Norwegian University of Life Sciences
Department of Chemistry, Biotechnology and
Food Science

Master Thesis 2015
30 credits

# Development of aromatic hop compounds and bitterness in beer during room temperature- and cold storage based on three different hopping methods

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# ACKNOWLEDGEMENTS

This thesis work has been carried out at the Department of Chemistry, Biotechnology and Food Science at the Norwegian University of Life Sciences in Ås, with Professor Trude Wicklund, Chief Engineer Ellen Skuterud and Senior Advisor Elisabeth Fjærvoll Olsen as supervisors.

First, I would like to thank the Nøgne Ø for suggesting the thesis, letting me come and spend a week brewing with them and for donating the hops used in the trials.

A special thanks go to Trude Wicklund, Ellen Skuterud and Elisabeth Fjærvoll Olsen for your support, and for letting me learn how to use the brewery.

I would also like to thank my family and friends for the support and patience you have shown. A big thank you to my tasters, and to my beer oracle, Matt. Finally, thank you Amber, for reading every word I wrote, and for believing in me. Thank you!

Ås, August 15th 2015

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# SAMMENDRAG

Hovedmålet med denne studien var å fastslå om lagringstemperatur eller humlingsmetode hadde innflytelse på aroma og bitterhet i øl. Fokus ble satt på aroma som kommer fra humle, og ikke fra gjær. Det sekundære målet for denne studien var å undersøke utviklingen av alkohol, $CO_2$ og bitterhet i øl etter priming og tapping. Avhandlingens perspektiv er sett fra hovedsakelig hjemmebryggingsperspektivet, og til en viss grad mikrobryggerier.

Øl ble brygget med 100 % pilsner malt og humlet med Cascade og Vic Secret humlepellets. Cascade ble brukt både til bitterhet, og aroma. Vic Secret ble bare brukt til aroma. Tre batcher øl ble brygget to ganger. Ølet ble brygget i 60 L bryggeriet i Pilotanlegget på NMBU. Alle tre batcher ble kokt i 90 minutter. De ble tilsatt den samme mengden Cascade humle for bitterhet i 60 og 30 minutter under koking. Brygg 1 og 4 ble tilsatt aromahumle i 5 minutter, brygg 2 og 6 ble tilsatt aromahumle ved 80 °C etter koking, brygg 3 og 6 ble tilsatt aromahumle (tørrhumlet) samme dag som ølet ble stukket om. Etter tapping ble halvparten av flaskene lagret ved 4 °C, og den andre halvparten ble lagret ved romtemperatur. Hele prosessen ble gjentatt en gang. Dette ga en total på seks batcher hvor brygg 1 og 4, 2 og 5 og 3 og 6 har gjennomgått samme humlebehandling. Seks brygg, og to forskjellige lagringstemperaturer ga 12 flasker som ble testet syv ganger i løpet av to måneder. På dag 1, 5, 10, 20, 30, 45 og 60 etter tapping ble prøvene tatt. Aroma ble målt i øl som var lagret ved begge temperaturer ved hjelp av GC-MS. GC-MS resulterte i påvisning av typiske humle og gjær aromaer, men et tydelig mønster i utviklingen av aromaer ikke ble funnet.

$CO_2$, prosent alkohol, farge og turbiditet ble målt hver prøvedag ved hjelp av Anton Paar Alcolyzer. Utviklingen av alkohol og $CO_2$ økte raskt de første 5 dagene etter tapping, for deretter å stabilisere seg etter prøvedag 20. Farge og turbiditet ble redusert under lagring, og lagringstemperaturen så ikke ha en betydelig effekt på disse.

Bitterhet ble målt ved hjelp av UV spektrofotometri. Ølet holdt en bitterhet på mellom 40 og 60 IBU etter tapping, noe som tyder på at utviklingen av bitterhet ikke er bestemt av den utregnete bitterheten, og mengde humle som er tilsatt.

Sensorisk evaluering ble utført på dag 30 og 60 etter tapping. Den sensoriske vurderingen ble utført ved anvendelse av en trekant test hvor romtemperatur lagret øl ble sammenlignet

med 4 °C kald lagret øl. Det semi-trente panelet var ikke i stand til å bestemme hvilke av øl hadde mer aroma.

# ABSTRACT

The main objective of this study was to determine whether storage temperature or hopping method had influence on the aroma and bitterness in beer. The focus was set on the aroma that comes from hops, and not from yeast. The secondary objective to this study was to explore the development of the alcohol, $CO_2$ and bitterness in the beer after priming and bottling. The thesis' main perspective is that of home brewers, and to some extent that of microbreweries.

Beer was brewed with 100 % pilsner malt and hopped with Cascade and Vic Secret hop pellets. Cascade was used both for bittering and for aroma. Vic Secret was only used for aroma. Three batches of beer were brewed two times. The beer was brewed in the 60 L brewery in the Pilot plant at NMBU. All three batches were boiled for 90 minutes. The same amount of Cascade hops were added to all three batches for bittering for 60 and 30 minutes boiling time. Aroma hops were added to brews 1 and 4 for 5 minutes, and to brews 2 and 6 at 80 °C post boiling. Aroma hops were added to brews 3 and 6 (dry hopping) on the day of racking into the secondary fermenter. After bottling, half of the bottles were stored at 4 °C and the other half were stored at room temperature. This process was repeated once ending with a total of six batches where batches 1 and 4, 2 and 5, and 3 and 6 have undergone the same treatment. Six brews and two different storage temperatures resulted in 12 bottles to be tested seven times over the course of two months. At day 1, 5, 10, 20, 30, 45 and 60 after bottling samples were gathered. Aroma was measured in the beers stored at both temperatures using GC-MS. The GC-MS resulted in detection of typical hop and yeast aromas, however a clear pattern in the development of the aromas could not be found.

