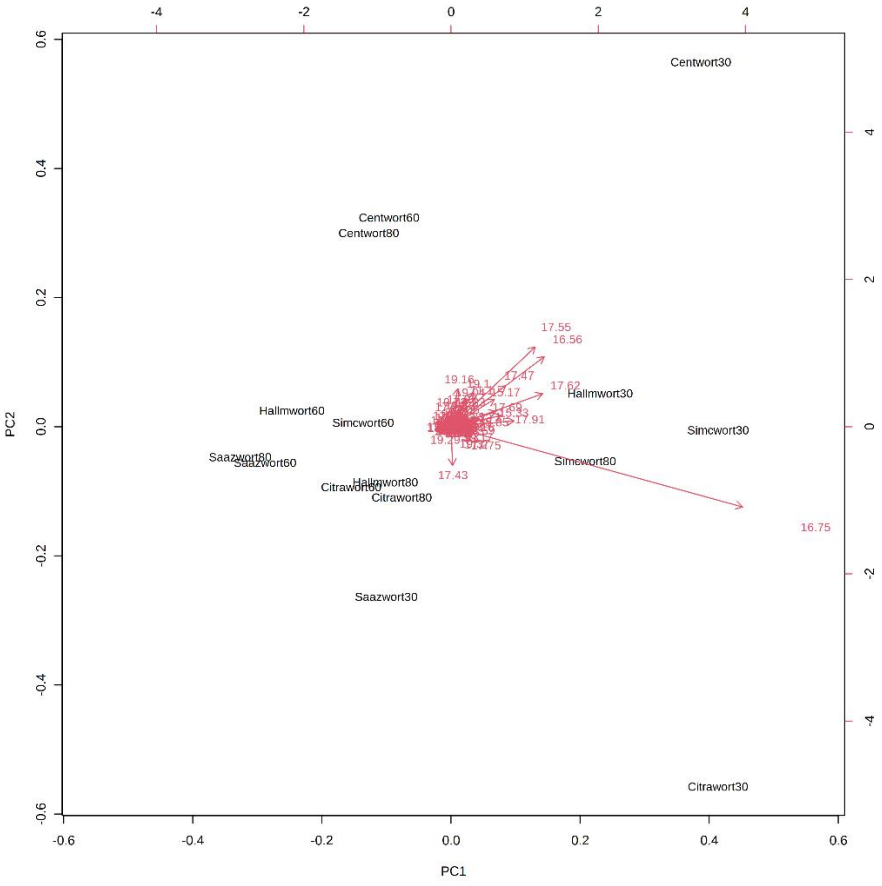


Figure 4.6: Biplot showing how strongly each compound (here their respective retention time is shown) influences the principal components, based on pareto scaled peak area data from all five hop varieties in wort at 30°C (low), 60°C (medium) and 80°C (high).

The biplot links the score plot of the wort samples and the loading plot of the retention times of the identified compounds. It shows how strongly each characteristic influences a principal component, so in this case, it can be used to figure out which compounds influences PC1 the most, i.e. the variation observed due to temperature.

In Figure 4.6, the vectors are pinned at the origin of the PCs (PC1 = 0 and PC2 = 0) and their project values on each PC shows how much weight they have on that PC. The loadings range from -1 to 1, with loadings close to -1 or 1 indicating that the variable strongly influences the component (54). For these data, it is clear that the compound that elutes at 16.75 (i.e. has a retention time of 16.75) strongly influences PC1, whereas the compounds that elute at 17.55 and 16.56 strongly influence PC2. These compounds are β–myrcene, isoamyl isobutyrate and β-pinene, respectively. It is not too easy to see many of the retention times from the biplot itself, so a table of loadings, arranged so that it shows the 22 variables that influences PC1 the most, can be found in Appendix F: Data Analysis with MetaboAnalyst 5.0 Table 4.2 shows the 10 first and most important compounds for differences related to temperature.

Table 4.2: Table of loadings for the 10 compounds with the highest loadings for PC1 when comparing wort samples for 30°C, 60°C and 80°C

Compound	RT	Loading
β–myrcene	16.75	0.804
β-pinene	16.56	0.257
D-limonene	17.62	0.253
Isoamyl isobutyrate	17.55	0.232
α-pinene	17.91	0.174
Propanoic acid, 2-methyl, 3-methylbutyl ester	17.47	0.151
Unidentified	15.33	0.138
3-carene	17.69	0.124
2-methylbutyl isobutyrate	15.17	0.120
Camphene	15.85	0.094

The focus here is put on PC1, since it as mentioned describes the greatest amount of variation in the data set (63.1 %), and these compounds may therefore be the most important for assessing differences between the temperatures. From Figure 4.6 and Table 4.2, we now know that these 10 compounds influence PC1 the most. This means that these compounds explain most of the variation between the samples at different temperatures.

As mentioned, the closer the loading is to -1 or 1, the stronger the influence is on the component. Since β–myrcene has such a large influence (0.804), it is interesting to see how

the peak area values of the compound, i.e. the amount that has evaporated, varies between the temperatures. This is illustrated in Figure 4.7.

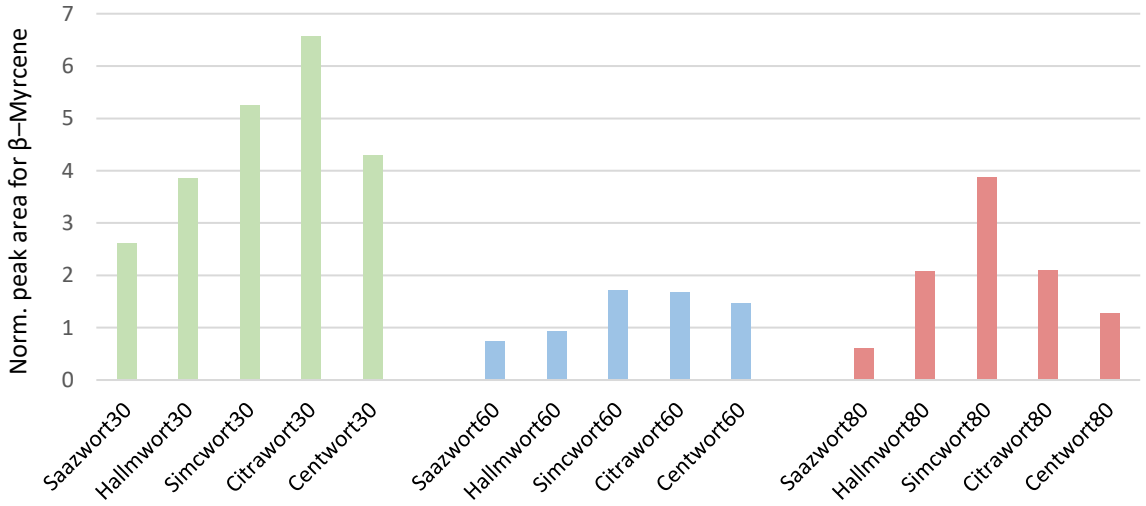


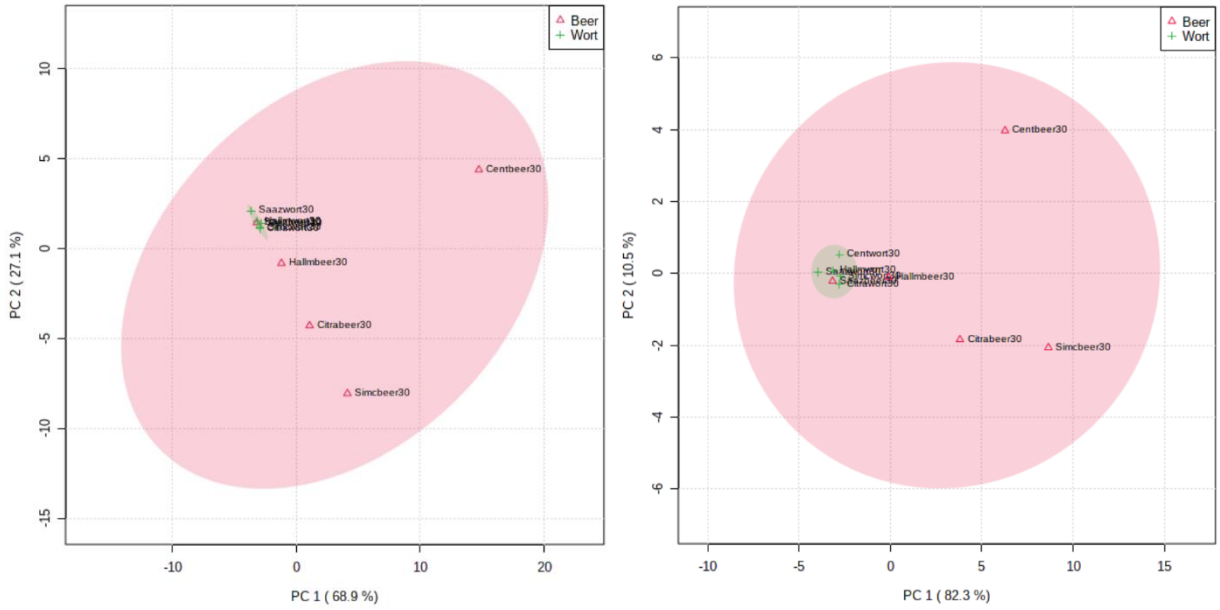
Figure 4.7: Bar graphs illustrating the amount of evaporated β–myrcene for all five hop varieties in wort at three temperatures (30°C, 60°C and 80°C). Sample names on the x-axis indicate hop variety, solvent state and temperature.

From this figure, it can be seen that for all five hop varieties in wort, it is actually at lower temperatures that the most β–myrcene evaporates. It can also be seen that for Saaz, it is generally not much that evaporates compared to the other varieties. Variation between the temperatures is also not too substantial for Saaz, which makes sense given the placement we saw of the Saaz samples in the score plot in Figure 4.5. The placement of Simcwort30, Citrawort30 and Centwort30 in the same plot also makes sense, as we in the above figure can see that for these three hop varieties, the variation in evaporation of β–myrcene between the temperatures is larger.

It can further be seen that it is in fact at medium temperatures that the general level of evaporation of β–myrcene is the smallest, while at high temperatures it is a bit higher. Still, it is not as much as at low temperatures. All 97 compounds were not taken a closer look at, but from these results, it is fair to assume that it might be a trend that more of the volatile compounds evaporate at lower and higher temperatures than at medium temperatures.

4.4 Differences in retainment of hop volatile compounds in wort versus beer

This last section of the results chapter will focus on the part of the aim that was to explore differences in retained hop volatile compounds in wort versus beer. To do this, the wort and beer samples (all tested at 30°C) for all hop varieties were used. In order to analyze these differences further, PCA was used again (Figure 4.8). In terms of data reduction, PCA was used to find the factors (i.e. volatile compounds) which had the greatest influence on the difference observed between the two solvent states. Information about scaling of the data and relevant code can be found in Appendix F: Data Analysis with MetaboAnalyst



5.0

Figure 4.8: Principle component analysis (PC1 and PC2) 2D score plot of pareto scaled peak area data from all five hop varieties in wort and beer at 30°C (left). Right shows the results when the peak area values of ethyl acetate were removed. PC1 and PC2 account for 92.8% of the total variation. Colored circles represent 95% c onfidence intervals.

The purpose of Figure 4.8 (right) is to visualize and compare the two solvent states with respect to the first two principal components, PC1 and PC2. As can be seen in Figure 4.8, two 2D score plots are included. The left plot contains all the peak area data with blanks excluded, while the right plot has is made form the same data, but with the peak area values for ethyl acetate (RT 5.97) removed, as the values were abnormally high for centennial and most likely due to an error rather than an actual difference. By removing ethyl acetate from the PCA, one can better understand the differences among the groups. The focus will be on the right plot from here..

Variation explained by PC1 accounts for 82.3%, while the variation explained by PC2 accounts for 10.5%. PC1 is anchored in the positive direction by three of the beer samples

and in the negative direction by a cluster of wort samples, indicating that the variation associated with PC1 is most likely explained by differences due to solvent. In addition to stretching horizontally, the beer data points also stretch vertically. Thus, PC1 does not seem to explain all of the variation between the beer data points. This variation can be explained by PC2, but it is not sure exactly what PC2 explains. Though, it can be assumed that the variation explained by PC2 is related to hop variety or errors in the sample preparation.

As can be seen from the figure, the groups are not clearly separated. The wort data points are clustered tightly together, but both the Saaz and Hallertau Mittelfrüh beer samples are grouped together with them. From earlier, we know that these two varieties are expected to be more similar. Standing out from the rest is the Citra, Simcoe and Centennial samples, with the latter standing out the most. All three spread along PC1, indicating that something is different with regards to volatile compounds when these hops are in beer rather than in wort. Centennial was also clearly vertically separated from the rest, indicating that not only is there a difference when it is in wort compared to beer, but there might be something with that specific hop variety that is unique from the rest. Generally, it seems from the results that at 30°C, the differences between the hop varieties with regards to volatile components and their retainment is not that large when added in wort, whereas in beer, the differences are clearly larger, especially for some hop varieties. To further assess which compounds are the cause of most of the observed variation, the associated biplot was taken a further look at (Figure 4.9)

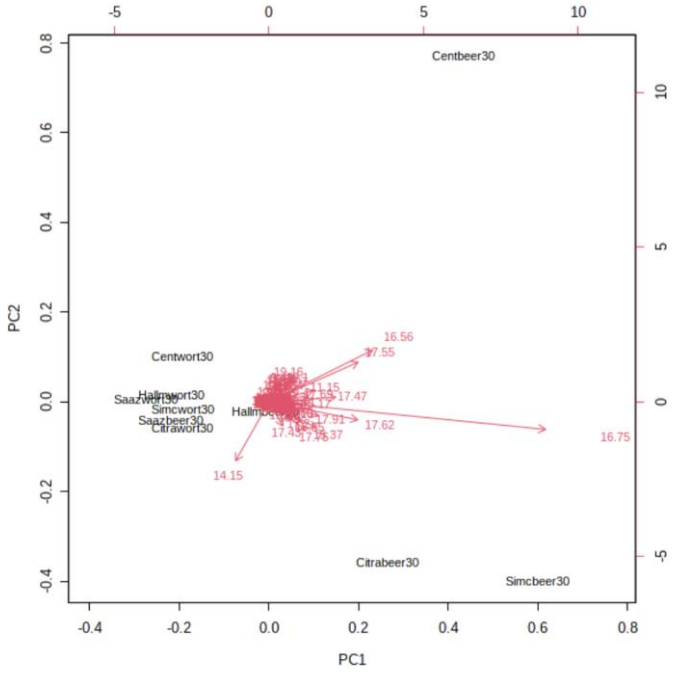


Figure 4.9: Biplot for PC1 and PC2 showing how strongly each compound (here their respective retention time is shown) influences the principal components., based of pareto scaled peak area data from all five hop varieties in wort and beer at 30 °C with ethyl acetate removed.

Since PC1 describes the greatest amount of variation in the data set, here 82.3%, it is interesting to look more into which compounds are the cause of this variation, as they are important for assessing differences between the solvent states. It is further interesting to look more into why Centennial, Citra and Simcoe stand out so much horizontally, as this is to a large extent will be due to these differences.

Most of the contributions in the loadings plot are not readily visible in the figure. Therefore, a table of loadings, arranged so that it shows the 22 variables that influence PC1 the most, can be found Appendix F: Data Analysis with MetaboAnalyst 5.0. Table 4.3 shows the 10 first compounds with the highest loadings; Hence, they are the most important compounds in assessing the cause of the observed the variation.

Table 4.3: Table of loadings for the 10 compounds with the highest loadings for PC1 when comparing wort and beer samples

Compound	RT	Loading
β–myrcene	16.75	0.775
β-pinene	16.56	0.290
Isoamyl isobutyrate	17.55	0.250
D-limonene	17.62	0.250
Propanoic acid, 2-methyl, 3-methylbutyl ester	17.47	0.187
α-pinene	17.91	0.138
Unidentified	15.37	0.131
Isoamyl alcohol	11.15	0.124
3-carene	17.69	0.110
2-methylbutyl isobutyrate	15.17	0.105

Now that we know which compounds influence PC1 the most, with β–myrcene again clearly having the largest influence, it is of interest to look more into what causes the observed variation. From the figures above, it seems that the difference is that volatile compounds are less retained in one or the other solvent. To look closer at these differences, bar graphs were made to compare percentage increase in evaporation of the 9 compounds with the highest loadings for PC1 from wort to beer (Figure 4.10).

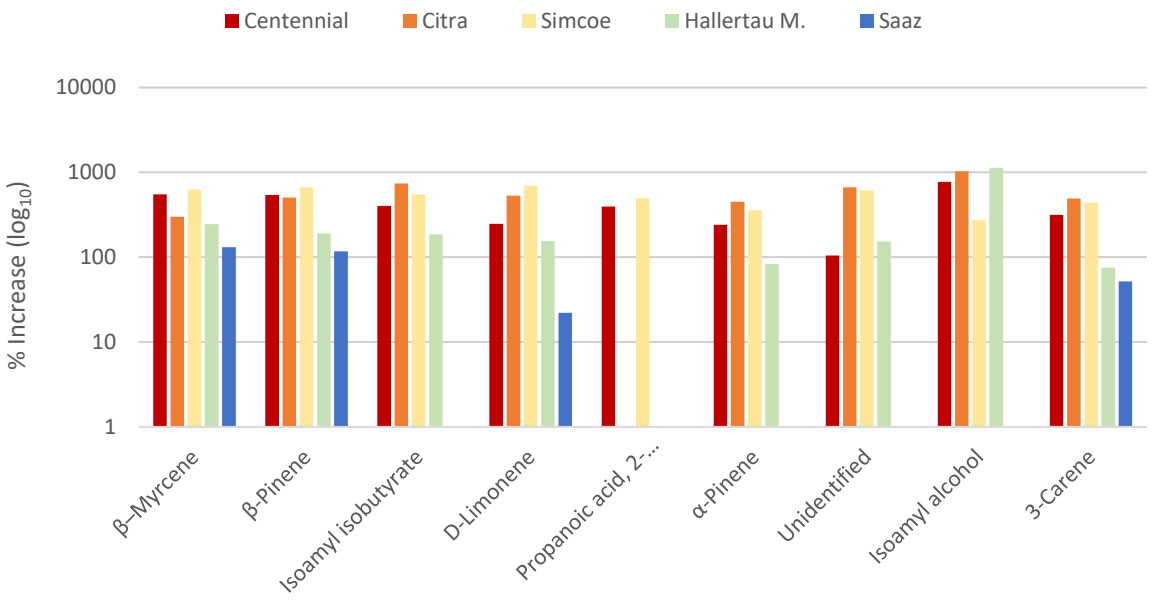


Figure 4.10: Bar graphs illustrating percentage increase in evaporation of 9 selected volatile compounds from wort to beer at 30°C for all five hop varieties.

From Figure 4.10, it can be clearly seen that there is a difference in retainment of volatile compounds in wort compared to beer. Generally, seen from these 9 volatile compounds that influence PC1 the most, there is a large increase in evaporation of them in beer compared to in wort. This makes it reasonable to assume that volatile compounds are not that well retained in beer, at least not when compared to in wort.

Not seen from this figure is that there was propanoic acid, 2-methyl, 3-methylbutyl ester present in the wort and beer samples from Hallertau Mittelfrüh, but peaks in that area were poorly separated and the peak area could therefore not be measured. Still, more evaporated in the beer sample here as well than the wort sample. There was also isoamyl alcohol in the Saaz beer sample, but this was also not measurable. Other places where there is no bars present indicate that the compound was not identified in the samples. In addition, α-pinene was present in Saaz, but it was the same amount that evaporated for both the wort and beer samples, hence there is no visible bar in the figure.

As seen previously in Figure 4.8, the wort samples are clustered tightly together, indicating that the variation in how volatile compounds are retained in wort is not too large between the hop varieties. In beer, however, the observed variation is larger, especially for Centennial, Citra and Simcoe. It seems that when in beer, differences in retainment of volatile compounds are larger between hop species. The fact that these differences are more apparent in the beer samples is most likely connected to the fact that differences will be more apparent when more of the volatile compounds evaporate. Centennial, Citra and

Simcoe all have high total oil contents (2.0, 2.3 and 2.0 ml/100 g, respectively), and in section 4.2 it was seen that these varieties generally had a larger amount some selected volatile compounds.

Overall, for the Saaz hop variety, the differences in retainment of volatile compounds in beer and wort are not too big compared to the other hop varieties. This is probably connected to the fact that the Saaz variety has the lowest average total oil content (0.7 ml/100 g). For Hallertau Mittelfrüh, the differences are not too big either, except from isoamyl alcohol. It therefore makes sense that these two hop varieties in beer are clustered with the wort samples in Figure 4.8. For both Simcoe and Citra, the difference between volatile compounds evaporated in wort compared to beer are generally a lot larger for most of the selected compounds, explaining their horizontal variation in the 2D score plot. Lastly, Centennial also has quite a difference in volatile compounds evaporated in wort versus in beer, explaining the horizontal variation observed for this variety as well. What causes the vertical variation, explained by PC2, is not clear.

5 Discussion

In this chapter, the results of the present study will be discussed in the light of the aims of the study, as well as in the light of previous studies. This study had two main aims: First, it was of interest to gain further insight into how different temperatures affect how well hop volatile compounds are retained in wort, as well as to explore differences in retainment of compounds between hop samples in wort and beer. Second, the study aimed to highlight the importance of hop addition timing in the brewing process with regards to retainment of volatile compounds. The study also sought to reveal analytical differences linked to hop varieties.

When preprocessing the data, pareto scaling was used. As mentioned in earlier chapters, pareto scaling keeps the data structure partially intact, and the relative importance of smaller quantities is increased. However, large fold changes may still show a dominating effect, and this must be taken into account. It is known that β-myrcene is the most abundant volatile compound in hop essential oil (14, 55). Therefore, when pareto scaling the data, one should be aware of that variations in β-myrcene will highly influence the results. For the purpose of this study, it was expected that this would still work well to highlight differences. Though, to make sure that the observed differences actually were trends and not just due to the chosen scaling method, auto scaling of the same data was also performed prior to the PCA. Figures corresponding to the ones in the results part, but from auto scaled data, can be found in Appendix G. Throughout this discussion, the pareto scaled results will be compared to these.

To look into the first aim and to gain further insight into how different temperatures affect the retainment of hop volatile compounds in wort, PCA was first used to make a 2D score plot to visualize that there in fact was differences between the sample treatments and hop varieties with respect to the first two principal components, PC1 and PC2. From Figure 4.5, which showed the score plot from peak area data from all five hop varieties in wort at 30°C (low), 60°C (medium) and 80°C (high), it was clear that there were differences in retainment of hop volatile compounds in wort at different temperatures.

By using the associated biplot and table of loadings to look further into which compounds contributed to most of this variation, it came as no surprise that β–myrcene had the biggest influence. It should be noted that β–myrcene had a quite extreme loading value compared to the other compounds when comparing the wort samples. β–myrcene had a loading of 0.804, whereas β-pinene, that was next on the list, had a loading of 0.257. This caused the comparisons to be largely influenced by variations in β–myrcene. To see if the observations remained the same if β–myrcene was less dominant, a look was taken at the PCA for the auto scaled data as well (Fig. G.1-G.3).

When the data was auto scaled, PC1 only accounted for 26.4%. On the other hand, when the data was pareto scaled, it accounted for 63.1%. High PC1 values often occur when one of the samples are a lot more dominant, so this proves again the dominance of β–myrcene when pareto scaling, and that when auto scaling, no compounds where as dominant. This can also be seen from the table of loadings for the auto scaled data of the PCA of the wort samples (Fig. G.3). Here, β–myrcene is the 18th most influential compound. The most influential compound is δ-cadinene, which was a compound with generally small peak areas. It had a loading of 0.201, with the next on the list being caryophyllene with a loading of 0.185. The gaps in the table of loadings is a lot smaller for the auto scaled data, which makes sense since there is no dominant features.

Even though the most influential compounds has changed, it is largely the same observations that can be made from the score plot of auto scaled data (Fig. G.1). Samples at medium temperatures still cluster together the most, and low temperatures spread more. Also, the Centennial samples still spread vertically from the rest. One difference is that with auto scaling, high temperatures cluster less than with pareto scaling, mostly due to the simcwort80 sample spreading a lot more vertically and horizontally. Generally, though, the trends were the same for both the pareto scaled data and the auto scaled data. It might be that the simcwort80 sample stands out more with the auto scaled data as Simcoe has a high total oil content, and likely contain higher levels of these more of these "smaller" compounds that have a bigger influence when auto scaled. Their evaporation will most likely vary more between the temperatures, hence highlighting the variation more than pareto scaling did.

By using data collected for β–myrcene to look into what these differences were, it was clear that they were due to reduced retainment of volatile compounds at lower temperatures (Figure 4.7). It was also clear that there were variations between the hop varieties, but generally, there was more evaporation for lower and higher temperatures, whereas more was retained at medium temperatures. Saaz was the hop variety with the least amount of variation, and in general, the least variation between the hop varieties was observed at medium temperatures. At higher temperatures, the variation was somewhere in between, but not as much as the observed variation for low temperatures. These results suggest that it is more critical to think of what hop variety you choose when doing hop additions at lower temperatures, at least in wort, as variation between the varieties is more is more apparent.

With β-myrcene being such an influential compound when the data is pareto scaled, one might then think that β-myrcene is also important for the overall finish of the beer; however, this is not the case. Linalool and β-myrcene are considered the most aroma-active volatiles in all analyzed hop varieties, but β-myrcene usually does not actually make a contribution to the hop aroma in beer (56). This is due to the fact that it evaporates readily

during the wort boiling, which leaves its concentration often far below the sensory threshold level (8). Hence, it works well for visualizing differences, as was the purpose of this study, but it is not possible to say much about the effects on the flavor and aroma of the beer from these results. If that was the purpose, it would have been even more important to handle the data in a way so that the contribution from β-myrcene was not as large – in other words, auto scale it. Generally, when data was pareto scaled, many of the same compounds did turn out to be important for differences both due to temperature, as well as when it came to differences between wort and beer, but this turned out to be mostly due to the scaling. For the auto scaled data, it could be seen from the table of loadings that different compounds were influential in each of the cases.

Then, if β-myrcene readily evaporates during the boil, would it not make sense to dry-hop if you want to maintain higher levels of β-myrcene? From the results in this study, it does not seem like that. In Figure 4.10, which showed the percentage increase in evaporation of 9 selected volatile compounds, including β-myrcene, from wort to beer at 30 °C for all of the five hop varieties, it was seen an increase in evaporation in beer compared to wort. The reason behind this is unclear, as it would be expected that non-polar compounds (e.g. β-pinene, β-myrcene and α-pinene) would be better retained in beer. As mentioned earlier, non-polar compounds will solubilize better in a high-alcohol environment (23). They do take longer time to solubilize in beer, so it may be that they did not get enough time to solubilize that well and be retained, or that the alcohol level was not high enough for sufficient solubilization of the non-polar compounds. Generally, from the results obtained in this study, it seems that hop volatile compounds are in fact better retained in wort than in beer and that hop additions should rather be done in wort if you want the volatile compounds to stay.

Again, to make sure that these observations were not due to the choice of scaling method, but due to actual trends, the results from wort to beer at 30 °C were compared with the same analyses done on auto scaled data (Fig. G.4-G.6). From the score plot, it can be seen that the trends are exactly the same as for the pareto scaled data. The wort samples cluster tightly, while the beer samples are more spread. Again, here it is the beer samples for Simcoe, Citra and Centennial that stand out. PC1 only accounts for 46.4% here though, which is almost half of what it did for the pareto scaled data. This again shows the dominance of β–myrcene when pareto scaling, and that when auto scaling, no compounds were as dominant. When further looking at the table of loadings for the auto scaled data (Fig. G.6), it can be seen that the loadings are pretty similar and there are no big gaps. Here, β-myrcene is the seventh most influential compound. Even with other compounds being more influential, the trends are still the same and not only due to scaling.

The study also sought to reveal analytical differences linked to hop varieties. Throughout the results shown in the previous chapter, it is clear that there were apparent differences between the hop varieties. Citra, Centennial and Simcoe tended to have similarities, as well as Saaz and Hallertau Mittelfrüh. This observation makes sense when thinking back to their total oil contents, which was 2.3, 2.0, 2.0, 0.7 and 1.0 ml/100 g, respectively. Another variation that was observed was in the retention of compounds between wort and beer. As seen in Figure 4.10, which shows the percentage increase in evaporation of volatile compounds from wort to beer, the "citrusy" hop varieties with higher total oil content tended to have a larger percentage increase in evaporation of volatile compounds in beer, than the "spicy" hop varieties with lower total oil content did. This may indicate that when using hop varieties with more citrusy characteristics and a higher total oil content, not much more ends up staying anyway.

Lastly, the findings in this study are put together to highlight the importance of hop addition timing in the brewing process with regards to retainment of volatile compounds. Given the findings, it seems that it is better to add your hops to wort than to beer, as more is generally retained, and that the retainment of volatile compounds is the best when the wort is at medium temperatures, i.e. around 60 °C. From the results, it also seems that if you want the variations between your hop varieties to be less apparent, they should be added at higher rather than lower temperatures. The results also indicate that when using hop varieties with more citrusy characteristics and higher total oil content, not much more ends up staying anyway compared to from hop varieties with a lower total oil content. However, this needs to be looked more into.

What is important to note about the results from this study is that they are not really applicable when compared to what is observed in the "real" brewing process. It does not really make sense to say that it is better to do the hop additions in wort than in beer, because the hop additions done to wort will be affected by the fermentation process in the brewing, which is not taken into account in this experiment. Previous studies have for example found that linalool increases during fermentation, and they have also shown that samples where the concentrations of linalool become lower, still score higher scores in aroma intensity (57). A possible explanation for this is the synergism between fermentation by-products and the hop aroma compounds, which is also important to consider. Studies have also shown that linalool is the only compound that has shown a direct contribution to the overall aroma, because final concentrations of most other compounds do not exceed their odor threshold concentrations (16). Another aspect this study did not focus on is the importance of compounds present at trace levels. Some compounds have thresholds so low that only small changes in the amount of them can lead to large changes in how the final beer is perceived. So, the fermentation process is critical to include in similar experiments, and much focus should be put on linalool and the odor thresholds.

This study did not take into account annual variation in hop oil composition either. It only compared different hop varieties, but it could also have been interesting to use the same hop varieties from different harvests, as annual variation in hop oil composition can lead to a different hop aroma from one year to another, which again will lead to changes in product quality (57).

6 Conclusion and outlook

In this study, the essential oils of five hop varieties were analyzed by HS GC-MS with the purpose to unravel differences in how the volatile compounds of the essential oil were retained in different solvent states, as well as at different temperatures. By doing this, the plan was to further highlight the importance of hop addition timing in the brewing process with regards to the retainment of these compounds, as well as to reveal analytical differences linked to the hop varieties. To ensure that the findings from the study were not only due the chosen scaling method, two scaling methods were used. The same trends were observed with both scaling methods.

The results indicate that if one wants the volatile compounds to be retained better, hop addition should be done in wort rather than in beer, and rather at temperatures around 60°C than at lower temperatures. The results also indicate that when using hop varieties with a higher total oil content, larger amounts of volatile compounds will evaporate than from hop varieties with a lower total oil content, and their flavor and aroma contribution may not be that different after all.

Due to the complexity of hop volatile compounds, and since the results from the PCA is only meant to guide continued investigation, it should be noted that no general conclusions could be drawn from the results in this study. The study is rather meant to highlight this complexity with regards to differences in how hop volatile compounds are retained in wort and beer, and also to show how temperature plays an important role in this complexity. This study is only one of many along the road to a better understanding of the complexity of hops and their volatile compounds, which again is only a part of the complexity of the entire brewing process. The road towards a complete understanding of hops and their role in the brewing process is still long due to the physical, biochemical and chemical changes that occur during brewing and fermentation.

For further work on the results from this study, liquid samples were made from the vials post-run and stored in a freezer. They can further be analyzed by nuclear magnetic resonance (NMR) to reveal what remained in the samples. For example, it is common that most of the β-myrcene evaporates, so it could be interesting to see how much stayed in the different samples. Also, it would be interesting to use the same hop varieties in the brewing process and take samples throughout it to see how these results would vary from the results in this study, as this study was stripped from many of the other factors that would be present in a "real" brewing process. This study did not look into how the volatile compounds persisted throughout the process either, which would also be interesting.

The data obtained from this study can also be used for numerous other analyses. One can for example choose to do analyses within the hop varieties themselves, or choose some of the varieties to compare. It is also a possibility to use the wet samples for some kind of comparison. It would also be interesting to see how different beer styles would affect the retainment of volatile compounds.

One thing that should be mentioned again is sensory thresholds and how important they have been shown to consider in previous studies. Now, it is still impossible to fully analyze the samples as they are without employing sensory techniques. It is therefore of high interest to relate the identification of chemical markers in hops to aroma changes in beer. The markers could then be related to certain sensory changes, and this could provide data for analysts to build statistical models that connect raw ingredient profiles to hops and could due to process developments track changes in beer.

To sum things up, this study reached its aims to gain further insight into how different temperatures affect how well hop volatile compounds are retained in wort, as well as to explore differences in retainment of compounds between hop samples in wort and beer. The study also revealed analytical differences linked to hop varieties, and it was attempted to highlight the importance of hop addition However, this experiment did not include all the factors that are included in the “real” brewing process, and it was therefore not possible to draw any conclusions that were of use for brewers.

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Appendix

Appendix A: Sample overview

Appendix B: IPA recipe

Appendix C: Headspace and GC paramters

Appendix D: MS parameters

Appendix E: Peak area values

Appendix F: Data Analysis with MetaboAnalyst 5.0

Appendix G: Results with auto scaling

Appendix A: Sample overview

1.0 g of ground hop pellets were weighed into headspace glass vials (20 ml). Samples were either left dry, or 10 ml of wort, beer or distilled water was added according to what sample it was. 30 µL 100x diluted 4-methyl-2-pentanol ($C_6H_{14}O$) was added as an internal standard to all samples, including the blanks. A total of 47 samples were made. For the Saaz variety, triplicates (x3) were made for each sample.

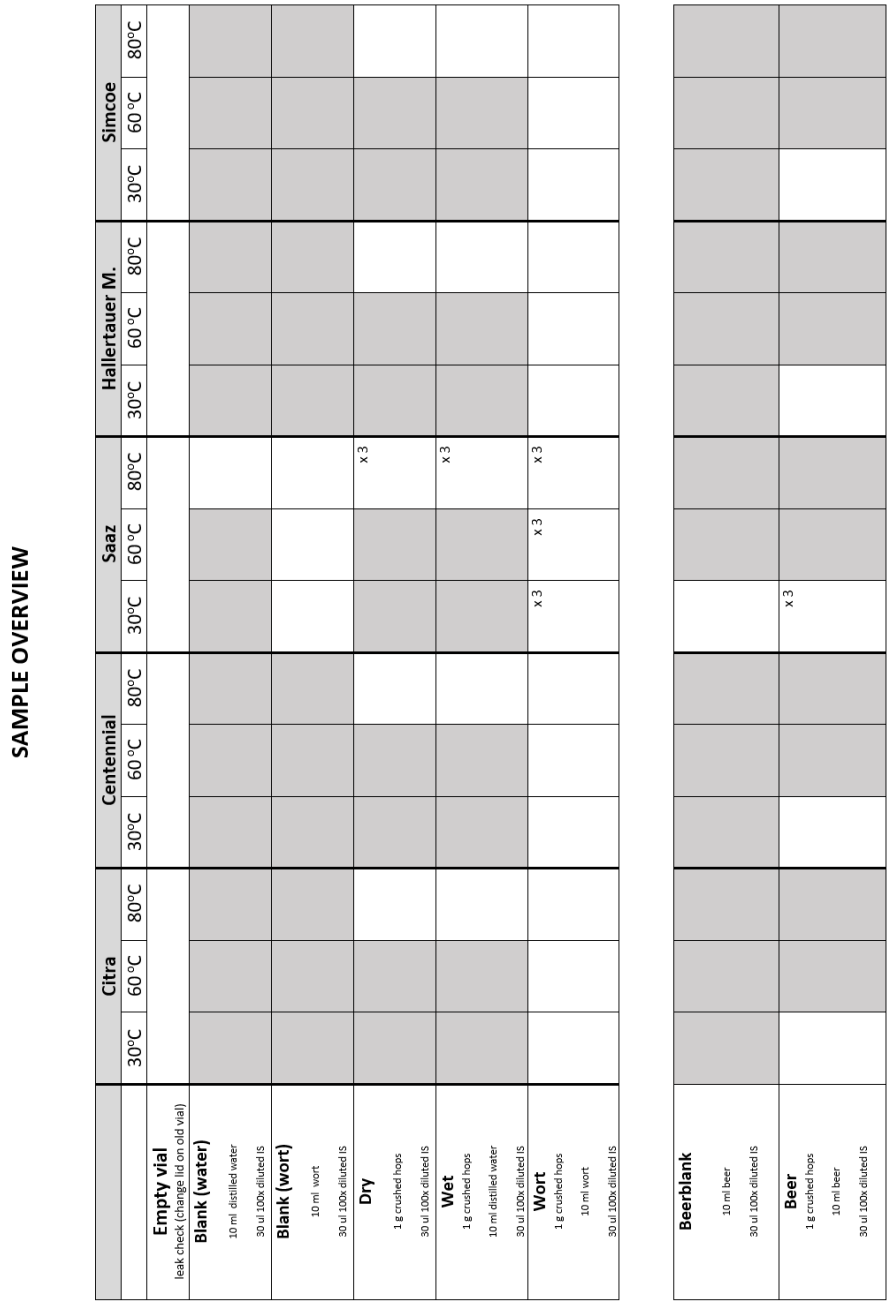


Figure A.1: Sample overview. The first column shows what the vials in each following row contains. White cells indicate that samples were made, grey cells indicate the opposite. "x3" indicates samples triplicates were made for.

Appendix B: IPA recipe

For all the samples that had beer as a solvent, the following IPA recipe was used to prepare this beer.