$CO_2$, percent alcohol, color and haze were measured on the test days using the Anton Paar Alcolyzer. The development of the alcohol and $CO_2$ increased fast the first 5 days after bottling and then seemed to stabilize after day 20. Color and haze both decreased during storage, the storage temperature did not have a significant effect on these.

Bitterness was measured using UV spectrophotometry. The bitterness kept fluctuating between 40 and 60 IBUs after bottling, suggesting that development of bitterness is not set by the calculated bitterness and the amount of bittering hops added.

Sensory evaluation was executed on days 30 and 60 after bottling. The sensory evaluation was carried out using a triangle test where room temperature stored beer was compared to 4 °C cold stored beer. The semi-trained panel was not able determine which of the beers had more aroma.

# Innhold

# INTRODUCTION

Beer is an alcoholic, often carbonated drink made from water, malt, and yeast, and very often also hops. There are many ways of tweaking the process of how beer is brewed and each little tweak gives a different beer. For example it is possible to count 79 different beer styles on CraftBeer.com (BrewersAssociation, 2015). The different styles are based on a variety of elements such as where they originate, the yeast, the combination of malts, type and amount of hops to mention a few.

The sudden growth and interest in home brewing and microbreweries, has developed new breeds of hops, crossbreeds, and breeds that have exceptionally high amounts of alpha acids and breeds with complex aroma profiles (Hieronymus, 2012). Hops have mainly two purposes in the beer. One is to create bitterness. As the alpha acids isomerizes and creates the bitter components alpha iso-acids. The bitterness does not just add flavors to the beer, it also prolongs the beers' shelf life. This again is useful and important in micro brewed beer as it is often not filtered or even pasteurized. The other purpose is to create aroma. However, Aroma is a volatile compound which gets lost while the wort boils. The longer the hop boils the more bitter the brew becomes (until a certain point), and the more aroma evaporates. To obtain the aroma components from the hop the method of hopping plays a substantial role. Some hopping methods are: finishing hops, adding hops when there are five minutes left of the boil, and whirlpool hopping, which is to add hops after the boil is over and the wort is transferred to the whirlpool tank. These methods can create different taste and aroma profiles in the beer. While the finishing hops method will isomerize some of the alpha acids to iso-alpha acids and thus make the wort even a little more bitter, the whirlpool added hops method may retain more of the aroma in the wort and give the beer more flavor.

Developing new hops, and not using hops for their complex aroma profiles, is fairly new, and has had an explosive development the last few years. The cultivation of hops and developing new breeds and cross breeds dates back to the early 1900s when Ernest S. Salmon, the head of hop breeding at Wye College, made crossbreeds between American wild hops and English

cultivated hops. One of these crossbreeds he named Brewers Gold, which may be the ancestor of several of the new popular American hops such as Citra and Mosaic (Hieronymus, 2012). When developing new, and improving old, hop breeds increased speed, the aim was first to develop hops that had higher amounts of alpha acids. This was solely for the big corporations and hop farmers. The more alpha acids the hops had, and the more bitterness the hops could produce per the weight of the hops, the better was the pay. At some point there was a shift and the interest in other traits became more evident. The resurgence of microbreweries which were on the hunt for different flavors other than the commercial beer flavors, gave a boost to local hop farmers to try something new. The "Shop Local"-movement also had a positive effect. Hop farmers started growing hops that had more exciting traits than just being high in alpha acids (Hieronymus, 2012).

As of 2010 the fastest growing beer in popularity has been the modern IPA with several variations. The classic IPA is an English invention stemming from the colonial age, the 1700's, when England colonized India. The Englishmen were already then quite dependent on their English beer. Beer was sent in barrels across the oceans, to quench the thirst of the English colonists, only to arrive ruined. This gave birth to the origin of the Indian Pale Ale, commonly known as IPA. Excessive amounts of hops were added to the boil of the wort, creating a very bitter beer. The bitter beer was once again sent to India and this time the beer survived all those months onboard the boat. The IPA has been developing ever since, but its real breakthrough came with the craft beer revolution (Steele, 2012). Craft brewers started wanting other hops, hops that tasted different and hops that had impressive amounts of alpha acids. Beers like the Crazy Bitch Double IPA (NorthwestBrewingCompany, 2012) with a calculated IBU of 100, and the Norwegian brewery Haandbryggeriet's Humlekanaon; an IPA with a calculated IBU of 160 provided a contrast to the IPAs with up to 10 different hops in the single hopped IPA. These are IPAs brewed with the use of one single hop, like Nøgne Ø's Single Hop Citra IPA (NøgneØ, n.n.), and Hopworks' IPX Single Hops Series (HopworksUrbanBrewery, 2014). Every brewery might have wished to stand out and be the first to come up with a new technique or to have a unique beer. This has caused the brewing of many different IPAs, not just in which hops have been used, but also in which hopping methods have been used. While some breweries have intricate ways of adding hops, for instance the 120 Minute IPA from Dogfish Head (DogfishHead, 2015), where hops are added

every 3 minutes of the 120 minute long boil and then dry hopped every 12 hours for two weeks, other breweries swears to using only bittering hops and dry hopping. The modern IPAs are still developing and brewers look to find hops with new flavor profiles. This desire has resulted in hops such as Mosaic with blueberry aromas and the very citrusy Meridian, the orange flavored Mandarina Bavaria and the Huell Melon that has aromas of honeydew melons (Woodske, 2013). The key to making a nice aromatic and balanced beer for the homebrewer is to experiment and carefully note every step, making it possible to repeat.

## 1.1 Developing a thesis

This master thesis came about through conversations with the Norwegian microbrewery Nøgne Ø's previous owner, Kjetil Jikiun. The thesis started developing in the fall of 2014 and came to its full content during a week-long internship at the Nøgne Ø's brewery in Grimstad in February of 2015. Through conversations with the brewmaster Edvard Hortemo, and the brewers and staff at Nøgne Ø, the idea formed. The IPA should be brewed with 100 % pilsner malt, two different hops and three hopping methods. The IPA should be stored at cold storage i.e. 4 °C, and at room temperature ~22°C. In addition to knowledge, time and hospitality, Nøgne Ø contributed with the hops that were used in the project, American Cascade pellets and Australian Vic Secret pellets.

## 1.2 INTENT

Contrary to the original IPA which was brewed very bitter to survive months on a boat sailing from England to India, the modern IPA is a beer sold and enjoyed as fresh as possible. This is due to exactly the use of more aroma hops in the beer. It is a known fact that aromas are volatile and will degrade with storage. The aim was to brew a modern IPA style beer using two aroma hops, like a IPA may be brewed in a microbrewery. Therefore this thesis is setting out to explore how the aroma changes with the use of three different hopping methods – 5 minute boil, 80 degrees post boil and dry hopping – at two different storing temperatures; cold storage (4 °C) and room temperature (~22°C). How beer tastes is a matter that concerns not just the breweries, but also the customers. In an attempt to decide whether or not modern IPA should be cold stored from the time of bottling/after developing $CO_2$, or if it is

the same being stored in room temperature the trial will also look into the sensory aspect of keeping the aroma. A semi trained panel will decide if there is a significant difference between cold stored and room temperature stored beer. In addition, this thesis explores how the alcohol, bitterness, color of the IPA and $CO_2$ develops during the first two months after brewing and during storing at these two temperatures.

# 2 THEORY

People have been successfully brewing beer for centuries. With the craft beer revolution, and people getting a taste for brewing, realizing anyone can do it, that's where the science comes in. "How does the process work and why? What happens when the brewing goes wrong? What makes one beer taste dry and bitter while another using the same ingredients taste fruity and sweet?"

## 2.1 Brewing

Brewing beer in short means steeping malted grains in hot water to make a wort, then boiling the wort, adding some hops, cooling the wort, then adding yeast and letting it sit and ferment.

Because of the flavor the most commonly used is malted barley. Malted barley has a husk that allows the sweet liquid wort to exit and the rest of the spent grain to remain in the husk (Steele, 2012). To access these starches the grains must be crushed, not to fine powder, but cracked just enough so when the malt is steeped in water the water can access the starches and gelatinize them. The bulk part of the starches in malted barley gelatinizes at temperatures between 60 and 65 °C. The aim with steeping the malt is to convert the starches into fermentable sugars. The conversion is mainly done by enzymes that work at certain temperatures. These enzymes are already present in the malt and will be active when the malt is steeped. As the starches gelatinize, the enzyme alpha-amylase starts working on cutting the long stranded starches into smaller chains called dextrins (Palmer, 2006). This happens during the mashing process. To help the enzymes break down the starches during the mash, different temperatures can be utilized. These are temperatures where the enzyme activity is optimized. A summary of the major enzyme groups found naturally in malted barley and their active range is listed below.

Phytase (30 – 52 °C) – Lowers the pH of the mash. The Phytase- or the acid rest is rarely used by modern brewers except in areas where the water is very pure and demineralized. Instead the mash usually reaches a lowered pH either with the use of darker, or specialty malts, or, for instance by adding calcium chloride (Palmer, 2006).

Beta-Glucanase (35 – 45 °C) – This enzyme helps to increase the solubility of beta-glucans, which are mostly broken down during malting, and this rest temperature is utilized when mashing with more than 20 % of unmalted grains. This rest temperature can be set for 20 minutes to break down the beta-glucan gums (Palmer, 2006).

Protinases and pepidases. (45 – 55 °C) – This is the protein rest temperature. During this rest temperature enzymes break down proteins, and produce free amino nitrogen (FAN). The FAN aids the fermentation as it provides a healthy yeast growth (Mallett, 2014).

Beta-Amylase (60 – 65 °C) – This is the enzyme that produces the bulk part of the maltose, which is the main sugar that is fermented in beer. Beta-amylase starts to denaturize at temperatures above 65 °C. A conversion rest set in the range of the beta-amylase activity temperature will give more fermentable sugars (maltose) and thus give more attenuable beer, i.e. more of the wort has sugars that ferment and this creates not only more alcohol but also a larger degree of fermentation making the beer less sweet and less viscous (Janson, 1996).