IPA Schnipa

American IPA (14 B)

Type: All Grain
Batch Size: 46,00 l
Boil Size: 54,92 l
Boil Time: 60 min
End of Boil Vol: 49,92 l
Final Bottling Vol: 45,00 l
Fermentation: Ale, Two Stage
Taste Notes:

Date: 21 Jan 2021
Brewer:
Asst Brewer:
Equipment: Braumeister 50L
Efficiency: 72,00 %
Est Mash Efficiency: 75,1 %
Taste Rating: 30,0

Ingredients

Amt	Name	Type	#	%/IBU
9,00 kg	Pale Malt (2 Row) UK (5,9 EBC)	Grain	1	66,7 %
3,00 kg	White Wheat Malt (4,7 EBC)	Grain	2	22,2 %
1,50 kg	Some light cara shit (19,7 EBC)	Grain	3	11,1 %
30,00 g	Chinook [13,00 %] - Boil 60,0 min	Hop	4	19,4 IBUs
30,00 g	Idaho 7 [12,40 %] - Boil 10,0 min	Hop	5	6,7 IBUs
30,00 g	Mosaic [12,70 %] - Boil 10,0 min	Hop	6	6,9 IBUs
30,00 g	Yellow Sub [7,20 %] - Boil 10,0 min	Hop	7	3,9 IBUs
80,00 g	Idaho 7 [12,40 %] - Steep/Whirlpool 15,0 min	Hop	8	12,2 IBUs
80,00 g	Mosaic [12,70 %] - Steep/Whirlpool 15,0 min	Hop	9	12,5 IBUs
80,00 g	Yellow Sub [7,20 %] - Steep/Whirlpool 15,0 min	Hop	10	7,1 IBUs
60,00 g	Idaho 7 [12,40 %] - Dry Hop 7,0 Days	Hop	11	0,0 IBUs
60,00 g	Mosaic [12,70 %] - Dry Hop 7,0 Days	Hop	12	0,0 IBUs
60,00 g	Yellow Sub [7,20 %] - Dry Hop 7,0 Days	Hop	13	0,0 IBUs
60,00 g	Idaho 7 [12,40 %] - Dry Hop 3,0 Days	Hop	14	0,0 IBUs
60,00 g	Mosaic [12,70 %] - Dry Hop 3,0 Days	Hop	15	0,0 IBUs
60,00 g	Yellow Sub [7,20 %] - Dry Hop 3,0 Days	Hop	16	0,0 IBUs

Gravity, Alcohol Content and Color

Est Original Gravity: 1,064 SG
Est Final Gravity: 1,017 SG
Estimated Alcohol by Vol: 6,3 %
Bitterness: 68,8 IBUs
Est Color: 12,8 EBC

Measured Original Gravity: 1,057 SG
Measured Final Gravity: 1,010 SG
Actual Alcohol by Vol: 6,2 %
Calories: 533,3 kcal/l

Mash Profile

Mash Name: Single Infusion, Medium Body
Sparge Water: 18,47 l
Sparge Temperature: 75,6 C
Adjust Temp for Equipment: TRUE

Total Grain Weight: 13,50 kg
Grain Temperature: 22,2 C
Tun Temperature: 22,2 C
Mash PH: 5,20

Mash Steps

Name	Description	Step Temperature	Step Time
Mash In	Add 32,54 l of water at 79,7 C	67,0 C	60 min
Mash Out	Add 17,94 l of water at 95,8 C	75,6 C	10 min

Sparge: Fly sparge with 18,47 l water at 75,6 C
Mash Notes: Simple single infusion mash for use with most modern well modified grains (about 95% of the time).

Carbonation and Storage

Carbonation Type: Bottle
Pressure/Weight: 264,69 g
Keg/Bottling Temperature: 21,1 C
Fermentation: Ale, Two Stage

Volumes of CO2: 2,3
Carbonation Used: Bottle with 264,69 g Corn Sugar
Age for: 30,00 days
Storage Temperature: 18,3 C

Figure B.1: IPA recipe used to brew the beer that was used as the solvent for all the samples in beer.

Appendix C: Headspace and GC parameters

For the headspace sampling, three methods were made with the only parameter varying being the platen/sample temp (Figure C.1). Following the headspace parameters are screenshots of the GC parameters used (Figure C.2, C.3, C.4 and C5).

Variable	Value
Valve Oven Temp.	100°C
Transfer Line Temp.	100°C
Standby Flow Rate	25 mL/min.
Trap Standby Temp.	30°C
Trap Sweep Temp.	0°C
Platen/Sample Temp.	30°C
Sample Preheat Time	20.00 min.
Preheat Mixer	Off
Preheat Mixing Level	Level 5
Preheat Mixing Time	2.00 min.
Preheat Mixer Stabilize Time	0.50 min.
Sweep Flow Rate	50 mL/min.
Sweep Flow Time	5.00 min.
Dry Purge Time	2.00 min.
Dry Purge Flow	50 mL/min.
Dry Purge Temp.	25°C
Desorb Preheat	240°C
Desorb Temp.	245°C
Desorb Time	2.00 min.
Trap Bake Temp.	260°C
Trap Bake Time	4.00 min.
Trap Bake Flow	450 mL/min.

Variable	Value
Valve Oven Temp.	100°C
Transfer Line Temp.	100°C
Standby Flow Rate	25 mL/min.
Trap Standby Temp.	30°C
Trap Sweep Temp.	0°C
Platen/Sample Temp.	60°C
Sample Preheat Time	20.00 min.
Preheat Mixer	Off
Preheat Mixing Level	Level 5
Preheat Mixing Time	2.00 min.
Preheat Mixer Stabilize Time	0.50 min.
Sweep Flow Rate	50 mL/min.
Sweep Flow Time	5.00 min.
Dry Purge Time	2.00 min.
Dry Purge Flow	50 mL/min.
Dry Purge Temp.	25°C
Desorb Preheat	240°C
Desorb Temp.	245°C
Desorb Time	2.00 min.
Trap Bake Temp.	260°C
Trap Bake Time	4.00 min.
Trap Bake Flow	450 mL/min.

Variable	Value
Valve Oven Temp.	100°C
Transfer Line Temp.	100°C
Standby Flow Rate	25 mL/min.
Trap Standby Temp.	30°C
Trap Sweep Temp.	0°C
Platen/Sample Temp.	80°C
Sample Preheat Time	20.00 min.
Preheat Mixer	Off
Preheat Mixing Level	Level 5
Preheat Mixing Time	2.00 min.
Preheat Mixer Stabilize Time	0.50 min.
Sweep Flow Rate	50 mL/min.
Sweep Flow Time	5.00 min.
Dry Purge Time	2.00 min.
Dry Purge Flow	50 mL/min.
Dry Purge Temp.	25°C
Desorb Preheat	240°C
Desorb Temp.	245°C
Desorb Time	2.00 min.
Trap Bake Temp.	260°C
Trap Bake Time	4.00 min.
Trap Bake Flow	450 mL/min.

Figure C.1: The three methods used for the headspace sampling. All parameters remained the same except from the platen/sample temp., which was set to 30 °C, 60° C and 80 °C, respectively.

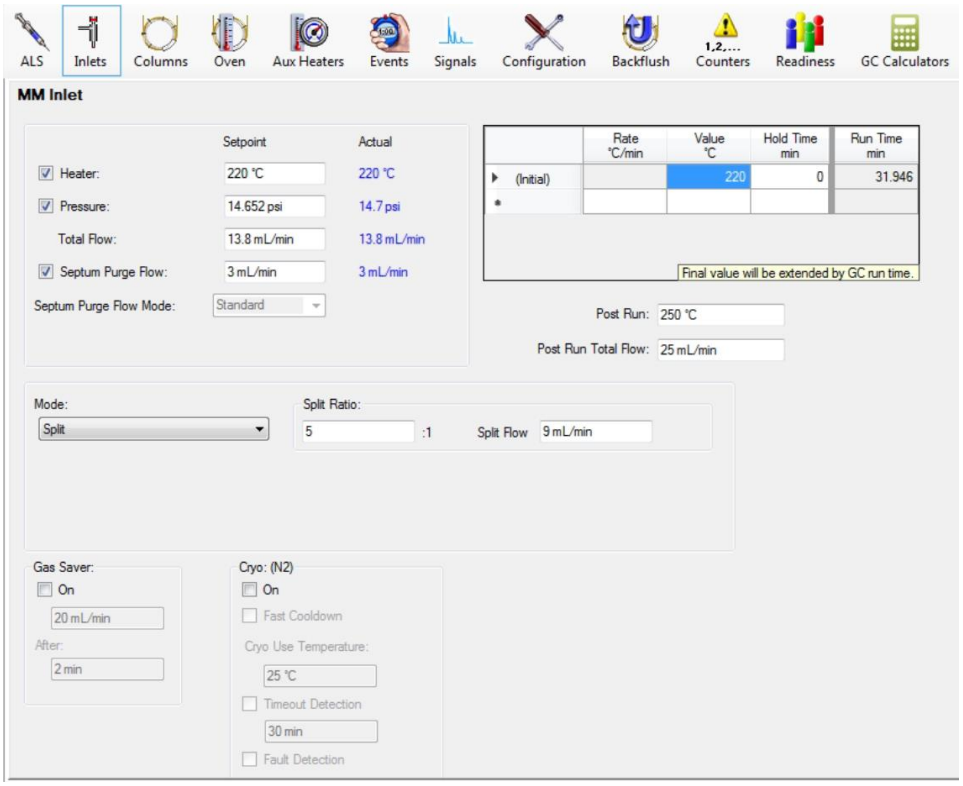


Figure C.2: GC inlet parameters

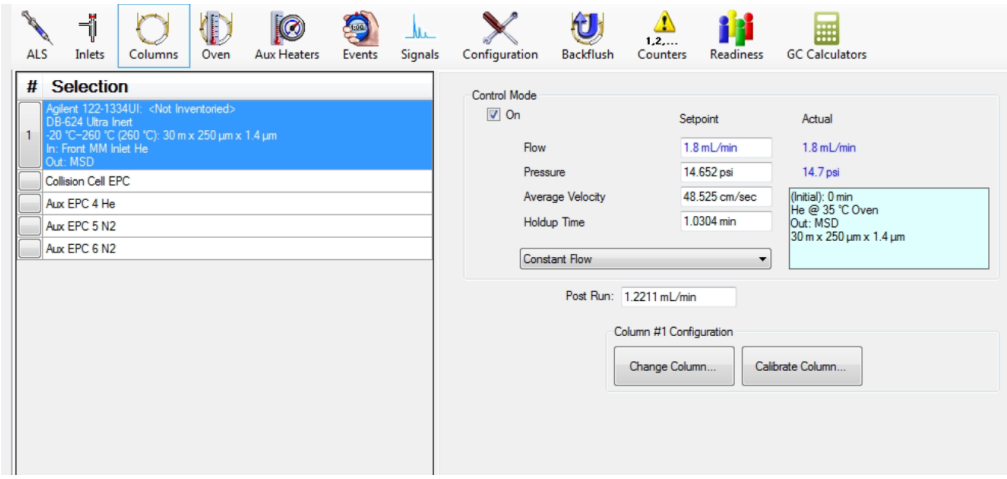


Figure C.3: GC column parameters

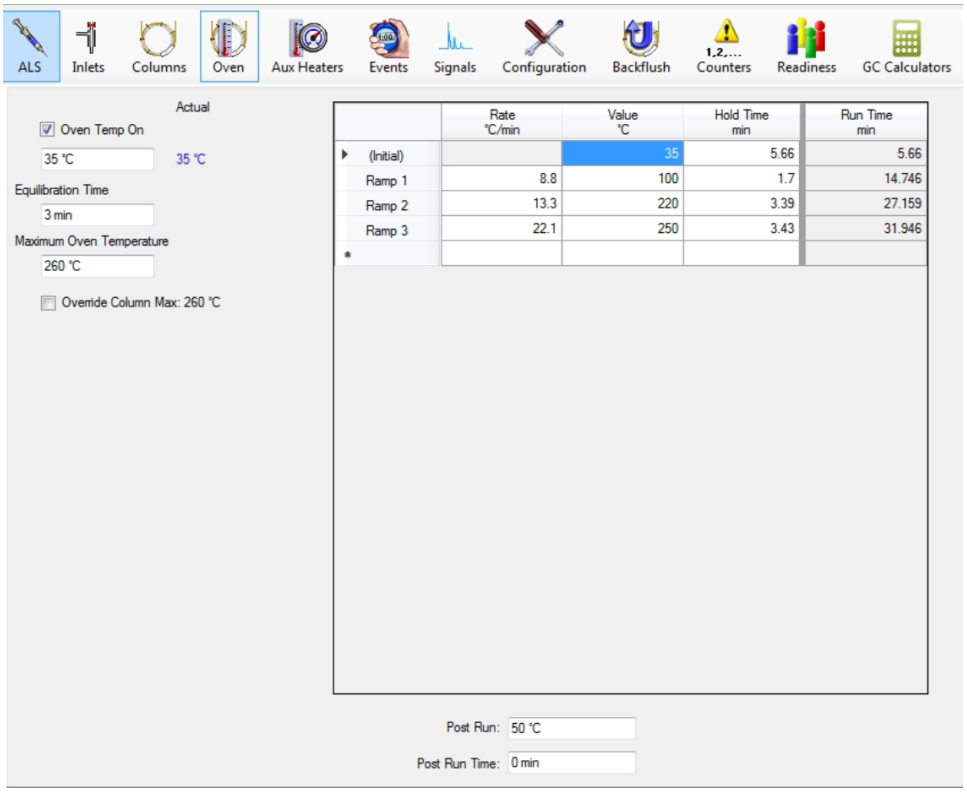


Figure C.4: GC oven parameters

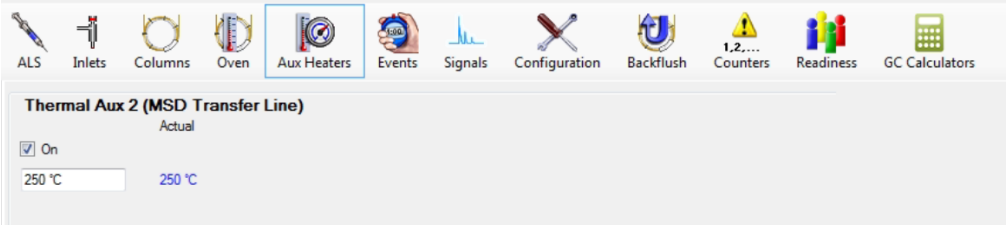


Figure C.5: GC Aux heater parameters

Appendix D: MS conditions

The parameters used for the MS are included in this appendix. Figure D.1 shows a screenshot of them.

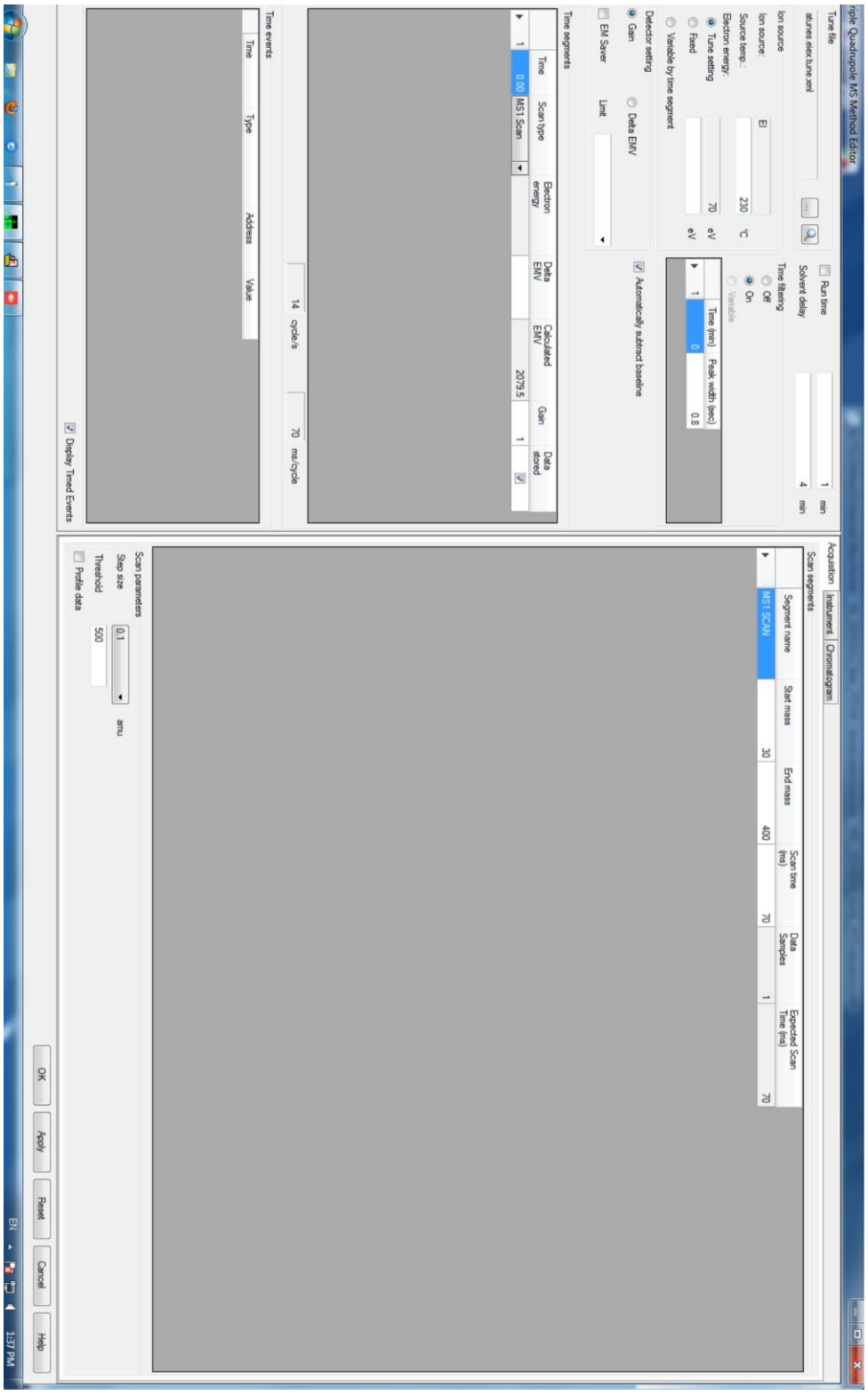


Figure D.1: MS conditions used for all samples

Appendix E: Peak area values

This appendix contains all the data used in the analyzes of this thesis. The first column for each sample represents the area measured for each volatile compounds respective peak. The second column shows the data normalized to the peak area of the internal standard of each respective sample with the normalized blanks subtracted from them. Blank spaces indicate places there were peaks, but where the "integrate chromatogram" function did not work. The second column of retention times is the same as the first column.