Alpha-Amylase (60 – 75 °C) – Produces a variety of sugars, including maltose and also some unfermentable sugars. Mashing at the higher end of this range produces more unfermentables and therefore more body in the finished beer. Because the higher temperatures favor the alpha-amylase, while beta-amylase starts to denaturize, there will be less maltose and more dextrins that will add viscosity and sweetness to the beer (Janson, 1996).

A step that is frequently used is the dough-in rest. This is the first step where the crushed malt encounters the water. Dough-in is useful to hydrate the malt before the conversion starts, to evenly distribute the grains in the water. The dough-in is usually performed at a temperature between 35 – 45 °C for a period of 20 minutes. During this time the pH might lower slightly since the low temperature allows for the phytase and other lower temperature enzymes to start working (Palmer, 2006). Both the beta-amylase and the alpha-amylase are included in the conversion rest, which is the main part of the mashing. For many single temperature infusion programs the mashing happens around 67 °C for 60-90 minutes before the mashout or lautering. Many home brewers use a single step mash and this will give a good result. The more advanced homebrewer, microbreweries and brewing with a

moderately modified malt[1] will often use a multi-rest mash. A multi-rest mash can be put together in various ways depending on what profile is wanted in the resulting beer. A good point of reference in a multi-rest infusion is the 40 to 60 to 70 °C mash schedule by George Fix (Fix, 1999).

Mashout usually is to increase the temperature to 77 °C before the lautering to deactivate the enzymes and to make the wort less viscous. In mashes where other grains are used, such as oats and wheat, and in thick mashes, this step is important so the grainbed will stay loose and favor a good flow. The mashout can last for 10-30 minutes.

Once mashout ends the mash is ready to be transferred to a lautering vessel where the mash is left to settle. After the settling of the grainbed, the wort must be recirculated. The wort is drawn from the bottom of the vessel which can be facilitated by having a false bottom or a container with a perforated bottom. The wort is poured back over the top of the grainbed; and the grainbed works as a filter. This way some of the cloudiness, husk and grain residue will be taken out of the wort. When the wort runs clear it is ready to be collected. The wort is again drawn from the bottom and transferred to the kettle. Collecting wort is a slow process and during this time the wort also needs to be sparged. The sparging rinses the grainbed for sugars and dilutes the wort. When sparging, the water should hold a temperature of 71.1 – 75.6 °C to avoid the extraction of silicates, tannins, fats and large proteins (Janson, 1996). The amount of water used for sparging may be calculated in advance. The easiest way is to use the same amount of sparge water as mash water. However it is also important to pay close attention to the gravity towards the end of sparging. The gravity can be adjusted by adjusting the amount of sparge water (Palmer, 2006).

There are a few different sparging methods. The one that is used by most breweries is the continuous sparge; where a small amount of water is constantly showered evenly over the grain bed till the wort has the desired gravity. Another sparging method is batch sparging. With batch sparging the grainbed is covered with a few centimeters of hot water and the

[1] Moderately modified malt is a malt where the germination has been stopped earlier in the process so these malts will yield less sugars and contain more proteins than a fully modified malt. MALLETT, J. 2014. *Malt : a practical guide from field to brewhouse,* Boulder, Colorado, Brewers Publications.

water is allowed to sink through as the wort is collected. Batch sparging is done several times, until the desired gravity is reached. When sparging with both continuous and especially with batch sparging it is important to pay close attention to the flow rate of the collecting wort. A too fast flow rate will cause the grainbed to set and be too dense for the sparge water to flow through. Therefore the grainbed and flow of wort must be monitored closely. If peaks of mashed grains are forming then either the wort is collecting too fast or the sparging is too slow (Palmer, 2006).

The gravity of the wort must be measured during sparging, usually more frequently towards the end of the sparge. To measure the gravity a hydrometer can be used or a refractometer that measures either °Brix or more commonly used °Plato. Measuring the gravity helps to predict the percent of alcohol that can be achieved in the beer. The wort should hold a lower gravity before the boil. Somewhat depending on the size of the kettle, the rule of thumb is that the loss of wort, which means mostly water, during the boil is 5 L/hour (Pierce, 2007). When water evaporates the wort becomes more concentrated. The pre boil gravity can be adjusted by adding more water to lower the gravity. In the case of over sparging and the wort getting too diluted, it is possible to get the pre boil gravity up by adding sugar (preferably DME – dry malt extract) to achieve higher gravity (Deeds, 2013).

To boil the wort correctly can have a great impact on the quality of the beer. The boil will sterilize the wort. In addition, bringing the wort to boiling will cease any enzyme activity and fixate the fermentable sugar content in the wort. A rolling or vigorous boil drives out oxygen, and oxygen can become a problem in the process. Oxygen can change the color darker than desired, and it may aid serious infections to develop in the beer. Even more important, the boiling process causes the breakdown of proteins. This can only happen when the boiling is vigorous. The boil will cause hot-break, which is the formation of proteins and phenols that denaturizes and sticks together. These particles then will concentrate on the wort-air-steam bubbles. Because of the high concentration they will aggregate into larger and larger masses. Proteins are unwanted in the beer as they cause haze and tannins can cause off flavors in the beer (Fix, 1999).