Saaz and blanks

		Samples for 30 degrees celsius								Samples for 60 degrees celsius				Samples for 80 degrees celsius									
		Saazwort 30 - 1		Saazbeer30 - 1		Blankbeer30		Blankwort30		Saazwort 60 - 1		Blankwort60		Saazwort 80 - 1		Saazwort80 - 1		Blankwort80		Blankwater80		Saaadry80 - 1	
	RT (min)	Area	Area [norm]	Area	Area [norm]	Area	Area [norm]	Area	Area [norm]	Area	Area [norm]	Area	Area [norm]	Area	Area [norm]	Area	Area [norm]	Area	Area [norm]	Area	Area [norm]	RT	Area	Area [norm]
Ethyl acetate	5.37	0	0	5.3E+07	-7.57556	6.5E+07	15.3977	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5.37	0	0
Acetic acid	9.20	0	0	0	0	0	0	0	0	29294	0.00052	0	0	0	0	0	0	0	0	0	0	9.20	0	0
Ethyl propionate	3.42	0	0	402945	-0.09588	625353	0.14887	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3.42	0	0
1,1-diethoxyethane	3.73	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3.73	0	0
Acetic acid	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	0	0
Dimethyl disulfide	10.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10.4	3654383	0.00636
Methyl isobutyl ketone	10.72	37937.3	0.00343	158798	0.02088	0	0	0	0	501833	0.00895	0	0	45640	0.00063	672282	0.00673	0	0	0	0	10.72	1.7E+07	0.0328
Isoamyl alcohol	11.15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11.15	0	0
3-methyl-2-pentanone	*11.65*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	636849	0.00637	0	0	0	0	*11.65*	4448076	0.00847
IS (4-methyl-2-pentanol)	11.66	2.8E+07	1	7604885	1	4204715	1	2.3E+07	1	5.6E+07	1	8791179	1	6.6E+07	1	1E+08	1	2.2E+07	1	1.3E+06	1	11.66	5.3E+08	1
2-hexanol (acikkur)	11.83	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11.83	0	0
Ethyl butanoate	12.01	0	0	462066	-0.21037	1140036	0.27113	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12.01	0	0
Hexanal	12.33	0	0	0	0	0	0	0	0	1171952	0.02083	0	0	0	0	6646225	0.06651	0	0	0	0	12.33	1.3E+07	0.02473
2-pentanol	12.54	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12.54	7057902	0.01344
Butanoic acid, 2-methyl-, ethyl ester	13.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13.3		
Ethyl isovalerate	13.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13.4		
Isobutyl propinoate	13.8	0	-0.00378	0	0	0	0	110668	0.00378	1931043	-0.00116	312732	0.03558	363362	-0.73986	0	0	1.7E+07	0.74544	0	0	13.8	715982	0.00136
Isoamyl acetate	14.15	0	0	1828732	-2.36456	1.1E+07	2.60502	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14.15	0	0
Isobutyl isobutyrate	14.55	0	0	0	0	0	0	0	0	385545	0.00687	0	0	274356	0.00415	527261	0.00528	0	0	0	0	14.55	4308120	0.0082
2-methylbutyl isobutyrate	15.17	152126	0.00542	0	0	0	0	0	0	311571	0.00555	0	0	201173	0.00304	613106	0.00614	0	0	0	0	15.17	1.6E+07	0.03064
Alpha-pinene	15.37	36553.2	0.0013	0	0	0	0	0	0	129800	0.00231	0	0	0	0	181990	0.00182	0	0	0	0	15.37	4073324	0.00777
Camphene	15.85	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15.85	371888	0.00071
Beta-pinene	16.56	1508618	0.05374	887192	0.11666	0	0	0	0	1748021	0.03116	0	0	1053577	0.01593	5433243	0.05437	0	0	0	0	16.56	8.1E+07	0.15364
Beta-myrcene	16.75	7.3E+07	2.60763	4.3E+07	6.0481	1505539	0.35807	214924	0.00734	4.3E+07	0.73011	307362	0.03436	4.2E+07	0.61142	1.4E+08	1.44553	470118	0.02113	0	0	16.75	6.6E+08	1.26562
Oximone	*17.07*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	*17.07*		
Benzaldehyde	*17.1*					0	0	0	0			163009	0.01922	1375246	-0.00143	1434631	0.01436	494350	0.02222	0	0	*17.1*	0	0
alpha-phellandrene	17.2			0	0	0	0	0	0	2838027	0.05059	0	0	0	0	0	0	0	0	0	0	17.2	7004561	0.01334
Ethyl hexanoate	17.21	0	0	0	-0.72018	3028160	0.72018	0	0	0	0	0	0	0	0	0	0	0	0	0	0	17.21		
1-octen-3-ol	17.23	0	0	0	0	0	0	0	0	0	0	0	0	2185905	0.03304	3188878	0.03191	0	0	0	0	17.23	0	0
6-methyl-5-hepten-2-one	17.35	1727891	0.06155	0	0	0	0	0	0	2372721	0.04229	0	0	3191667	0.04825	4866475	0.0487	0	0	0	0	17.35	5829006	0.0111
a-Terpinene	17.43	3043982	0.10864	1217630	0.16012	0	0	0	0	3145170	0.05606	0	0	1216408	0.01842	3363050	0.03372	0	0	0	0	17.43	1.4E+07	0.02719
Propanoic acid, 2-methyl, 3-methylbutyl ester	17.47	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	17.47	0	0
Isoamyl isobutyrate	17.55	581158	0	618555	-0.13269	1152220	0.27403	660837	0.02256	966861	0.01723	0	0	630428	0.01044	2516935	0.02519	0	0	0	0	17.55	1.2E+07	0.02343
Limonene	17.62	2605481	0.08364	1211726	0.16311	2086640	0.49362	86752	0.00296	3088883	0.05506	0	0	1120273	0.01634	5511062	0.05521	0	0	0	0	17.62	4E+07	0.07653
3-carene	17.69	2377612	0.08314	1207253	0.12635	136240	0.0324	45398.7	0.00155	3639836	0.06595	0	0	916244	0.01388	4662011	0.04665	0	0	0	0	17.69	1.7E+07	0.03151
Beta-phellandrene	17.75	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	17.75	7339432	0.01409
Alpha-pinene	17.91	3331508	0.11867	961769	0.11813	35041.2	0.00833	0	0	4435902	0.07887	11117.6	0.00126	1980738	0.02394	6745342	0.0675	0	0	0	0	17.91	2.3E+07	0.05592
Gamma-terpinene	18.16	703687	0.02473	413720	0.05125	13251.5	0.00315	9755.39	0.00033	1083536	0.01918	1160.88	0.00013	216520	0.00327	1445715	0.01447	0	0	0	0	18.16	3316478	0.00746
Methyl 2,4-dimethylhexanoate	18.38	102349	0.00367	42403.4	0.00558	0	0	0	0	211775	0.00377	0	0	63658.2	0.00096	526460	0.00527	0	0	0	0	18.38	1341385	0.00255
Geraniol?	18.49	81496.4	0.0029	7333.97	0.00097	0	0	0	0	487396	0.00869	0	0	703463	0.01063	952590	0.00953	0	0	0	0	18.49	491368	0.00094
Unidentified	18.62	35373.6	0.00128	37000	0.00487	0	0	0	0	297044	0.00529	0	0	110357	0.00167	522624	0.00523	0	0	0	0	18.62	733623	0.0014
alpha-terpinene	18.69	1139002	0.04271	567327	0.07088	15913.3	0.0038			2117754	0.03775	0	0	1238002	0.01872	0	0	0	0	0	0	18.69	6484639	0.01235
Benzeneacetaldehyde	*18.74*	0	0					0	0		-0.00929	81676.5	0.00929	2356120	-0.01717	0	0	2389491	0.10739	0	0	*18.74*	0	0
Octanoic acid, methyl ester	18.82	163296	0.00603	77386.1	0.01018	0	0	0	0	403721	0.0072	0	0	0	0	698261	0.00699	0	0	0	0	18.82	743461	0.00142
Cis linalool oxide	18.9	12237.6	0.00044	0	0	0	0	0	0	126672	0.00226	0	0	815315	0.01233	1043401	0.01044	0	0	0	0	18.9	224955	0.00043
Heptanoic acid, ethyl ester (ethyl heptanoate)	18.97	0	0	0	0	0	0	0	0	82188.7	0.00146	0	0	123366	0.00187	280333	0.00281	0	0	0	0	18.97	0	0
Perillene	19.04	105842	0.00377	76205.6	0.01002	0	0	0	0	239171	0.00426	0	0	201860	0.00305	923684	0.0093	0	0	0	0	19.04	2933951	0.0056
Butanoic acid, 2-methyl-, 2-methylbutyl ester	19.1	0	0	0	0	0	0	0	0	0	0	0	0	243548	0.00368	660364	0.00664	0	0	0	0	19.1	210316	0.0004
n-Amyl isovalerate	19.16	835188	0.02966	43284.5	0.00469	4195.35	0.001	2603.81	8.9E-05	1579579	0.02577	20927.5	0.00238	1966280	0.02972	3878090	0.03881	0	0	0	0	19.16	1187656	0.00226
2-nonanone	19.17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	19.17	0	0
Linalool	19.29	843360	0.03006	44825.7	0.00327	11055.1	0.00263	0	0	3383794	0.05237	70828.3	0.00806	1.5E+07	0.19652	2E+07	0.19638	747009	0.03357	0	0	19.29	356203	0.00182
Nonanal	19.33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	19.33	1122533	0.00214
Myrtenol	19.37	1003222	0.03552	389427	0.04354	7009.8	0.00167	12339.6	0.00042	1552458	0.02767	0	0	0	0	2931775	0.02934	0	0	0	0	19.37	1443054	0.00275
Methyl octanoate	19.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	19.4	0	0
Cosmene	19.54	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	19.54	0	0
Allo-ocimene	19.61	1543787	0.0545	606683	0.07714	11101.2	0.00264	14454.8	0.00049	2224459	0.03945	1721	0.0002	421858	0.00573	2213795	0.02215	14354	0.00065	0	0	19.61	1450663	0.00276
Unidentified	19.89	0	0	0	0	0	0	0	0	0	0	0	0									19.89		
Phenylethyl alcohol	20.01	0	0	26458.6	-0.01001	56737.7	0.01349	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20.01	0	0
Cis-p-mentha-2,8-dien-1-ol	20.26	0	0	0	0	0	0	0	0	0	0	0	0	331934	0.00502	726040	0.00727	0	0	0	0	20.26	0	0
Ethyl octanoate	20.4	0	0	42288	-0.01086	69056.3	0.01642	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20.4	0	0
2-decanone	20.62	143910	0.00534	5347.26	0.00077	0	0	0	0	347515	0.00381	20920.6	0.00238	839059	0.00347	1254274	0.01255	317815	0.00412	0	0	20.62	187169	0.00036
3-nonenoic acid, methyl ester	20.68	250007	0.00831	0	0	0	0	0	0	31572.4	0.00163	0	0	363536	0.00398	645682	0.00646	33620	0.00151	0	0	20.68	40049.7	7.6E-05
Unidentified	20.74	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20.74	0	0
Nonanoic acid, methyl ester	20.77	14718.8	0.00052	0	0	0	0	0	0	80144.2	0.00143	0	0	396076	0.00599	727331	0.00728	0	0	0	0	20.77	21954.2	4.2E-05
2-undecanone	21.43									0				0								21.43	35038.3	6.7E-05
Nerol acetate	21.47	0	0	0	0	0	0	0	0	5760.4	0.0001	0	0	369007	0.00558	801141	0.00802	0	0			21.47	0	0
Unidentified	21.56	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	21.56	0	0
Unidentified	21.71	0	0	0	0	0	0	0	0	0	0	0	0	115543	0.00044	266422	0.00267	29141.1	0.00131	0	0	21.71	65505.8	0.00012
methyl dec-4-enoate	21.85	0	0	0	0	0	0	0	0	63361	0.00113	0	0	243310	0.00377	1008297	0.01009	0	0	0	0	21.85	345025	0.00066
Unidentified	21.92	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	21.92	0	0
Methyl decanoate (decanoic acid, methyl ester)	21.98	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	21.98	0	0
Methyl geranate	22.06	51458.1	0.00183	33613.9	0.00521	0	0	0	0	429617	0.00766	0	0	241566	0.00365	1328440	0.01329	0	0	0	0	22.06	7284.5	1.4E-05
gamma-himachalene	22.18	21516	0.00077	19294	0.00254	0	0	0	0	256535	0.00457	0	0	107261	0.00162	610386	0.00611	0	0	0	0	22.18	0	0
δ-Cadinol	22.32	233805	0.00854	107329	0.01322	3746.59	0.00089	0	0	1167510	0.02007	6486.48	0.00074	376088	0.00325	1939786	0.02001	54277.2	0.00244	0	0	22.32	29003	5.5E-05
δ-Guaiene	22.6	80107.3	0.00285	14391.4	0.00197	0	0	0	0	479347	0.00855	0	0	125602	0.0019	285401	0.00286	0	0	0	0	22.6	0	0
Ylangene	22.63	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	22.63	36666.1	7E-05
Copaene	22.68	78782.1	0.00281	0	0	0	0	0	0	403606	0.00719	0	0	143822	0.00226	711873	0.00712	0	0	0	0	22.68	205760	0.00039
2-dodecanone	22.98	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	22.98	0	0
Unidentified	23.22	1927.9	6.9E-05	0	0	0	0	0	0	50465.6	0.0009	0	0	20748	0.00031	181352	0.00181	0	0	0	0	23.22	583920	0.00112
Caryophyllene	23.33	127650	0.00455	133158	0.01652	4163.59	0.00099	0	0	436582	0.00601	15609	0.00178	764289	0.00591	3086437	0.03089	125535	0.00564	0	0	23.33	4416343	0.00841
Alpha-humulene	23.71	223392	0.00798	231084	0.02751	12095	0.00288	0	0	0	-0.00154	13501.6	0.00154	56515	0.00306	4231549	0.04235	122003	0.00548	0	0	23.71	1.6E+07	0.03071
γ-Cadinene	23.76	0	0	0	0	0	0	0	0	21083.3	0.00038	0	0	115320	0.00174	1171099	0.01171	0	0	0	0	23.76	435639	0.00083
Alpha-farnesene	23.83	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	23.83	111139	0.00021
Unidentified	23.92	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	23.92	0	0
alpa-muurolene	23.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	23.99	212066	0.0004
β-Selinene	24.04	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	24.04	126660	0.00024
Alpha-Selinene	24.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	24.1	74206.3	0.00014
δ-Cadinene	24.2	7192.32	0.00026	1488.2	0.0002	0	0	0	0	34405.2	0.00061	0	0	0	0	132740	0.00133					24.2	717940	0.00137
Unidentified	24.31	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	24.31		
Unidentified	24.37	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	24.37		
Unidentified	24.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	24.4		
alfa-cadinene	24.44	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	24.44	0	0
Unidentified	24.53	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	24.53	0	0
Unidentified	24.61	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	24.61		
Alfa-calacorene	24.69	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	24.69	0	0
Sanalol	25.63	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	25.63	0	0
Caryophyllene oxide	26.09	0	0	0	0	0	0	0	0	0	0	0	0	68032.4	0.00103	392591	0.00393	0	0	0	0	26.09	0	0
Unidentified	26.44	0	0	0	0	0	0	0	0	0	0	0	0	13811	0.00202	514048	0.00514	0	0	0	0	26.44	0	0
Unidentified	26.56	26852.7	0.00096	25424	-0.00435	34859	0.00823	0	0	37470.6	-0.00475	57064.2	0.00649	179184	-0.00231	0	0	111614	0.00502	0	0	26.56	100965	0.00019

Figure E.1: Peak areas for all 97 compounds, as well as the internal standard, for all Saaz samples and all blanks. The first column for each sample represents the area measured for each volatile compounds respective peak. The second column shows the data normalized to the peak area of the internal standard of each respective sample with the normalized blanks subtracted from them. Blank spaces indicate places there were peaks, but where the "integrate chromatogram" function did not work. The second column of retention times is the same as the first column.

Hallertau Mittelfrüh

		30 degrees celsius				55 degrees celsius		80 degrees celsius					
		Hallmwort30		Hallmbeer30		Hallmwort60		Hallmwort80		Hallmwet80		Hallmdry80	
	RT	Area	Area [no	Area	Area [no	Area	Area [no	Area	Area [no	Area	Area [no	RT	Area
Ethyl acetate	5.37	0	0	1.2E+08	-10.4838	0	0	0	0	0	0	5.37	0
Acetic acid	9.20	217365	0.00254	0	0	1038708	0.00391	0	0	0	0	9.20	12324334.4
Ethyl propionate	9.42	0	0	2152785	-0.06002	0	0	0	0	0	0	9.42	0
1,1-diethoxyethane	9.73	0	0	869372	0.03588	0	0	0	0	0	0	9.73	0
Acetic acid	10	0	0	0	0	0	0	0	0	0	0	10	0
Dimethyl disulfide	10.4	819330	0.00957	1038094	0.04284	4003579	0.01511	810024	0.00744	2386644	0.02136	10.4	2.2E+07
Methyl isobutyl ketone	10.72	1057049	0.01235	661184	0.02729	5364396	0.02255	858532	0.00788	1620371	0.0145	10.72	1.8E+07
Isoamyl alcohol	11.15	3303166	0.03859	1.2E+07	0.47747	2.1E+07	0.07895	1374126	0.01261	4243304	0.03799	11.15	
3-methyl-2-pentanone	*11.16*	0	0	0	0	0	0	0	0	0	0	*11.16*	
IS (4-methyl-2-pentanol)	11.66	8.6E+07	1	2.4E+07	1	2.7E+08	1	1.1E+08	1	1.1E+08	1	11.66	5.1E+08
2-hexanol (usikker)	11.83	0	0	0	0	0	0					11.83	0
Ethyl butanoate	12.01	0	0	2075799	-0.18546	0	0	0	0	0	0	12.01	0
Hexanal	12.33	1511250	0.01766	1364671	0.05632	5537919	0.02087					12.33	8120885
2-pentenal	12.54	1849350	0.02161	0	0	6392264	0.02408	1.2E+07	0.10573	9265700	0.08294	12.54	3913047
Butanoic acid, 2-methyl-, ethyl ester	13.3	0	0	0	0	0	0	0	0	0	0	13.3	0
Ethyl isovalerate	13.4	0	0	0	0	0	0	0	0	0	0	13.4	0
Isobutyl propinoate	13.8	1433134	0.01304	1331159	0.05494	1952851	-0.02822	5644381	-0.63363	476015	0.00426	13.8	1.6E+07
Isoamyl acetate	14.15	0	0	4513730	-2.41873	0	0	0	0	0	0	14.15	0
Isobutyl isobutyrate	14.55	0	0	1095069	0.0452	0	0	0	0	0	0	14.55	0
2-methylbutyl isobutyrate	15.17	2.6E+07	0.23932	2.4E+07	0.97834	2.5E+07	0.09268	1.2E+07	0.11263	1.8E+07	0.15925	15.17	1.5E+08
Alpha-pinene	15.37	5513133	0.06448	3966384	0.1637	2333380	0.00879	2146861	0.01971	4132540	0.03639	15.37	3.9E+07
Camphene	15.85	3121533	0.03647	1834382	0.07571	631005	0.0026	870101	0.00799	1704662	0.01526	15.85	2.3E+07
Beta-pinene	16.56	4E+07	0.464	3.3E+07	1.35239	1.8E+07	0.06807	1.7E+07	0.15627	3E+07	0.266	16.56	2.8E+08
Beta-myrcene	16.75	3.3E+08	3.84823	3.3E+08	13.3284	2.5E+08	0.9223	2.3E+08	2.07134	2.8E+08	2.49991	16.75	7.2E+08
Ocimene	*17.07*											*17.07*	
Benzaldehyde	*17.1*											*17.1*	
alpha-phellandrene	17.2	2423364	0.02831	2413394	0.0936	1.1E+07	0.04024	2416354	0.02218	2154185	0.01928	17.2	6341264
Ethyl hexanoate	17.21	0	0			0	0	0	0	0	0	17.21	0
1-octen-3-ol	17.23											17.23	
6-methyl-5-hepten-2-one	17.35	2211236	0.02584	0	0	3139765	0.01183	1E+07	0.09409	1889709	0.01691	17.35	1520934
α-Terpinene	17.43					0	0			1.1E+07	0.10188	17.43	0
Propanoic acid, 2-methyl, 3-methylbutyl ester	17.47					2.1E+07	0.07917			3133947	0.02811	17.47	1.2E+07
Isoamyl isobutyrate	17.55	4.3E+07	0.55419	4.5E+07	1.5835	3.4E+07	0.12663	2E+07	0.18021	2.2E+07	0.20082	17.55	3.7E+07
Limonene	17.62	3.3E+07	0.37849	2.5E+07	0.96441	1.8E+07	0.06734	1.3E+07	0.11972	1.5E+07	0.1331	17.62	3.4E+07
3-carene	17.69	2.6E+07	0.30433	1.4E+07	0.53538	1.4E+07	0.05376	1.2E+07	0.10837	8504627	0.07612	17.69	0
Beta-phellandrene	17.75											17.75	2E+07
Alpha-pinene	17.31	3.5E+07	0.4141	1.9E+07	0.76202	2.6E+07	0.09824	1.7E+07	0.15189	1.5E+07	0.13067	17.31	1.2E+07
Gamma-terpinene	18.16	6029737	0.07012	3645487	0.1473	2421471	0.00899	3728697	0.03423	4503275	0.04036	18.16	1393784
Methyl 2,4-dimethylhexanoate	18.38	1343103	0.01576	533559	0.02202	464276	0.00175	585311	0.00537	670540	0.006	18.38	550530
Geraniol?	18.43	1321726	0.01544	491802	0.0203	705780	0.00266	1063668	0.00976	813965	0.00729	18.43	38135
Unidentified	18.62	1144432	0.01337	487928	0.02014	266425	0.001	894342	0.00821	1005757	0.009	18.62	0
alpha-terpinene	18.69	9347281	0.11622	5900336	0.23912	4522360	0.01704	7044950	0.06467	6356215	0.05689	18.69	730933
Benzeneacetaldehyde	*18.74*											*18.74*	
Octanoic acid, methyl ester	18.82	3000040	0.03505	1194709	0.04331	1620007	0.0061	2091418	0.0132	2064131	0.01848	18.82	876666
Cis linalool oxide	18.9	203837	0.00238	0	0	402726	0.00152					18.9	
Heptanoic acid, ethyl ester (ethyl heptanoate)	18.97	0	0	519831	0.02146	1400306	0.00528					18.97	54233.9
Perillene	19.04	2543257	0.02979	1773914	0.07346	1115652	0.0042	866306	0.00795	967543	0.00866	19.04	1127776
Butanoic acid, 2-methyl-, 2-methylbutyl ester	19.1	4253504	0.04977	2115380	0.08733	2314325	0.00872	1336593	0.01227	1394586	0.01248	19.1	784824
n-Amyl isovalerate	19.16			2862366	0.11714	0	-0.00238	0	0	0	0	19.16	0
2-nonanone	19.17	7576553	0.08852	0	0	6167111	0.02324	3676418	0.03375	3688933	0.03302	19.17	2111198
Linalool	19.29	8267665	0.0966	2283552	0.09162	2.2E+07	0.07542	2.5E+07	0.1963	3.1E+07	0.27638	19.29	1.2E+07
Nonanal	19.33	0	0	0	0	0	0	0	0	0	0	19.33	0
Myrtenol	19.37	7704665	0.0896	3260438	0.1329			3136948	0.08387	1.1E+07	0.1007	19.37	1403182
Methyl octanoate	19.4	0	0	0	0	0	0	0	0	0	0	19.4	0
Cosmene	19.54	541813	0.00633	270786	0.01118	467612	0.00176					19.54	0
Allo-ocimene	19.61	7314160	0.08496	2163490	0.08665	1581382	0.00576	6212789	0.05638	6795937	0.06083	19.61	0
Unidentified	19.89	783013	0.00915	286308	0.01182	745447	0.00281	1170797	0.01075	735079	0.00658	19.89	410257
Phenylethyl alcohol	20.01	0	0	481396	0.00637	0	0	0	0	0	0	20.01	0
Cis-p-mentha-2,8-dien-1--ol	20.26	166884	0.00195	296364	0.01223	612034	0.00231	612034	0.00562	2343354	0.0264	20.26	792784
Ethyl octanoate	20.4	126120	0.00147	122594	-0.01136	378624	0.00143	0	0	0	0	20.4	136620
2-decanone	20.62	454406	0.00531	156092	0.00644	319689	-0.00117	468003	0.00017	563218	0.00504	20.62	343573
3-nonanoic acid, methyl ester	20.68	240113	0.00281	176392	0.0073	87852.5	0.00033	0	-0.00151	0	0	20.68	0
Unidentified	20.74	0	0	0	0	0	0	0	0	0	0	20.74	0
Nonanoic acid, methyl ester	20.77	148577	0.00174	124347	0.00516	236071	0.00089	1068868	0.00981	1281116	0.01147	20.77	541289
2-undecanone	21.43		0		0		0					21.43	430158
Nerol acetate	21.47	180387	0.00211	135029	0.00557	162824	0.00061	546398	0.00502	1128331	0.01011	21.47	234561
Unidentified	21.56	0	0	0	0	0	0	0	0	0	0	21.56	196479
Unidentified	21.71	93736.8	0.0011	46714.6	0.00193	92333.3	0.00035		-0.00131			21.71	312045
methyl dec-4-enoate	21.85	470601	0.0055	440287	0.01817	378858	0.00143	456735	0.00419	315903	0.0082	21.85	6028383
Unidentified	21.92	53065.5	0.00062	75090	0.0031	59454	0.00022					21.92	1532738
Methyl decanoate (decanoic acid, methyl ester)	21.98	104666	0.00122	0	0	0	0	0	0	0	0	21.98	
Methyl geranate	22.06	1332712	0.01557	659548	0.02722	243780	0.00092	804306	0.00738	1055255	0.00945	22.06	1430271
gamma-himachalene	22.18	512862	0.00599	121855	0.00503	64752.8	0.00024	408316	0.00375	416321	0.00373	22.18	25113.4
δ-Cadinol	22.32	3080114	0.03599	831952	0.03344	485330	0.00109	1823020	0.01429	1484373	0.01329	22.32	371882
δ-Guaiene	22.6	1170825	0.01368	279502	0.01154	203817	0.00077	429092	0.00394	122103	0.00109	22.6	0
Ylangene	22.63	0	0	0	0	0	0	0	0	0	0	22.63	717306
Copaene	22.68											22.68	2630385
2-dodecanone	22.98	0	0	0	0	0	0	0	0	0	0	22.98	430955
Unidentified	23.22											23.22	
Caryophyllene	23.33	2356703	0.03455	2258931	0.09224	1280306	0.00305	2302903	0.0155	4120705	0.03688	23.33	5.3E+07
Alpha-humulene	23.71	6703054	0.07839	5143029	0.20938	1611563	0.00454	4933618	0.03386	9362536	0.08917	23.71	1.2E+08
γ-Cadinene	23.76	325555	0.0038	206169	0.00851	76143.3	0.00029	243774	0.00229	518924	0.00464	23.76	6331925
Alpha-farnesene	23.83	0	0	0	0	0	0	0	0	0	0	23.83	866674
Unidentified	23.92	0	0	0	0	0	0	0	0	0	0	23.92	850407
alpa-muurolene	23.99											23.99	3973660
β-Selinene	24.04	114388	0.00134	82291.7	0.0034	34021.3	0.00013			370700	0.00332	24.04	3017087
Alpha-Selinene	24.1	102681	0.0012	73593.2	0.00304	24028.3	9.1E-05	0	0			24.1	2826803
δ-Cadinene	24.2	303960	0.00362	216755	0.00895	68468.2	0.00026	203528	0.00187	573091	0.00513	24.2	5421556
Unidentified	24.31	0	0	0	0	0	0	0	0	0	0	24.31	797141
Unidentified	24.37	0	0	0	0	0	0	0	0	0	0	24.37	
Unidentified	24.4											24.4	
alfa-cadinene	24.44	0	0	0	0	0	0	0	0	0	0	24.44	800481
Unidentified	24.53	0	0	0	0	0	0	0	0	0	0	24.53	326769
Unidentified	24.61	0	0	0	0	0	0	0	0	0	0	24.61	303941
Alfa-calacorene	24.69	0	0	0	0	0	0	0	0	0	0	24.69	283204
Sanslol	25.63											25.63	
Caryophyllene oxide	26.09	47106.9	0.00055	0	0	5069.43	1.9E-05	148770	0.00137	348365	0.00312	26.09	273460
Unidentified	26.44	428037	0.005	80233.2	0.00331	88143.3	0.00033	705675	0.00648	767352	0.00687	26.44	89195.6
Unidentified	26.56	868493	0.01015	588332	0.01602	604624	-0.00421	533850	-0.00012	531552	0.00476	26.56	1366613

Figure E.2: Peak areas for all 97 compounds, as well as the internal standard, for all Hallertau Mittelfrüh samples. The first column for each sample represents the area measured for each volatile compounds respective peak. The second column shows the data normalized to the peak area of the internal standard of each respective sample with the normalized blanks subtracted from them. Blank spaces indicate places there were peaks, but where the "integrate chromatogram" function did not work. The second column of retention times is the same as the first column.

Simcoe

	30 degrees celsius				18 degrees celsius		Samples for 80 degrees celsius						
	Simcwort30		Simcbeer30		Simcwort60		Simcwort80		Simcwet80		Simcdry80		
	RT	Area	Area (no	Area	Area (no	Area	Area (no	Area	Area (no	Area	Area (no	RT	Area	Area (corre
Ethyl acetate	5.37	0	0	1.1E+08	-8.00232	0	0	0	0	0	0	5.37	0	0
Acetic acid	9.20	204280	0.00231	0	0	131475	0.00083	0	0	0	0	9.20	36096.1	0.00015
Ethyl propionate	9.42	263578	0.00298	2135535	-0.01128	737348	0.0032	0	0	0	0	9.42	0	0
1,1-diethoxyethane	9.73	0	0	278571	0.01795	0	0	0	0	0	0	9.73	0	0
Acetic acid	10	0	0	0	0	0	0	0	0	0	0	10	0	0
Dimethyl disulfide	10.4	1973395	0.02238	3081256	0.19851	2877459	0.01243	1382064	0.01529	2410680	0.01534	10.4	2.3E+07	0.03972
Methyl isobutyl ketone	10.72	345750	0.00391	316260	0.02038	1206214	0.00524	565662	0.00626	701879	0.00446	10.72		
Isoamyl alcohol	11.15	1.6E+07	0.17653	**1E+07**	0.65388	2.5E+07	0.10901	1.7E+07	0.19179	2.8E+07	0.17774	11.15		
3-methyl-2-pentanone	*11.16*		0									*11.16*		
IS (4-methyl-2-pentanol)	11.66	8.8E+07	1	1.6E+07	1	2.3E+08	0	9E+07	1	1.6E+08	1	11.66	2.3E+08	1
2-hexanol (usikker)	11.83	0	0		0	0	0	0	0	0	0	11.83	0	0
Ethyl butanoate	12.01	0	0	482548	-0.24004	0	0	0	0	0	0	12.01	0	0
Hexanal	12.33	1490337	0.01685	396183	0.02552	3635226	0.01578	1.6E+07	0.18047	8785652	0.05589	12.33	7006239	0.02997
2-pentenal	12.54	0	0	0	0	0	0	0	0	0	0	12.54	0	0
Butanoic acid, 2-methyl-, ethyl ester	13.3	0	0	0	0	0	0	0	0	0	0	13.3	4236526	0.01838
Ethyl isovalerate	13.4	0	0	0	0	0	0	0	0	0	0	13.4	0	0
Isobutyl propinoate	13.8	1613002	0.01446	1483536	0.09558	2262192	-0.02576	7750726	-0.65911	1463346	0.00931	13.8	4522705	0.01934
Isoamyl acetate	14.15	1937086	0.0219	3573790	-2.37478	2553379	0.01111	2267050	0.02508	4713537	0.02998	14.15	6.4E+07	0.27165
Isobutyl isobutyrate	14.55	704022	0.00796	1139390	0.07341	1083492	0.0047	4163182	0.04605	2941244	0.01871	14.55	2972384	0.01271
2-methylbutyl isobutyrate	15.17	1.5E+07	0.16653	1.5E+07	0.93721	1.4E+07	0.05363	8031508	0.08884	2.4E+07	0.15244	15.17	2.6E+07	0.11107
Alpha-pinene	15.37	1.3E+07	0.21443	2.4E+07	1.52441	1.2E+07	0.05044	3583172	0.10607	4.6E+07	0.29577	15.37	1.5E+08	0.62702
Camphene	15.85	3837621	0.11124	1.3E+07	0.84478	6017359	0.02612	4724115	0.05225	2554399	0.01625	15.85	6.3E+07	0.26861
Beta-pinene	16.56	5.2E+07	0.53175	7.1E+07	4.54442	3.1E+07	0.13375	2.7E+07	0.29538	9.5E+07	0.60537	16.56	1.2E+08	0.50019
Beta-myrcene	16.75	4.6E+08	5.24761	6E+08	38.1417	4E+08	1.71032	3.5E+08	3.87692	5.3E+08	3.76559	16.75	7.5E+08	3.21307
Ocimene	*17.07*											*17.07*		
Benzaldehyde	*17.1*											*17.1*		
alpha-phellandrene	17.2	6013615	0.068	1.2E+07	0.77405	7405668	0.03214	2488474	0.02753	7316704	0.05036	17.2	8459110	0.03618
Ethyl hexanoate	17.21											17.21		
1-octen-3-ol	17.23											17.23		
6-methyl-5-hepten-2-one	17.35	1851840	0.02094	1783091	0.11488	2071881	0.00899	0	0	1975763	0.01257	17.35	1365053	0.00584
α-Terpinene	17.43			0	0	0	0	0	0	0	0	17.43	0	0
Propanoic acid, 2-methyl, 3-methylbutyl ester	17.47	4.7E+07	0.52843	4.9E+07	3.15047	5.5E+07	0.23691	4.3E+07	0.48073	8.1E+07	0.51616	17.47	2.3E+07	0.12503
Isoamyl isobutyrate	17.55	4.9E+07	0.52666	5.7E+07	3.42134	4.5E+07	0.19734	3.1E+07	0.3434	5.9E+07	0.37683	17.55	3.7E+07	0.15722
Limonene	17.62	4.7E+07	0.52439	6.6E+07	4.19326	2.8E+07	0.12286	2.9E+07	0.32481	7.2E+07	0.45615	17.62	4.4E+07	0.18393
3-carene	17.69	1.1E+07	0.12443	1.1E+07	0.67376	7315333	0.03176	1.3E+07	0.14091	2.1E+07	0.13669	17.69	0	0
Beta-phellandrene	17.75	1.3E+07	0.14697	2E+07	1.30418	8314633	0.03869	6478846	0.07166	1.8E+07	0.11503	17.75	2.7E+07	0.11471
Alpha-pinene	17.91	2.6E+07	0.29172	2.1E+07	1.33047	2.1E+07	0.09152	2.1E+07	0.22735	3.6E+07	0.23215	17.91	1.2E+07	0.05113
Gamma-terpinene	18.16	4071926	0.04571	5413620	0.34601	2435594	0.0107	3583904	0.03971	6373331	0.04436	18.16	2353416	0.01266
Methyl 2,4-dimethylhexanoate	18.38											18.38		
Geraniol?	18.43	473756	0.00536			642352	0.00279	370121	0.00409	473001	0.00301	18.43	271615	0.00116
Unidentified	18.62											18.62		
alpha-terpinene	18.69	5290679	0.05982	5290679	0.33706	2342753	0.01277	6517503	0.07209	3423206	0.05394	18.69	2681193	0.01147
Benzeneacetaldehyde	*18.74*		0	0	0	0	-0.00329	0	-0.10739	0	0	*18.74*	0	0
Octanoic acid, methyl ester	18.82	1526339	0.01726	1526339	0.09834	2352572	0.01021	2030378	0.02312	2371209	0.01508	18.82	5041370	0.02156
Cis linalool oxide	18.9	117373	0.00133	0	0	256035	0.00111					18.9		
Heptanoic acid, ethyl ester (ethyl heptanoate)	18.97	457193	0.00517	362001	0.06198	804205	0.00349	0	0	0	0	18.97	0	0
Perillene	19.04	1393946	0.01583	1588631	0.10235	1515897	0.00658	1287709	0.01424	1878031	0.01195	19.04	3416624	0.01461
Butanoic acid, 2-methyl-, 2-methylbutyl ester	19.1	4151822	0.04695	3293020	0.21216	4316014	0.01873	3224510	0.03567	4183195	0.02661	19.1	6031645	0.0258
n-Amyl isovalerate	19.16	0	-8.3E-05	0	-0.001	0	-0.00238	0	0	0	0	19.16	0	0
2-nonanone	19.17	7373655	0.08345	4477420	0.28846	9236188	0.04035	6458688	0.07144	6832546	0.04385	19.17	1.3E+07	0.05653
Linalool	19.29	4543591	0.05144	2076616	0.13116	7054814	0.02257	1.6E+07	0.13837	7213152	0.04592	19.29	1.8E+07	0.07614
Nonanal	19.33	0	0	0	0	0	0	0	0	0	0	19.33	0	0
Myrtenol	19.37	3551794	0.03974	2439700	0.15551	3348575	0.01453	2.1E+07	0.22796	3357935	0.05953	19.37	2272727	0.00972
Methyl octanoate	19.4	0	0	0	0	0	0	0	0	0	0	19.4	3258510	0.01394
Cosmene	19.54	3847773	0.04351	220227	0.01419	1108840	0.00481	1667759	0.01845	882199	0.00561	19.54	0	0
Allo-ocimene	19.61	1841184	0.02033	343919	0.05817	907544	0.00374	3270511	0.03553	1812901	0.01153	19.61	593044	0.00256
Unidentified	19.89											19.89		
Phenylethyl alcohol	20.01	0	0	464365	0.01642	0	0	0	0	0	0	20.01	0	0
Cis-p-mentha-2,8-dien-1-ol	20.26	450035	0.00509	474210	0.03055	1006533	0.00437	1.3E+07	0.14514	3991225	0.02539	20.26	2011743	0.0086
Ethyl octanoate	20.4	246533	0.00279	311836	0.00367	492783	0.00214	828846	0.00917	313470	0.00199	20.4	1674753	0.00716
2-decanone	20.62	136763	0.00222	165782	0.01068	316051	-0.00101	1255511	0.00976	655466	0.00417	20.62	2551312	0.01091
3-nonenoic acid, methyl ester	20.68	88248.3	0.001	90353.2	0.00582	143878	0.00065	227814	0.00101	67714.2	0.00043	20.68	807284	0.00345
Unidentified	20.74											20.74		
Nonanoic acid, methyl ester	20.77	51016.8	0.00058	86550.6	0.00558	169267	0.00073	1806436	0.01998	1190748	0.00757	20.77	1677817	0.00718
2-undecanone	21.43	0	0	43022.3	0.00277	0	0	0	0	0	0	21.43	1517335	0.00649
Nerol acetate	21.47	120157	0.00136	176292	0.01136	535390	0.00233	4913163	0.05435	3732233	0.02374	21.47	4014588	0.01717
Unidentified	21.56											21.56		
Unidentified	21.71											21.71		
methyl dec-4-enoate	21.85	777813	0.0088	683617	0.04404	760683	0.0033	1624308	0.01797	2890363	0.01839	21.85	2E+07	0.08568
Unidentified	21.92	28088.7	0.00032	93351	0.00601	64783.4	0.00028		0	393227	0.0025	21.92	3126702	0.01337
Methyl decanoate (decanoic acid, methyl ester)	21.98	37383.4	0.00042	25175.8	0.00162	12485.5	5.4E-05	70138.4	0.00078	113370	0.00072	21.98	1190770	0.00509
Methyl geranate	22.06	1111539	0.01257	827637	0.05333	854415	0.00371	1124272	0.01244	2614363	0.01663	22.06	1.5E+07	0.06603
gamma-himachalene	22.18	100631	0.00114	103046	0.00703	57619.7	0.00025	93365.8	0.00104	132877	0.00085	22.18	1745872	0.00747
δ-Cadinol	22.32	783475	0.00893	391047	0.0243	230843	0.00052	414410	0.00214	148370	0.00094	22.32	668998	0.00286
δ-Guaiene	22.6	282988	0.0032	162442	0.01175	172757	0.00075	124784	0.00138	0	0	22.6	0	0
Ylangene	22.63	0	0	0	0	0	0	0	0	150173	0.00096	22.63	2163727	0.00925
Copaene	22.68	771900	0.00873	605886	0.03903	696636	0.00302	457186	0.00506	812253	0.00517	22.68	8584244	0.03672
2-dodecanone	22.98	35903	0.00041	19203.6	0.00124	29356.5	0.00013		0	251522	0.0016	22.98	1058803	0.00453
Unidentified	23.22	0	0	40678.2	0.00262	0	0	0	0	110053	0.0007	23.22		
Caryophyllene	23.33	2704025	0.03058	3140112	0.20131	1846063	0.00624	3121971	0.02889	1E+07	0.06648	23.33	8.9E+07	0.38094
Alpha-humulene	23.71	4365512	0.04936	4853580	0.30982	2030536	0.00728	4643497	0.04595	1.7E+07	0.10849	23.71	1.2E+08	0.492
γ-Cadinene	23.76	252577	0.00286	300007	0.01933	138506	0.0006	430403	0.00476	1454965	0.00926	23.76	8663907	0.03706
Alpha-farnesene	23.83	0	0	12102.7	0.00078	57123.4	0.00025	84413.2	0.00093	336793	0.00214	23.83		
Unidentified	23.92	6330.02	7.8E-05	18396	0.00119	0	0	80684	0.00089	362243	0.0023	23.92	1126265	0.00482
alpa-muurolene	23.99	124373	0.00141	128080	0.00825			0	0	0	0	23.99	4811175	0.02058
β-Selinene	24.04	36658.8	0.00109	31789.9	0.00531	234537	0.00102	313597	0.01017	3728838	0.02372	24.04	3454668	0.01478
Alpha-Selinene	24.1	63482.7	0.00072	67875.8	0.00437	27772.7	0.00012	200474	0.00222	849612	0.0054	24.1	2870393	0.01228
δ-Cadinene	24.2	257360	0.00291	272501	0.01756	138050	0.0006	464045	0.00513	2418280	0.01538	24.2	1.1E+07	0.04899
Unidentified	24.31	9276.7	0.0001	14437	0.00093	0	0	5211.38	5.8E-05	230643	0.00147	24.31	828893	0.00355
Unidentified	24.37	0	0	0	0	0	0	0	0	0	0	24.37	0	0
Unidentified	24.4	22251.8	0.00025	55409	0.00357	8155.73	3.5E-05	102170	0.00113	543283	0.00349	24.4	1083833	0.00466
alfa-cadinene	24.44	10684.2	0.00012	0	0	3730.25	1.6E-05	51125.6	0.00057	379870	0.00242	24.44	664026	0.00284
Unidentified	24.53	0	0	0	0	0	0	0	0	0	0	24.53	0	0
Unidentified	24.61	0	0	0	0	0	0	0	0	0	0	24.61	0	0
Alfa-calacorene	24.69	11044	0.00012	0	0	0	0	0	0	288256	0.00183	24.69	48333.4	0.00021
Sanalol	25.63	0	0	0	0	0	0			364644	0.00232	25.63		
Caryophyllene oxide	26.09	0	0	0	0	0	0			138683	0.00088	26.09		
Unidentified	26.44	60204.3	0.00068	36811.4	0.00237	87513	0.00038	853134	0.00944	639663	0.00407	26.44	304697	0.0013
Unidentified	26.56	655411	0.00741	486028	0.02302	652885	-0.00366	848728	0.00437	1286285	0.00818	26.56	1252665	0.00536