Boiling should not take less than 90 minutes. The minimum time is explained by the following; Sterilization requires about 5 minutes; 15 minutes will denature the enzymes and

thus deactivate them. Another 15 minutes are required to eliminate tannins which originate from the malt husks. This first half hour is to decompose and precipitate some of the proteins. This should be accomplished before hops are added to avoid the sticky hop resins from combining with the protein flocks and precipitating out of solution. Except for high gravity beers, the total boiling time should not last longer than 2 hours. Boiling the hops longer than one hour will start generating sharp, undesirable and unpleasant flavors. During a long boil a greater percentage of the hops' bittering and preservative qualities are carried into the finished beer (Janson, 1996).

Dimethyl sulphide (DMS) is an intensely aromatic compound present in most beers. However if it is present in large amounts, it can be tasted and smelled. When this happens it is considered a major defect. At low levels it smells of corn or sweet corn. When it is more intense it can resemble cooked cabbage or even garlic. DMS is formed from s-methyl-methionine (SMM), which in turn is produced from amino acids during malting. SMM is converted to DMS by heat. DMS is volatile and most of this component will evaporate during the boil, given that home brewers boil the wort without a lid and commercial breweries have ventilation that will suck the vapor off during boiling. Unless the precursor is all removed, more DMS can be formed during wort clarification and this DMS will survive to the final beer. Usually this can be a problem in commercial breweries during the use of whirlpools. This is one of the reasons why wort should be cooled as quickly as possible after the boil is complete (Fix, 1999).

Another event that happens during the boil is a color change, the color gets darker caused by a combination of several factors. The caramelization of wort sugars darkens the wort as it boils, due to the breakdown of the sugars to simpler carbon structures. The caramelization of wort can also bring new flavors to the beer which may not be favorable in the finished beer. To add both color and taste to the beer it is easier to control the desired flavor and color by adding darker malts than to caramelize the wort itself (Janson, 1996).

During the boil is when the bittering hops are added. The alpha-acid oils in hops need to be heated to isomerize, which causes them to be soluble in wort and creates the bitter taste in beer. Hops also contain a plethora of volatile flavor and aroma compounds. The longer the hops are boiled, the more of these components are boiled off. Thus, adding the hops at the

end of the boil will result in more of these volatiles being present. As long as the wort is hot the volatiles will still be vaporizing slowly.

Hops contain two kinds of aromatic material: bitter resins and essential oils. Bitter resins require vigorous boiling and relatively longer time for dissolution. During this, most of the essential oils leave the kettle with the steam. Another important requirement for hops processing is the pH level of the wort: it is supposed to be around 5.5 and 5.8 initially. Vigorous boiling will reduce this by 0.2-0.3 to the near minimum level of 5.2 under which no coagulation of proteins takes place. Although hop utilization increases at higher pH, a finer or less harsh bitterness can be achieved at lower pH. As tannin combines with proteins in the unoxidized state, these protein-tannin complexes form with other proteins that do not coagulate and precipitate during the boil, causing chill-haze. These proteins are soluble in hot wort, but will precipitate in chilled wort. Although these proteins are not as heavy as the hot-break proteins, they will float and will not totally form sedimentation. Hot break will reduce the chances of chill-haze development. The oxidized tannin on the other hand, is called phlobaphene, and it combines with protein. Phlobaphene-protein complexes are insoluble in water, therefore precipitate in the hot break. Since most of the essential oils of hops are lost in the boiling process, a beer with a good hop aroma requires additional hops after boiling. Finishing hops are usually added within the last minutes of boiling, or as the wort is struck from the kettle. There are several components that can form haze in beer. The most common haze is made of proteins, other compounds that can form haze is polyphenols, glucans, inorganic matter and calcium oxalate. The polyphenols come from hops and malt. Glucans come from starch in the barley that have not been fully degraded. Inorganic matter is usually compounds from dirt and dust. And the calcium oxalate originate from oxalic acid and calcium, the oxalic acid is present in the malt and calcium is present in water, or added during the brewing as calcium chloride (Steiner et al., 2010).

At the end of the boil, it is important to cool the wort quickly. While it is still hot, (above 60 °C) bacteria and wild yeasts are inhibited. Because the wort is rich in nutrients, once the wort starts to cool below 60 °C it will become vulnerable to infections. A way of reducing the risk of spoilage is to have sterilized equipment, and make the fermentation start quickly. The fermentation serves to stabilize the wort against most contaminants. The most frequent bacteria to spoil wort are Gram-negative enterobacteria, especially species of *Klebsiella,*

*Citrobacter, Enterobacter, Obesumbacterium,* and *Escherichia*. These bacteria can produce DMS, organic acids, and 2,3-butanediol. Growth of enterobacteria can inhibit the growth of *Saccharomyces* (Bokulich and Bamforth, 2013). The wort is also susceptible to oxidation damage as it cools. The objective is to rapidly cool the wort to below 27 °C before oxidation or contamination can occur. During rapid cooling, the cold-break forms. The cold-break is a group of proteins which must to be thermally shocked into precipitating out of the wort (Fix, 1999). The lack of a cold-break will leave these proteins in the beer and end up causing chill haze. When beer is chilled for drinking, these proteins partially precipitate and form a haze.