Figure E.3: Peak areas for all 97 compounds, as well as the internal standard, for all Simcoe samples. The first column for each sample represents the area measured for each volatile compounds respective peak. The second column shows the data normalized to the peak area of the internal standard of each respective sample with the normalized blanks subtracted from them. Blank spaces indicate places there were peaks, but where the "integrate chromatogram" function did not work. The second column of retention times is the same as the first column.

Centennial

		30 degrees celsius				60 degrees celsius		Samples for 80 degrees celsius						
		Citrawort30		Citrabeer30		Citrawort60		Citrawort80		Citrawet80		Citradry80		
	RT	Area	Area [no	Area	Area [no	Area	Area [no	Area	Area [no	Area	Area [no	RT	Area	Area [norm
Ethyl acetate	5.37	0	0	1.5E+08	-6.23655	0	0	0	0	0	0	5.37	0	0
Acetic acid	9.20	55529.7	0.00037	0	0	0	0	218179	0.0009			9.20	2356677	0.00542
Ethyl propionate	9.42	9345.8	0.00016	883459	-0.03419	2100232	0.00829	0	0	0	0	9.42	0	0
1,1-diethoxyethane	9.73	0	0	278343	0.01723	0	0	0	0	0	0	9.73	0	0
Acetic acid	10	0	0			0	0	0	0	0	0	10	0	0
Dimethyl disulfide	10.4	854289	0.01434	2458937	0.1522	3787974	0.01496	4476978	0.01844	943029	0.0082	10.4	3.9E+07	0.08983
Methyl isobutyl ketone	10.72	321395	0.00562	0	0	1370141	0.00778	1083872	0.00446	1083235	0.00342	10.72	1.3E+07	0.03027
Isoamyl alcohol	11.15	4524063	0.07912	1.4E+07	0.8917	1.6E+07	0.06429	2E+07	0.0813	1.2E+07	0.10596	11.15		
3-methyl-2-pentanone	*11.16*		0									*11.16*		
IS (4-methyl-2-pentanol)	11.66	5.7E+07	1	1.6E+07	1	2.5E+08	1	2.4E+08	1	1.2E+08	1	11.66	4.4E+08	1
2-hexanol (usikker)	11.83			0	0	0	0	0	0	4.2E+07	0.36605	11.83	0	0
Ethyl butanoate	12.01	0	0	300391	-0.25254	0	0	0	0	0	0	12.01	0	0
Hexanal	12.33	1932453	0.03379	240529	0.01489			1750593	0.00721	1.8E+07	0.16032	12.33	7169311	0.01648
2-pentenal	12.54	1238506	0.02166	0	0			6253957	0.02575	0	0	12.54	0	0
Butanoic acid, 2-methyl-, ethyl ester	13.3	426415	0.00746	0	0	1017705	0.00402	728155	0.003	1189757	0.01034	13.3	3051223	0.00701
Ethyl isovalerate	13.4	0	0	733491	0.0454	0	0	0	0	0	0	13.4	0	0
Isobutyl propinoate	13.8	766953	0.00963	504751	0.03124	825321	-0.03232	842351	-0.74197	1309215	0.01138	13.8	1.8E+07	0.04108
Isoamyl acetate	14.15	305542	0.00534	820793	0	85634	0.00034	574976	0.00237	0	0	14.15	5291630	0.01216
Isobutyl isobutyrate	14.55	820231	0.01434	271310	0.01679	1340833	0.0053	1515386	0.00624	2350432	0.02565	14.55	7758450	0.01783
2-methylbutyl isobutyrate	15.17	4542686	0.07944	7077825	0.43808	1.3E+07	0.05315	1.6E+07	0.06413	1.5E+07	0.12616	15.17	6.2E+07	0.14257
Alpha-pinene	15.37	6778327	0.11855	1.5E+07	0.91181	1.6E+07	0.06475	2.1E+07	0.08498	2.8E+07	0.24371	15.37	2.9E+08	0.6663
Camphene	15.85	3186930	0.05573	5931609	0.37085	8220376	0.03247	1.1E+07	0.04421	1.4E+07	0.12141	15.85	1.3E+08	0.29119
Beta-pinene	16.56	1.7E+07	0.29728	2.3E+07	1.79665	3E+07	0.11906	4.6E+07	0.19019	5E+07	0.43323	16.56	1.8E+08	0.40391
Beta-myrcene	16.75	3.8E+06	6.56034	4.3E+08	26.1953	4.3E+08	1.674	5.1E+08	2.0951	5.8E+08	5.0077	16.75	8.4E+08	1.93337
Ocimene	*17.07*											*17.07*		
Benzaldehyde	*17.1*											*17.1*		
alpha-phellandrene	17.2	5438603	0.09511			3147228	0.03613	4760735	0.01961	5961399	0.05184	17.2	3434973	0.02168
Ethyl hexanoate	17.21	345454	0.01653			2330714	0.00921	2801529	0.01154	0	0	17.21	0	0
1-octen-3-ol	17.23											17.23		
6-methyl-5-hepten-2-one	17.35	1295399	0.02265			5663610	0.02237	7852841	0.03234	7143788	0.06211	17.35	2818990	0.00648
α-Terpinene	17.43	1.3E+07	0.22113	2.3E+07	1.78263	4.4E+07	0.17567	6.4E+07	0.26391	3.8E+07	0.33247	17.43	0	0
Propanoic acid, 2-methyl, 3-methylbutyl ester	17.47	0	0	0	0	0	0	0	0	0	0	17.47	1.6E+07	0.03759
Isoamyl isobutyrate	17.55	1.2E+07	0.18619	3E+07	1.56221	3.6E+07	0.14388	4.2E+07	0.17406	3.2E+07	0.28165	17.55	3.4E+07	0.07794
Limonene	17.62	2.3E+07	0.40406	4.2E+07	2.55652	4.2E+07	0.16594	6.2E+07	0.25604	5.3E+07	0.46234	17.62	7.4E+07	0.17091
3-carene	17.69	8365088	0.14474	1.4E+07	0.85637	1.3E+07	0.05267	2.3E+07	0.11864	1.5E+07	0.12744	17.69	0	0
Beta-phellandrene	17.75	4183898	0.07327	5772178	0.35727	6331235	0.02761	1E+07	0.04122	3162760	0.07967	17.75	3.2E+07	0.07452
Alpha-pinene	17.91	1.4E+07	0.25333	2.3E+07	1.39849	2.3E+07	0.09084	3.2E+07	0.13227	2.2E+07	0.18768	17.91	8660929	0.0199
Gamma-terpinene	18.16	2006051	0.03475	3998732	0.24435	1368125	0.00527	7707437	0.03174	4231127	0.03731	18.16	1477670	0.0034
Methyl 2,4-dimethylhexanoate	18.38	171768	0.003	472619	0.02925	970933	0.00383	875211	0.0036	445433	0.00387	18.38	292241	0.00067
Geraniol?	18.49	174802	0.00306	686757	0.04251	1735502	0.00685	2907219	0.01197	2250606	0.01957	18.49		
Unidentified	18.62	207429	0.00363					2064700	0.0085	713779	0.00621	18.62	39192.3	9E-05
alpha-terpinene	18.69	3106865	0.05433	5903724	0.36161	4748204	0.01875	1.2E+07	0.04838	5707452	0.04962	18.69	2337734	0.00537
Benzeneacetaldehyde	*18.74*	0	0	0	0	0	-0.00329	0	-0.10739	0	0	*18.74*	0	0
Octanoic acid, methyl ester	18.82	711600	0.01244	1634082	0.10114	1756564	0.00694	3936322	0.01646	1375355	0.01718	18.82	2464514	0.00566
Cis linalool oxide	18.9	**261603**	0.00457	0	0	168087	0.00066	0	0	2373777	0.02064	18.9	241046	0.00055
Heptanoic acid, ethyl ester (ethyl heptanoate)	18.97	0	0	2624939	0.16247	2403070	0.00951	0	0	0	0	18.97	231301	0.00053
Perillene	19.04	634304	0.01109	907427	0.05617	1406544	0.00556	2622655	0.0108	2121355	0.01844	19.04	3521207	0.00809
Butanoic acid, 2-methyl-, 2-methylbutyl ester	19.1	427623	0.00748	1111156	0.06878	2761422	0.01093	3433350	0.01416	2338673	0.02033	19.1	1664682	0.00383
n-Amyl isovalerate	19.16	0	-8.9E-05	2654733	0.16332	0	-0.00238	0	0	0	0	19.16	0	0
2-nonanone	19.17	1304723	0.03331	0	0	7563451	0.02987	9333116	0.03868	6134733	0.05334	19.17	5458309	0.01254
Linalool	19.29	6326684	0.11064	2081535	0.12621	1.2E+07	0.03933	1.5E+07	0.03008	3.5E+07	0.30179	19.29	2.2E+07	0.05037
Nonanal	19.33	0	0	0	0	0	0	0	0	0	0	19.33	0	0
Myrtenol	19.37	2391303	0.0414	3631014	0.22308	4553286	0.01798	1.2E+07	0.04972	4.2E+07	0.3644	19.37	2374145	0.00684
Methyl octanoate	19.4	0	0	0	0	0	0	0	0	0	0	19.4	3747240	0.00861
Cosmene	19.54	391063	0.00684	1086099	0.06722	1855535	0.00733	4183811	0.01725	2393563	0.02081	19.54	0	0
Allo-ocimene	19.61	1120437	0.0191	2676174	0.163	1539119	0.00588	355037	0.00082	2683062	0.02333	19.61	158550	0.00036
Unidentified	19.83	192640	0.00337	329099	0.02037	1800561	0.00711	1775049	0.00731	3277863	0.0285	19.83	662246	0.00152
Phenylethyl alcohol	20.01	0	0	677865	0.02846	0	0	0	0	0	0	20.01	0	0
Cis-p-mentha-2,8-dien-1-ol	20.26	637941	0.01116	371097	0.02297	996138	0.00393	3074655	0.01266	2.5E+07	0.21676	20.26	2541041	0.00584
Ethyl octanoate	20.4	25708	0.00045	322232	0.00352	542308	0.00214	361259	0.00157	0	0	20.4	458518	0.00105
2-decanone	20.62	190799	0.00334	125244	0.00775	235094	-0.00121	476144	-0.00216	1935744	0.01683	20.62	1433963	0.0033
3-nonenoic acid, methyl ester	20.68	53701.8	0.00094	46830	0.0029	192169	0.00076	207996	-0.00065	837747	0.00728	20.68	724574	0.00167
Unidentified	20.74	0	0	0	0	0	0			1053696	0.00916	20.74	0	0
Nonanoic acid, methyl ester	20.77	382364	0.00669	72152.9	0.00447	231891	0.00092	308832	0.00374	1625626	0.01413	20.77	2126952	0.00489
2-undecanone	21.43	0	0	11305.4	0.00074	21630	8.6E-05	0	0	0	0	21.43	230499	0.00067
Nerol acetate	21.47	528352	0.00924	49069	0.00304	129126	0.00051	365030	0.0015	2267081	0.01971	21.47	976522	0.00224
Unidentified	21.56	8451.78	0.00015	5504	0.00034	37523.1	0.00015	22554.3	9.3E-05	243098	0.00217	21.56	316708	0.00211
Unidentified	21.71	26136.6	0.00046	25846	0.0016	50688.8	0.0002	93148.3	-0.00093	232640	0.00254	21.71	888072	0.00204
methyl dec-4-enoate	21.85	381548	0.00667	643309	0.03982	830826	0.00328	1439294	0.00617	2966951	0.0258	21.85	1.8E+07	0.0417
Unidentified	21.92	96783.9	0.00169	104453	0.00647	150469	0.00059	317623	0.00131	782722	0.00681	21.92	4357521	0.01139
Methyl decanoate (decanoic acid, methyl ester)	21.98	52828.7	0.00092	58105.2	0.0036	85324.6	0.00034	186672	0.00077	417508	0.00363	21.98	3908359	0.00898
Methyl geranate	22.06	550588	0.00963	757571	0.04689	1225065	0.00484	1340896	0.00739	3150403	0.02739	22.06	1.7E+07	0.03824
gamma-himachalene	22.18	43681.7	0.00087	112728	0.00698	74483	0.00029	143628	0.00059	78031.4	0.00068	22.18	445480	0.00102
δ-Cadinol	22.32	262033	0.00458	680354	0.04126	396128	0.00083	613879	0.00011	123048	0.00107	22.32	222217	0.00051
δ-Guaiene	22.6	104878	0.00183	262826	0.01627	207485	0.00082	123861	0.00051	0	0	22.6	0	0
Ylangene	22.63	0	0	0	0	0	0	0	0	53835.3	0.00052	22.63	1441406	0.00331
Copaene	22.68	236535	0.00519	811808	0.05025	624573	0.00247	503639	0.00207	208877	0.00182	22.68	4375399	0.01143
2-dodecanone	22.98	24453.4	0.00043	78884.4	0.00488	10642.4	4.2E-05	43848.4	0.00018	131618	0.00167	22.98	351365	0.00081
Unidentified	23.22	0	0	0	0	0	0	0	0	0	0	23.22	1E+08	0.23633
Caryophyllene	23.33	1169693	0.02046	2932708	0.18053	1453988	0.00399	2702484	0.00549	4127352	0.03589	23.33	7.7E+07	0.1762
Alpha-humulene	23.71	1383636	0.03463	3839478	0.23477	1831518	0.0057	3714642	0.00981	5322543	0.05143	23.71	3.6E+07	0.22042
γ-Cadinene	23.76	97706	0.00171	284983	0.01764	136130	0.00054	333443	0.00137	545206	0.00474	23.76	6336128	0.01456
Alpha-farnesene	23.83	71493	0.00125	100207	0.0062	41480.4	0.00016	180608	0.00074	284487	0.00247	23.83	3857274	0.00886
Unidentified	23.92	13620.2	0.00024	33336.9	0.00206			100394	0.00042	183779	0.0016	23.92	1037245	0.00238
alpa-muurolene	23.99	78883.4	0.00138	163281	0.01011	123064	0.00049			683665	0.006	23.99	4446180	0.01022
β-Selinene	24.04	168705	0.00295	428122	0.0265	207631	0.00082	363970	0.00397	1336572	0.01162	24.04	1.1E+07	0.02526
Alpha-Selinene	24.1	164825	0.00288	463757	0.0287	185540	0.00073	571732	0.00235	1050011	0.00913	24.1	1.1E+07	0.02605
δ-Cadinene	24.2	132713	0.00232	247877	0.01534	115329	0.00046	308906	0.00127	663128	0.00577	24.2	6858122	0.01576
Unidentified	24.31	0	0	11491.2	0.00071	5340.1	2.1E-05	28002.4	0.00012	71950.4	0.00063	24.31	671524	0.00154
Unidentified	24.37	0	0	0	0	0	0	0	0	0	0	24.37	0	0
Unidentified	24.4	31483.1	0.00055	480315	0.00297	40116	0.00016	135458	0.00056	193875	0.00169	24.4	1106425	0.00254
alfa-cadinene	24.44	0	0	15027.3	0.00093	13857.7	5.5E-05	93543.8	0.00039	97633.4	0.00085	24.44	465308	0.00107
Unidentified	24.53	45405.7	0.00079	0	0	0	0	0	0	0	0	24.53	0	0
Unidentified	24.61	0	0	0	0	0	0	0	0	0	0	24.61	0	0
Alfa-calacorene	24.69	2802.78	4.9E-05	853.63	5.3E-05	13336.6	5.3E-05	50543.9	0.00021	44764.4	0.00039	24.69	104346	0.00024
Sanalol	25.63	10701	0.00019	12288.4	0.00076	20978.4	8.3E-05	112176	0.00046	232082	0.00202	25.63	150209	0.00035
Caryophyllene oxide	26.09									107528	0.00093	26.09		
Unidentified	26.44	0	0	0	0	42957.3	0.00017	278370	0.00115	314273	0.00273	26.44	39805.6	0.00023
Unidentified	26.56	203846	0.00367	353528	0.01359	708354	-0.00369	1037719	-0.00074	446157	0.00388	26.56	1308349	0.00301

Figure E.4: Peak areas for all 97 compounds, as well as the internal standard, for all Centennial samples. The first column for each sample represents the area measured for each volatile compounds respective peak. The second column shows the data normalized to the peak area of the internal standard of each respective sample with the normalized blanks subtracted from them. Blank spaces indicate places there were peaks, but where the "integrate chromatogram" function did not work. The second column of retention times is the same as the first column.