### 2.1.1 Malt

Malt is the essential part of brewing, without malt, no beer. Barley is malted to release the starch in the grain so it can be converted to the sugar maltose. This sugar is what gives the malty flavor to beer and also whiskey. Barley is a member of the grass family and is one of the most cultivated grains in the world. There are three different types of barley, two row-, four row- and six row barley where the two row barley is considered the best, and the four row barley is useless as malt. Briefly, the barley becomes malt by being harvested, sorted, dried, cleaned and stored. Furthermore the actual malting process begins when barley is soaked in water to start germination of the grain. The germination process is stopped when the grains have sprouted and the sprouts have attained a length that is three quarters of the grain (Mallett, 2014). The sprouted grains are tumbled to get rid of the sprout itself as this contains large amounts of proteins and amino acids. The malt is then dried to a water content of 4 % (Mallett, 2014) and kilned. The malt, kilned at 80-85 ° C becomes either pilsner malt or pale malt. The malt may also be kilned at higher temperatures to achieve darker colors and other flavor profiles. There exist a vast number of malts and all of them have a specific attribute that makes them different from each other. Pilsner- and pale malt are a group called standard processed malts, this group also contains Vienna malt, Munic Malt, and Melanoidin Malt (Mallett, 2014). The darker of the standard processed malts are aromatic malts. These are a group of toasted malts that are dried at a higher temperature than the pale base malts, ~ 50 ° C, and with less ventilation, so that drying of aromatic malt takes about twice as long as drying of the pale malts. After 24 hours the moisture content will be about 20 %. This way of drying the malt provides a greater loss of enzymes, but it

gives a higher amount of soluble sugars. When these malts are kilned Maillard reactions will take place and provide melanoidins which are large, colored polymeric compounds (Mallett, 2014). It is these which give a caramel-like flavor and golden color to the aromatic malts. Roasted malts are the darkest types of malts. These malts are widely used in darker ales such as porters and stouts. Black malt is burned at approximately 230 ° C for 2 to 2.5 hours. Chocolate Malt is burned at about the same temperature, but for 1 to 1.5 hour. The enzymes in chocolate and black patent have all been destroyed during roasting. These grains are only used to darken, and impart a roasted, coffee-like or burnt taste to beer. Crystal malts or caramel malts are malts where the initial drying is skipped and the green malt is directly heated to 60-70 ° C for 1 to 1.5 hour without ventilation and humidity is kept high so that the starch in the grain is converted to sugars. The liquid sugars furthermore undergoes caramelization while subsequently increasing the temperature to 150 ° C for 1-2 hours, depending on how dark the caramelization should be (Mallett, 2014).

### 2.1.2 Hops

Hops are the strobiles of the climbing vine growing hop plant. In Latin their name is *Humulus lupulus* which refers to their lupulin glands found at the base of the bracts, and these glands contains α-acid humulone resins (Palmer, 2006). Their leaves resemble those of grape plants, and the strobiles look like yellow to light green soft pine cones. The hop plant is a hardy plant that can grow in poor soil and cold weather climates, but they thrive in more temperate areas (Hieronymus, 2012). Hops have been used to add bitterness and aroma and also to increase the shelf life of the beer. Figure 1 shows a cross section of a hop cone. The resins and essential oils that the hop is wanted for are found in the lupulin glands.

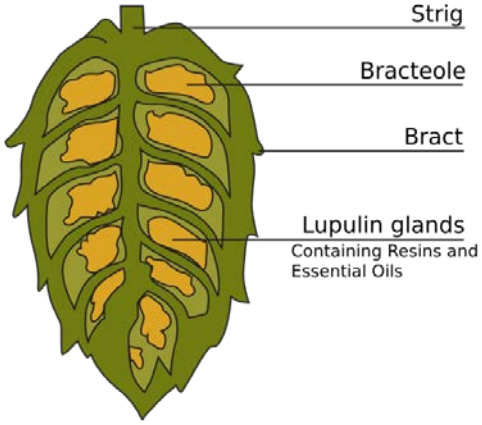



**Figure 1: Cross-section of a hop cone with its' main components.(Wikipedia, 2008)**

Hop bitterness is derived from the alpha acids contained in the lupulin glands. These are known as humulones. Humulones typically make up 2 to 14 percent of the hop's dry weight. When the label on the hop bag says 10 % alpha acids this means that 10 percent of the hops' weight is made up of humulones. The alpha acids are measured at harvest and decreases with storage, as alpha acids degrade with time, and how they are stored plays an important part in keeping the hops fresh. Alpha acids are not soluble in wort however, boiling the wort causes a chemical reaction called isomerization that transforms the alpha acids into iso-alpha acids. Iso-alpha acids are soluble in the wort, and these are what create the bitterness. The longer hops are boiled in the wort the more alpha acids isomerize, thus increasing the bitterness. Iso-alpha acids not only add bitterness, they also inhibit the growth of certain bacteria and aid in foam retention and cling. They are also the source for skunkiness in green- and clear-bottled beers. When light reaches an iso-alpha acid, it will react with sulfur that is present in the beer. To avoid skunky beer the easiest solution is to use brown beer bottles. Bitterness in hops also comes from the beta acids called; lupulones. The beta acids have little significance in the brewing process, however as the beta acids age they become more bitter. During storage, beta acids degrade due to oxidation. Unlike alpha acids, the beta acids' oxidation products are bitter. Oxidized beta acids smell like cheese. This is due to volatile fatty acids that are released from oxidized beta acids. These volatile fatty acids are identical to compounds found in aged cheeses, such as parmesan (Hieronymus, 2012).

When using hops for brewing, the quality of the hops needs to be excellent. Hop quality is affected by seasonal variations, hop packaging, hop storage, and the age of stored hops. Hops must be stored cold as hop deterioration is directly related to temperature. Cold storage greatly extends the hops' shelf life. Hops should not be exposed to air as oxygen breaks down alpha acids and causes beta acids to smell like cheese. Hops are compressed into hop plugs, hop bales, or pellets to minimize oxygen damage. Even compressed, the hops need proper packaging. Some of the best packages on the market are vacuum packing- or nitrogen packing bags that do not allow oxygen to enter. Plastic storage bags, for example, allow oxygen to enter the package (Hieronymus, 2012).

Hops are generally available to home brewers in three forms whole hop cones, pellets and plugs. $CO_2$ hop extracts have also entered the market. Whole hops consist of the hop cones dried and pressed into bales. The other two forms are both pelletized as Type 90[2] and Type 100 pellets. The Type 90s are typically called pellets and Type 100s are called plugs. The Type 90 pellets are made by breaking up a bale of hops, pulverizing the hops in a hammer mill, and forcing the resulting gummy powder through an extrusion die. In the pelletizing process the lupulin sacs are ruptured and release the resins. The resin acts as both the binder that holds the pellets together, and as the seal that protects the pellet's interior. Hardened resin protects the pellets from oxidation. Pellets are therefore stored better than whole hops (Steele, 2012). The plugs resemble the shape of a hockey puck and weighs 15 grams. Although these are a type of pellet they have not undergone the same treatment as the Type 90 pellets. The plugs are compressed whole cones that will leave more of a residue than the pellets do (Steele, 2012).

#### 2.1.2.1 Aroma

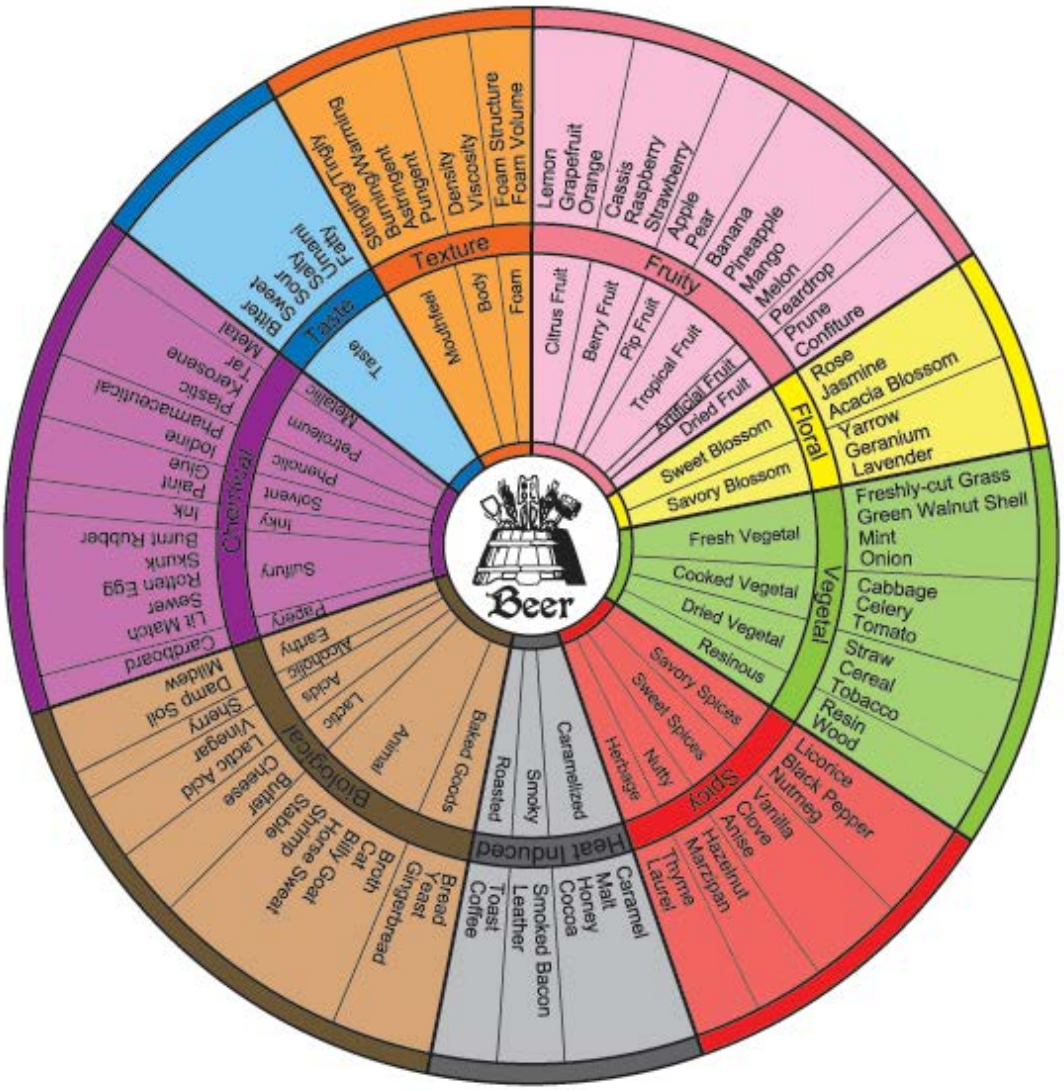
Aromas are volatile compounds that are connected to flavor. While the basal tastes; salt, sweet, sour, bitter and umami are tasted on the tongue, aromas are tasted through the nose (Lawless and Heymann, 2010). There are many more aroma compounds than the basal