Citra

		30 degrees celsius				60 degrees celsius		Samples for 80 degrees celsius					
		Citrawort30		Citrabeer30		Citrawort60		Citrawort80		Citrawet80		Citradry80	
	RT	Area	Area [no	Area	Area [no	Area	Area [no	Area	Area [no	Area	Area [no	RT	Area
Ethyl acetate	5.37	0	0	1.5E+08	-6.23655	0	0	0	0	0	0	5.37	0
Acetic acid	9.20	55523.7	0.00037	0	0	0	0	216179	0.0009	0	0	9.20	2356677
Ethyl propionate	9.42	3345.8	0.00016	883459	-0.03419	2100232	0.00829	0	0	0	0	9.42	0
1,1-diethoxyethane	9.73	0	0	276343	0.01723	0	0	0	0	0	0	9.73	0
Acetic acid	10	0	0	0	0	0	0	0	0	0	0	10	0
Dimethyl disulfide	10.4	854289	0.01434	2458337	0.1522	3787974	0.01496	4476978	0.01844	943029	0.0082	10.4	3.3E+07
Methyl isobutyl ketone	10.72	321395	0.00562	0	0	1370141	0.00778	1083872	0.00446	1083235	0.00342	10.72	1.3E+07
Isoamyl alcohol	11.15	4524069	0.07912	1.4E+07	0.8317	1.6E+07	0.06429	2E+07	0.0813	1.2E+07	0.10596	11.15	
3-methyl-2-pentanone	*11.16*	0										*11.16*	
IS (4-methyl-2-pentanol)	11.66	5.7E+07	1	1.6E+07	1	2.5E+08	1	2.4E+08	1	1.2E+08	1	11.66	4.4E+08
2-hexanol (usikker)	11.83			0	0	0	0	0	0	4.2E+07	0.36605	11.83	0
Ethyl butanoate	12.01	0	0	300391	-0.25254	0	0	0	0	0	0	12.01	0
Hexanal	12.33	1932453	0.03379	240529	0.01483			1750593	0.00721	1.8E+07	0.16032	12.33	7163911
2-pentenal	12.54	1238506	0.02166	0	0			6253357	0.02575	0	0	12.54	0
Butanoic acid, 2-methyl-, ethyl ester	13.3	426415	0.00746	0	0	1017705	0.00402	728155	0.003	1169757	0.01034	13.3	3051223
Ethyl isovalerate	13.4	0	0	733431	0.0454	0	0	0	0	0	0	13.4	0
Isobutyl propinoate	13.8	766353	0.00963	504751	0.03124	825321	-0.03232	842351	-0.74197	1309215	0.01138	13.8	1.8E+07
Isoamyl acetate	14.15	305542	0.00534	820793	0	85634	0.00034	574976	0.00237	0	0	14.15	5231630
Isobutyl isobutyrate	14.55	820231	0.01434	271310	0.01679	1340833	0.0053	1515386	0.00624	2950432	0.02565	14.55	7758450
2-methylbutyl isobutyrate	15.17	4542686	0.07944	7077825	0.43808	1.3E+07	0.05315	1.6E+07	0.06413	1.5E+07	0.12616	15.17	6.2E+07
Alpha-pinene	15.37	6778327	0.11855	1.5E+07	0.91181	1.6E+07	0.06475	2.1E+07	0.08438	2.8E+07	0.24371	15.37	2.9E+08
Camphene	15.85	3186930	0.05573	5931609	0.37085	8220376	0.03247	1.1E+07	0.04421	1.4E+07	0.12141	15.85	1.3E+08
Beta-pinene	16.56	1.7E+07	0.29728	2.9E+07	1.79665	3E+07	0.11906	4.6E+07	0.19019	5E+07	0.43323	16.56	1.8E+08
Beta-myrcene	16.75	3.8E+08	6.56034	4.3E+08	26.1953	4.3E+08	1.674	5.1E+08	2.0951	5.8E+08	5.0077	16.75	8.4E+08
Ocimene	*17.07*											*17.07*	
Benzaldehyde	*17.1*											*17.1*	
alpha-phellandrene	17.2	5438603	0.09511			9147228	0.03613	4760735	0.01961	5361999	0.05184	17.2	9434973
Ethyl hexanoate	17.21	345454	0.01653			2330714	0.00921	2801529	0.01154	0	0	17.21	0
1-octen-3-ol	17.23											17.23	
6-methyl-5-hepten-2-one	17.35	1295399	0.02265			5663610	0.02237	7852841	0.03234	7143788	0.06211	17.35	2818990
α-Terpinene	17.43	1.3E+07	0.22113	2.3E+07	1.78263	4.4E+07	0.17567	6.4E+07	0.26391	3.8E+07	0.33247	17.43	0
Propanoic acid, 2-methyl, 3-methylbutyl ester	17.47	0	0	0	0	0	0	0	0	0	0	17.47	1.6E+07
Isoamyl isobutyrate	17.55	1.2E+07	0.18619	3E+07	1.56221	3.6E+07	0.14388	4.2E+07	0.17406	3.2E+07	0.28165	17.55	3.4E+07
Limonene	17.62	2.3E+07	0.40406	4.2E+07	2.55652	4.2E+07	0.16594	6.2E+07	0.25604	5.3E+07	0.46234	17.62	7.4E+07
3-carene	17.69	8365088	0.14474	1.4E+07	0.85637	1.3E+07	0.05267	2.3E+07	0.11864	1.5E+07	0.12744	17.69	0
Beta-phellandrene	17.75	4183898	0.07327	5772178	0.35727	6931235	0.02761	1E+07	0.04122	3162760	0.07967	17.75	3.2E+07
Alpha-pinene	17.91	1.4E+07	0.25333	2.3E+07	1.39849	2.3E+07	0.09084	3.2E+07	0.13227	2.2E+07	0.18768	17.91	8660929
Gamma-terpinene	18.16	2006051	0.03475	3998732	0.24435	1368125	0.00527	7707437	0.03174	4231127	0.03731	18.16	1477670
Methyl 2,4-dimethylhexanoate	18.38	171768	0.003	472619	0.02925	970933	0.00383	875211	0.0036	445433	0.00387	18.38	292241
Geraniol?	18.49	174802	0.00306	686757	0.04251	1735502	0.00685	2307219	0.01137	2250606	0.01357	18.49	
Unidentified	18.62	207429	0.00363					2064700	0.0085	713779	0.00621	18.62	39192.3
alpha-terpinene	18.69	3106865	0.05433	5903724	0.36161	4748204	0.01875	1.2E+07	0.04838	5707452	0.04962	18.69	2337734
Benzeneacetaldehyde	*18.74*	0	0	0	0	0	-0.00329	0	-0.10739	0	0	*18.74*	0
Octanoic acid, methyl ester	18.82	711600	0.01244	1634082	0.10114	1756564	0.00694	3396322	0.01646	1975355	0.01718	18.82	2464514
Cis linalool oxide	18.3	**261603**	0.00457	0	0	168087	0.00066	0	0	2373777	0.02064	18.3	241046
Heptanoic acid, ethyl ester (ethyl heptanoate)	18.97	0	0	2624939	0.16247	2403070	0.00951	0	0	0	0	18.97	231301
Perillene	19.04	634304	0.01109	907427	0.05617	1406544	0.00556	2622655	0.0108	2121355	0.01844	19.04	3521207
Butanoic acid, 2-methyl-, 2-methylbutyl ester	19.1	427623	0.00748	1111156	0.06878	2781422	0.01099	3433350	0.01416	2338679	0.02033	19.1	1664682
n-Amyl isovalerate	19.16	0	-8.9E-05	2654733	0.16332	0	-0.00238	0	0	0	0	19.16	0
2-nonanone	19.17	1904723	0.03331	0	0	7563451	0.02987	9393116	0.03868	6134733	0.05334	19.17	5458309
Linalool	19.29	6326634	0.11064	2081535	0.12621	1.2E+07	0.03933	1.5E+07	0.03008	3.5E+07	0.30179	19.29	2.2E+07
Nonanal	19.33	0	0	0	0	0	0	0	0	0	0	19.33	0
Myrtenol	19.37	2391303	0.0414	3631014	0.22308	4553286	0.01798	1.2E+07	0.04972	4.2E+07	0.3644	19.37	2974145
Methyl octanoate	19.4	0	0	0	0	0	0	0	0	0	0	19.4	3747240
Cosmene	19.54	331063	0.00684	1086099	0.06722	1855535	0.00733	4189811	0.01725	2393563	0.02081	19.54	0
Allo-ocimene	19.61	1120497	0.0191	2676174	0.163	1539119	0.00588	355037	0.00082	2683062	0.02333	19.61	158550
Unidentified	19.83	192640	0.00337	329099	0.02037	1800561	0.00711	1775043	0.00731	3277863	0.0285	19.83	662246
Phenylethyl alcohol	20.01	0	0	677865	0.02846	0	0	0	0	0	0	20.01	0
Cis-p-mentha-2,8-dien-1-ol	20.26	637341	0.01116	371097	0.02297	996138	0.00393	3074655	0.01266	2.5E+07	0.21676	20.26	2541041
Ethyl octanoate	20.4	25708	0.00045	322292	0.00352	542308	0.00214	361259	0.00157	0	0	20.4	458518
2-decanone	20.62	190799	0.00334	125244	0.00775	235094	-0.00121	476144	-0.00216	1935744	0.01683	20.62	1433963
3-nonenoic acid, methyl ester	20.68	53701.8	0.00094	46830	0.0029	132163	0.00076	207996	-0.00065	837747	0.00728	20.68	724574
Unidentified	20.74	0	0	0	0	0	0			1053696	0.00916	20.74	0
Nonanoic acid, methyl ester	20.77	382364	0.00669	72152.9	0.00447	231891	0.00092	308832	0.00374	1625626	0.01413	20.77	2126952
2-undecanone	21.43	0	0	11305.4	0.00074	21630	8.6E-05	0	0	0	0	21.43	290439
Nerol acetate	21.47	528352	0.00924	43063	0.00304	129126	0.00051	365030	0.0015	2267081	0.01971	21.47	376522
Unidentified	21.56	8451.78	0.00015	5504	0.00034	37529.1	0.00015	22554.3	9.3E-05	243038	0.00217	21.56	316708
Unidentified	21.71	26136.6	0.00046	25846	0.0016	50688.8	0.0002	33148.3	-0.00093	232640	0.00254	21.71	888072
methyl dec-4-enoate	21.85	381548	0.00667	643309	0.03982	830826	0.00328	1493294	0.00617	2966951	0.0258	21.85	1.8E+07
Unidentified	21.92	96783.9	0.00169	104453	0.00647	150463	0.00059	317623	0.00131	782722	0.00681	21.92	4357521
Methyl decanoate (decanoic acid, methyl ester)	21.98	52628.7	0.00092	58105.2	0.0036	85324.6	0.00034	186672	0.00077	417508	0.00363	21.98	3308359
Methyl geranate	22.06	550588	0.00963	757571	0.04689	1225065	0.00484	1340896	0.00799	3150403	0.02739	22.06	1.7E+07
gamma-himachalene	22.18	43681.7	0.00087	112728	0.00698	74483	0.00029	143628	0.00059	78031.4	0.00068	22.18	445480
δ-Cadinol	22.32	262033	0.00458	680954	0.04126	336128	0.00083	619879	0.00011	123048	0.00107	22.32	222217
δ-Guaiene	22.6	104878	0.00183	262826	0.01627	207485	0.00082	123861	0.00051	0	0	22.6	0
Ylangene	22.63	0	0	0	0	0	0	0	0	53835.3	0.00052	22.63	1441406
Copaene	22.68	236535	0.00519	811808	0.05025	624573	0.00247	503639	0.00207	208877	0.00182	22.68	4375393
2-dodecanone	22.98	24453.4	0.00043	78884.4	0.00488	10642.4	4.2E-05	43848.4	0.00018	191618	0.00167	22.98	351365
Unidentified	23.22	0	0	0	0	0	0	0	0	0	0	23.22	1E+08
Caryophyllene	23.33	1169693	0.02046	2932708	0.18053	1453988	0.00599	2702484	0.00543	4127352	0.03589	23.33	7.7E+07
Alpha-humulene	23.71	1983636	0.03469	3833478	0.23477	1831518	0.0057	3714642	0.00981	5922543	0.05149	23.71	9.6E+07
γ-Cadinene	23.76	37706	0.00171	284983	0.01764	136130	0.00054	333443	0.00137	545206	0.00474	23.76	6336128
Alpha-farnesene	23.83	71499	0.00125	100207	0.0062	41480.4	0.00016	180608	0.00074	284487	0.00247	23.83	3857274
Unidentified	23.92	13620.2	0.00024	33336.9	0.00206			100934	0.00042	183779	0.0016	23.92	1037245
alpa-muurolene	23.99	78883.4	0.00138	163281	0.01011	123064	0.00049			689665	0.006	23.99	4446180
β-Selinene	24.04	168705	0.00295	428122	0.0265	207631	0.00082	363970	0.00337	1336572	0.01162	24.04	1.1E+07
Alpha-Selinene	24.1	164825	0.00288	463757	0.0287	185540	0.00073	571732	0.00235	1050011	0.00913	24.1	1.1E+07
δ-Cadinene	24.2	132713	0.00232	247877	0.01534	115329	0.00046	308906	0.00127	663128	0.00577	24.2	6858122
Unidentified	24.31	0	0	11491.2	0.00071	5340.1	2.1E-05	28002.4	0.00012	71350.4	0.00063	24.31	671524
Unidentified	24.37	0	0	0	0	0	0	0	0	0	0	24.37	0
Unidentified	24.4	31483.1	0.00055	480315	0.00297	40116	0.00016	135458	0.00056	193875	0.00169	24.4	1106425
alfa-cadinene	24.44	0	0	15027.3	0.00093	13857.7	5.5E-05	33543.8	0.00039	37633.4	0.00085	24.44	465308
Unidentified	24.53	45405.7	0.00079	0	0	0	0	0	0	0	0	24.53	0
Unidentified	24.61	0	0	0	0	0	0	0	0	0	0	24.61	0
Alfa-calacorene	24.69	2802.78	4.3E-05	853.63	5.3E-05	13336.6	5.3E-05	50543.9	0.00021	44764.4	0.00039	24.69	104346
Sanalol	25.63	10701	0.00019	12288.4	0.00076	20978.4	8.3E-05	112176	0.00046	232082	0.00202	25.63	150209
Caryophyllene oxide	26.09									107528	0.00093	26.09	
Unidentified	26.44	0	0	0	0	42357.3	0.00017	278370	0.00115	314279	0.00273	26.44	93805.6
Unidentified	26.56	203846	0.00367	353528	0.01359	708954	-0.00369	1037719	-0.00074	446157	0.00388	26.56	1308349

Figure E.5: Peak areas for all 97 compounds, as well as the internal standard, for all Citra samples. The first column for each sample represents the area measured for each volatile compounds respective peak. The second column shows the data normalized to the peak area of the internal standard of each respective sample with the normalized blanks subtracted from them. Blank spaces indicate places there were peaks, but where the "integrate chromatogram" function did not work. The second column of retention times is the same as the first column.

Appendix F: Data Analysis with MetaboAnalyst 5.0

For further preprocessing, modeling and statistical analysis of the normalized peak area data, MetaboAnalyst 5.0 was used. This appendix contains some extra information on the preprocessing of the pareto scaled data used in the results part, as well as the tables of loadings referred to in part 4.3 and 4.4. It also includes the R command history.

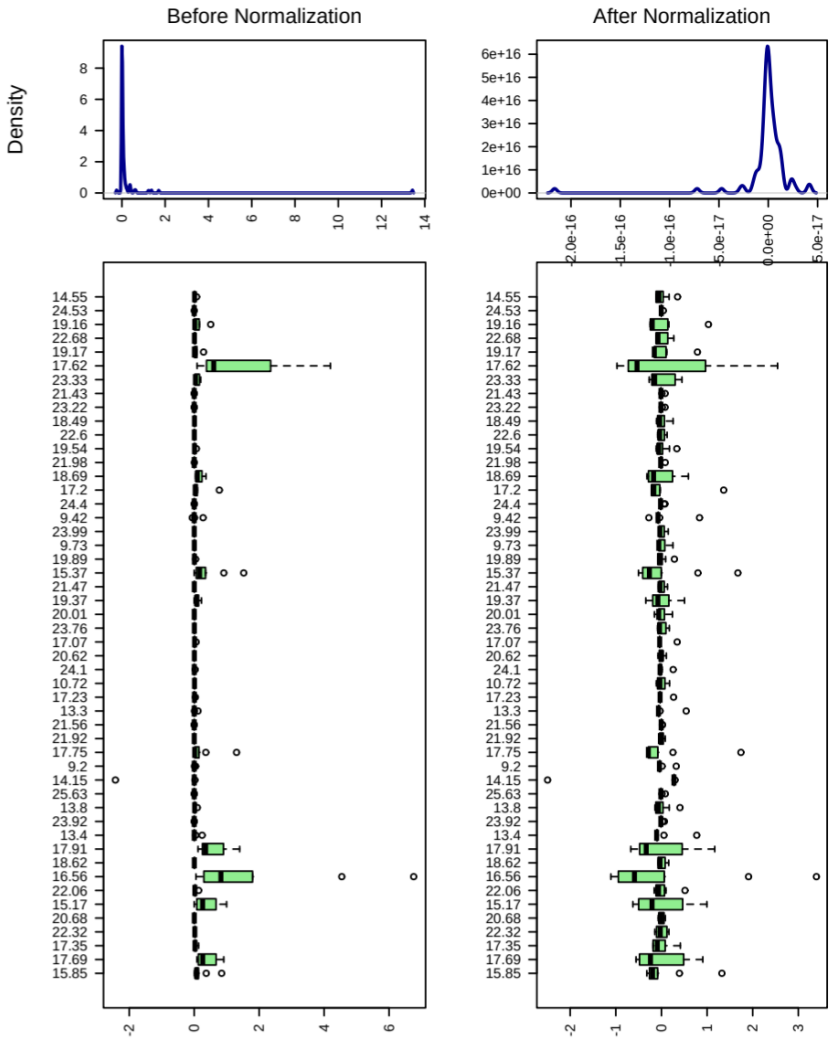


Figure F.1: Box plots and kernel density plots before and after pareto scaling normalization. The boxplots show at most 50 features due to space limit. The density plots are based on all samples. Selected methods: Row-wise normalization: N/A; Data transformation: N/A; Data scaling: Pareto Scaling

Name	Loadings 1	Loadings 2
16.75	0.80417	-0.45646
16.56	0.25694	0.39693
17.62	0.25256	0.18739
17.55	0.23216	0.45305
17.91	0.17397	0.032617
17.47	0.15069	0.23226
15.33	0.1384	0.063469
17.69	0.12409	0.087968
15.17	0.11975	0.15677
15.85	0.094325	0.019804
23.71	0.077721	0.044222
17.75	0.077546	-0.085201
11.1	0.067915	0.16614
18.69	0.063988	-0.017584
18.16	0.061968	-6.5096E-4
19.1	0.060552	0.19474
19.17	0.059718	-0.04918
23.33	0.05766	0.039822
17.2	0.05538	-0.081576
19.37	0.051958	-0.078368
12.33	0.042659	0.11152
19.04	0.042165	0.155

Figure F.2: Table of loadings for the pareto scaled data of wort samples at different temperatures. It is rearranged so that the variables, i.e. compounds (here their retention time is shown), that contribute strongly to principal component 1 are listed in order.

Name	Loadings 1	Loadings 2
16.75	0.77461	-0.21573
16.56	0.29005	0.40646
17.55	0.24929	0.31219
17.62	0.24829	-0.14048
17.47	0.1866	0.036745
17.91	0.13794	-0.10647
15.37	0.13122	-0.20406
11.15	0.12381	0.088985
17.69	0.11025	0.048409
15.17	0.10543	-0.013791
17.75	0.10007	-0.21871
14.15	-0.093152	-0.46064
15.85	0.091128	-0.15755
23.71	0.071217	0.0050572
17.2	0.066667	-0.13902
18.69	0.066188	-0.066099
18.16	0.065844	-0.074051
19.1	0.062147	0.15246
23.33	0.058217	-0.016628
18.97	0.052474	0.12487
10.4	0.046421	-0.094528

Figure F.3: Table of loadings for the pareto scaled data of wort versus beer samples. It is rearranged so that the variables, i.e. compounds (here their retention time is shown), that contribute strongly to principal component 1 are listed in order.

```
 [1] "mSet<-InitDataObjects(\"pktable\", \"stat\", FALSE)"
 [2] "mSet<-Read.TextData(mSet, \"Replacing_with_your_file_path\", \"colu\", \"disc\");"
 [3] "mSet<-SanityCheckData(mSet)"
 [4] "mSet<-ContainMissing(mSet)"
 [5] "mSet<-ReplaceMin(mSet);"
 [6] "mSet<-SanityCheckData(mSet)"
 [7] "mSet<-ContainMissing(mSet)"
 [8] "mSet<-PreparePrenormData(mSet)"
 [9] "mSet<-Normalization(mSet, \"NULL\", \"NULL\", \"ParetoNorm\", ratio=FALSE, ratioNum=20)"
[10] "mSet<-PlotNormSummary(mSet, \"norm_0_\", \"png\", 72, width=NA)"
[11] "mSet<-PlotSampleNormSummary(mSet, \"snorm_0_\", \"png\", 72, width=NA)"
[12] "mSet<-PCA.Anal(mSet)"
[13] "mSet<-PlotPCAPairSummary(mSet, \"pca_pair_0_\", \"png\", 72, width=NA, 5)"
[14] "mSet<-PlotPCAScree(mSet, \"pca_scree_0_\", \"png\", 72, width=NA, 5)"
[15] "mSet<-PlotPCA2DScore(mSet, \"pca_score2d_0_\", \"png\", 72, width=NA, 1,2,0.95,0,0)"
[16] "mSet<-PlotPCALoading(mSet, \"pca_loading_0_\", \"png\", 72, width=NA, 1,2);"
[17] "mSet<-PlotPCABiplot(mSet, \"pca_biplot_0_\", \"png\", 72, width=NA, 1,2)"
[18] "mSet<-PlotPCA3DLoading(mSet, \"pca_loading3d_0_\", \"json\", 1,2,3)"
[19] "mSet<-PlotPCA2DScore(mSet, \"pca_score2d_1_\", \"png\", 72, width=NA, 1,2,0.95,1,0)"
[20] "mSet<-PlotHCTree(mSet, \"tree_0_\", \"png\", 72, width=NA, \"euclidean\", \"ward.D\")"
[21] "mSet<-PlotHeatMap(mSet, \"heatmap_0_\", \"png\", 72, width=NA, \"norm\", \"row\", \"euclidean\
[22] "mSet<-PlotHeatMap(mSet, \"heatmap_1_\", \"png\", 72, width=NA, \"norm\", \"row\", \"euclidean\
[23] "mSet<-Kmeans.Anal(mSet, 3)"
[24] "mSet<-PlotKmeans(mSet, \"km_0_\", \"png\", 72, width=NA, \"default\", \"F\")"
[25] "mSet<-PlotClustPCA(mSet, \"km_pca_0_\", \"png\", 72, width=NA, \"default\", \"km\", \"F\")"
[26] "mSet<-Kmeans.Anal(mSet, 5)"
[27] "mSet<-PlotKmeans(mSet, \"km_1_\", \"png\", 72, width=NA, \"default\", \"F\")"
[28] "mSet<-PlotClustPCA(mSet, \"km_pca_1_\", \"png\", 72, width=NA, \"default\", \"km\", \"F\")"
[29] "mSet<-SaveTransformedData(mSet)"
[30] "mSet<-PreparePDFReport(mSet, \"guest2105930823588437394\")\n"
```

Figure F.4: R command history

Appendix G: Results with auto scaling

For the main results part of this study, results and figures based on pareto scaled data was included. However, it was also of interest to see how much the scaling can affect the interpretation of the data and to see how different auto scaling was compared to pareto scaling. The auto scaling will render all features equally important, and larger variables will not be as dominant. Therefore, 2D score plots, tables of loadings and biplots from the PCA of data from the same samples used in section 4.3 and 4.4 with the data auto scaled instead of pareto scaled, are included here.

First comes the corresponding figures from the auto scaled data to the ones in section 4.3: "The effect of temperature on retainment of hop volatile compounds in wort" (Fig. G.1 - G.3), followed by the ones corresponding to the figures in section 4.4: "Differences in retained hop volatile compounds in wort versus beer" (Fig. G.4 – G.6). The figures included in this appendix are discussed in Chapter 5: Discussion. For information on data preprocessing and modeling, see section 3.6: "Data processing and scaling".

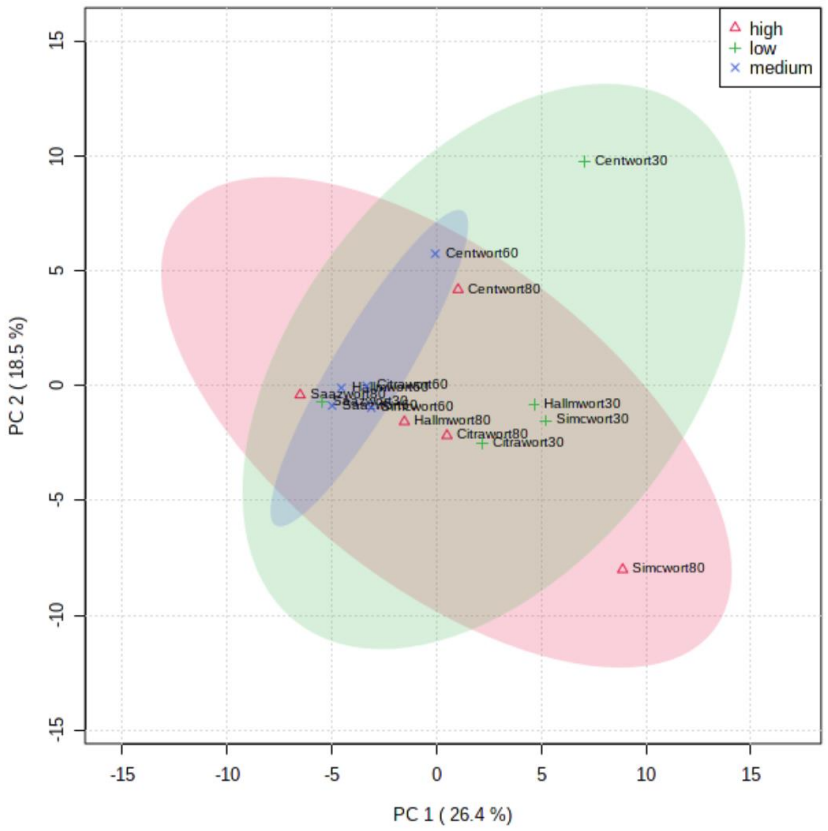


Figure G.1: Principle component analysis (PC1 and PC2) 2D score plot from pareto scaled peak area data from all five hop varieties in wort at 30°C (low), 60°C (medium) and 80°C (high). PC1 and PC2 account for 44.9% of the total variation. Colored circles represent 95 % confidence intervals.

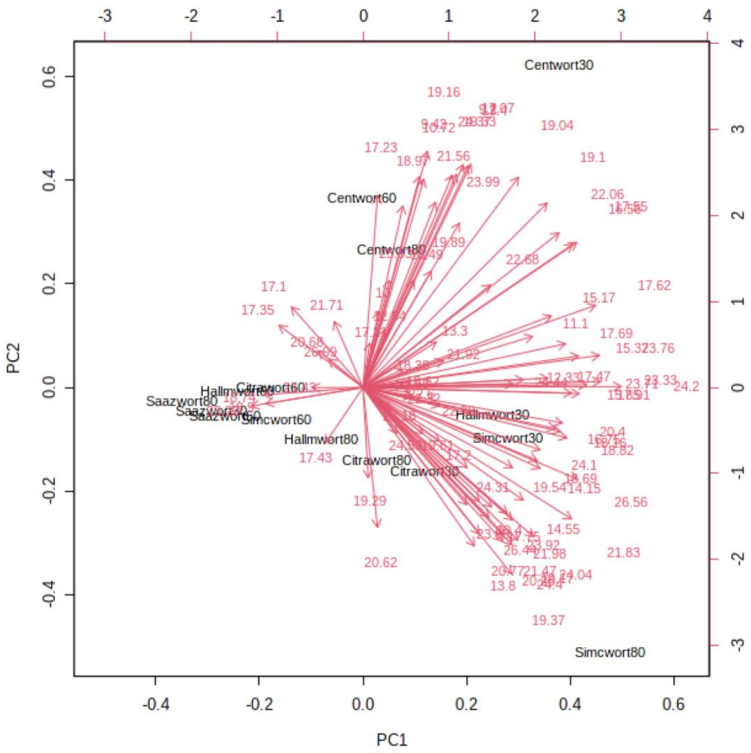


Figure G.2: Biplot showing how strongly each compound (here their respective retention time is shown) influences the principal components, based on auto scaled peak area data from all five hop varieties in wort at 30°C (low), 60°C (medium) and 80°C (high).

Name	Loadings 1	Loadings 2
24.2	0.2012	9.1442E-4
23.33	0.18506	0.0055699
23.76	0.18413	0.029861
17.62	0.18117	0.076511
23.71	0.17424	0.002541
17.91	0.1684	-0.0057067
15.37	0.16786	0.029409
26.56	0.16707	-0.085269
17.55	0.16662	0.13489
16.56	0.16336	0.13311
15.85	0.16252	-0.0051624
21.83	0.16244	-0.1223
18.82	0.15885	-0.046811
17.69	0.15783	0.04076
20.4	0.15533	-0.032942
18.16	0.15366	-0.040875
22.06	0.15265	0.14391
16.75	0.14995	-0.038175

Figure G.3: Table of loadings for the auto scaled data of wort samples at different temperatures. It is rearranged so that the variables, i.e. compounds (here their retention time is shown), that contribute strongly to principal component 1 are listed in order. Respective compounds for the retention times can be found in appendix E.

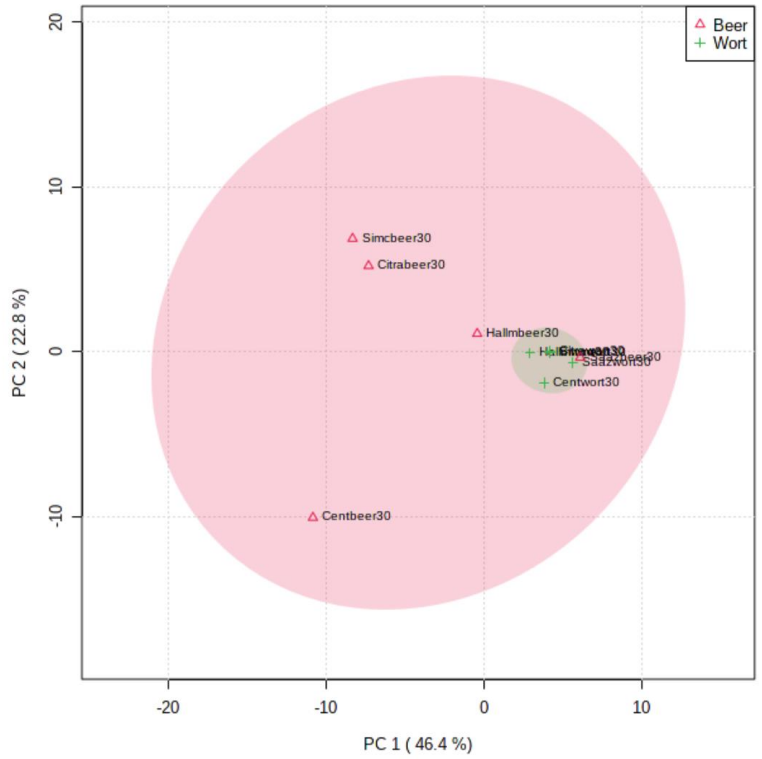


Figure G.4: Principle component analysis (PC1 and PC2) 2D score plot of auto scaled peak area data from all five hop varieties in wort and beer at 30°C with ethyl acetate removed. PC1 and PC2 account for 69.2% of the total variation. Colored circles represent 95% confidence intervals.

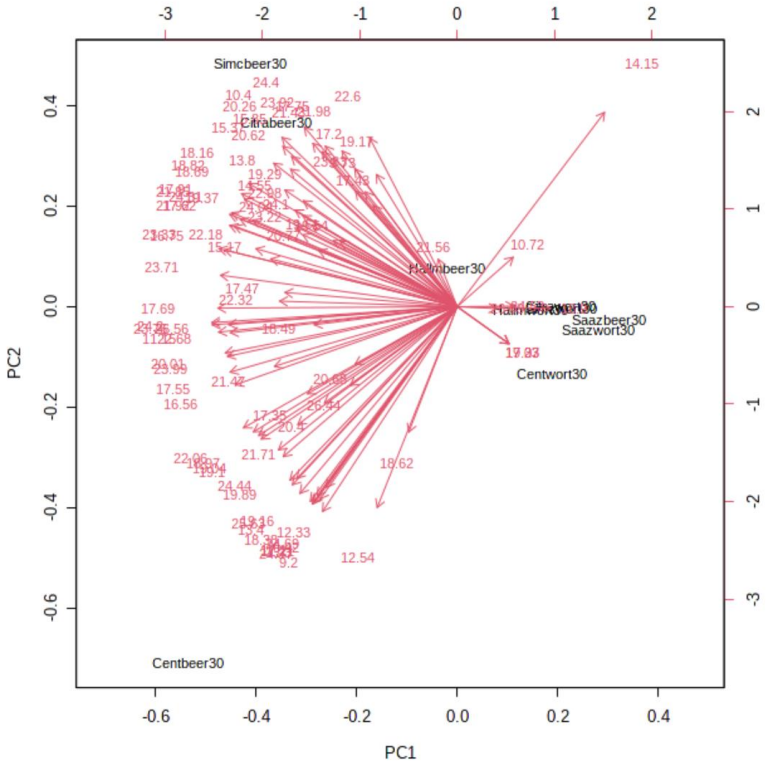
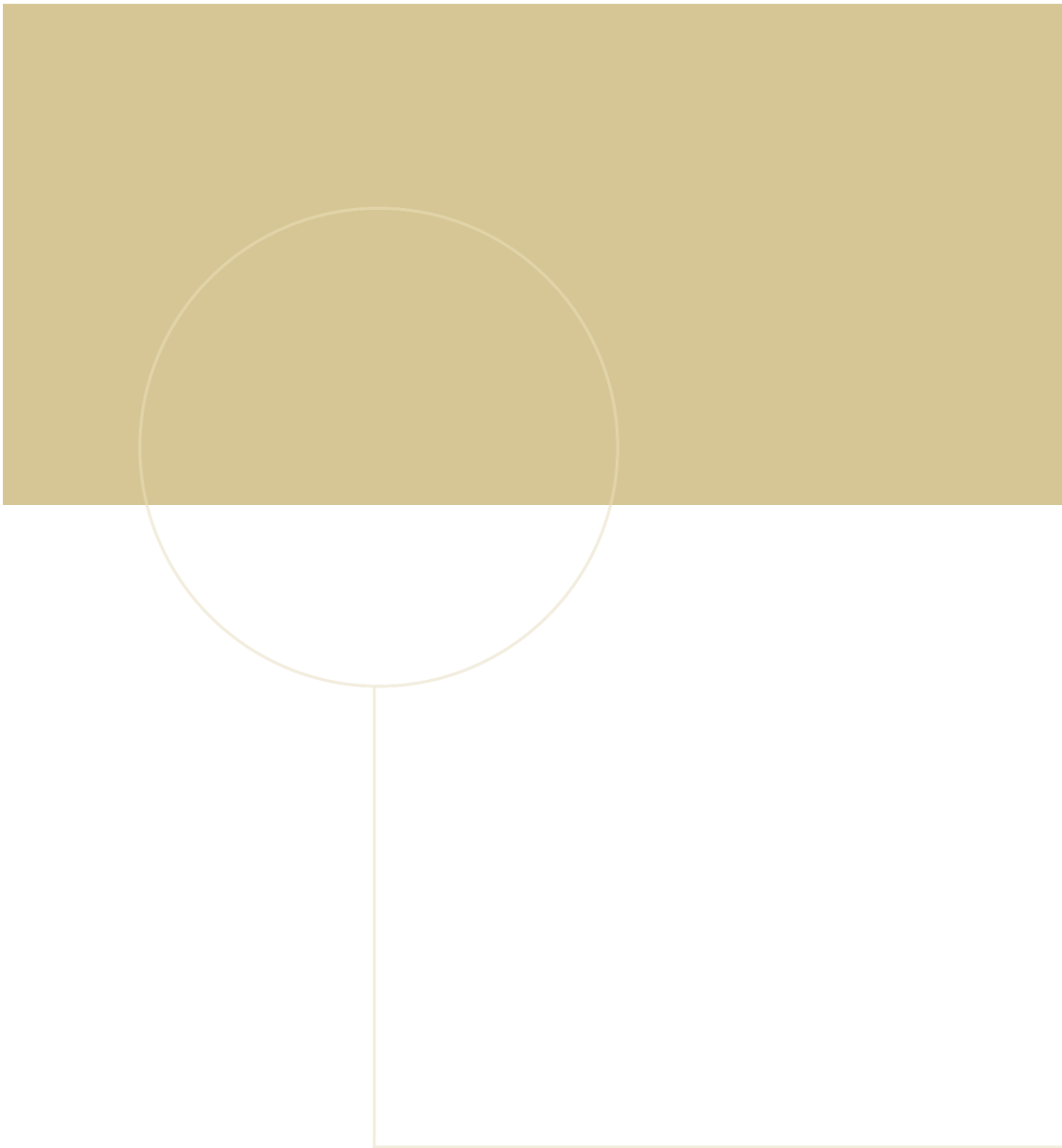


Figure G.5: Biplot showing how strongly each compound (here their respective retention time is shown) influences the principal components, based on pareto scaled peak area data from all five hop varieties in wort at 30°C (low), 60°C (medium) and 80°C (high).

24.2	-0.1558	-0.014365
23.76	-0.1556	-0.016419
17.69	-0.15197	-0.0013251
11.15	-0.15152	-0.023011
23.33	-0.15133	0.052445
23.71	-0.15031	0.028462
16.75	-0.14785	0.051413
20.01	-0.14726	-0.041793
23.99	-0.14589	-0.044978
26.56	-0.14519	-0.016233
21.92	-0.1445	0.073524
21.85	-0.14442	0.083549
17.55	-0.14418	-0.059231
22.68	-0.14406	-0.023675
17.91	-0.14316	0.085043
17.62	-0.14155	0.072877
16.56	-0.14081	-0.070588
24.31	-0.13779	0.07924
18.82	-0.13692	0.10232

Figure G.6: Table of loadings for the auto scaled data of wort versus beer samples. It is rearranged so that the variables, i.e. compounds (here their retention time is shown), that contribute strongly to principal component 1 are listed in order. Respective compounds for the retention times can be found in appendix E.



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