[2] The number 90 comes from the fact that 90 % of the original hop weight is retained in the pellet, type 100 therefore means 100 % of the hop is retained. STEELE, M. 2012. *IPA : brewing techniques, recipes, and the evolution of India pale ale,* Boulder, Colo., Brewers Publications.

tastes, ranging from grassy and floral flavors to rotten and rancid. Aroma compounds can be detected doing chemical analyses using gas chromatography. Gas chromatography (GC) is a commonly used technique in analytical chemistry. The method displays good resolution and an ability to distinguish very similar chemical compounds. The principle behind all chromatographic methods is the same. A sample is dissolved in a mobile phase and transported through a stationary phase. Separation is achieved because the various components in the sample have different affinity for the stationary phase, and move at different speeds through it (Ahuja, 2003). In gas chromatography, the mobile phase is an inert gas, while the stationary phase can be a solid or a liquid attached to an inert solid inside a column. The data that is collected from the detector is shown visually in a chromatogram. The peaks in a chromatogram are displayed usually in a coordinate system with retention time plotted on the x-axis and the signal on the y-axis (Stuart and Royal Society of Chemistry (Great Britain), 2003). Retention time is the time a compound uses through the column from injection to detection (Ahuja, 2003).

Hop aroma compounds stems from the essential oils found in the hops. These oils make up 1 to 1.5 percent of the hop's dry weight. There are more than 200 essential oils found in hops. How these oils are distributed makes up the fingerprint of the hop variety. Four of the essential oils important in beer are known as the terpenes: myrcene, humulene, caryophyllene, and farnesene. Terpenes are also found in fruits, flowers and herbs. This is why some hop varieties give aromas that for instance resemble certain fruit aromas. The terpenes are very volatile and most are lost during the kettle boil. The most abundant of the terpenes is Myrcene with 30-60 % of the total amount of essential oils in the hop. This is also one of the more volatile compounds and will for the most part be lost during boil. Therefore, this compound is of importance when it comes to late addition hopping and post boil hopping (Steele, 2012).

The vast amounts of flavors that may occur in beer are described in figure 2. *The flavor wheel of beer* has been developed by Hochschule RheinMain University of Applied Science (Hieronymus, 2012). Although this is a flavor wheel for beer, it is not for hops specifically, but these flavors all occur from the hops, the yeast, the malt and the interactions between them.




# THE BEER FLAVOR WHEEL

**Figure 2: The beer flavor wheel, a graphic representation of the flavors that can occur in beer. This flavor wheel makes it easier for brewers to communicate with for instance consumers about different flavor profiles of different beers.**

Several of the aromas that come from hops have been found and described. A full compendium of hops and their aromas is still under development; the *Hop Aroma Compendium* by the Barth-Haas Group. About the Hop Aroma Compendium they say: *"This book offers the detailed aroma and flavor descriptions of 48 different hop varieties from the USA, Australia and Europe. No other raw material used in brewing has such a great influence on the aroma, flavor and bitterness of the beer as hops. And no other raw material has as many aromas as hops. In the last few years, there has been a growth in the number of brewers who wish to rediscover hops in order to better differentiate their beers. They want to know not only the alpha or oil content; they are interested not only in technical values. What they want to know is how the hops smell, what aromas they have and what effect these aromas may have on the finished beer."*

In beer, aroma does not only come from hops, it also come from the malt itself and from the yeast. In addition, certain aromas will also come from hop-yeast interactions. Some of the aroma compounds that can be found in hopped beer are listed in table 1 from the book *For the Love of Hops* by Stan Hieronymus, 2012.

Table 1: Various aroma compounds that stems from hops.

| Aroma Compounds Found in Hops and Hopped Beers | |
|---|---|
| 2-methylbutyric acid | cheesy |
| 3-methylbutyric acid (isovaleric acid) | cheesy |
| 3-mercaptohexan-1-ol (3MH) | black currant, grapefruit |
| 3-mercaptohexyl acetate (3MHA) | black currant, grapefruit |
| 3-mercapto-4-methylpentan-1-ol (3M4MP) | grapefruit, rhubarb |
| 4-mercapto-4-methylpentan-2-one (4MMP) | black currant |
| Alpha-pinene | pine, herbal |
| Beta-pinene | pine, spicy |
| Beta-ionone | floral, berry |
| Caryophylla-3,8-dien-(13)-dien-5-beta-ol | cedarwood |
| Caryophyllene | woody |
| Cis-3-hexenal | green, leafy |
| Cis-rose oxide | fruity, herbal |
| Citral | sweet citrus, lemon |
| Citronellol | citrusy, fruity |
| Ethyl-2-methylbutyrate | fruity |
| Ethyl-2-methylpropanoate | pineapple |
| Ethyl-3-methylbutonate | fruity |
| Ethyl-4methylpentanoate | fruity |
| Eudesmol | spicy |
| Farnesene | floral |
| Geraniol | floral, sweet, rose |
| Gumulene | woody, pine |
| Isobutyl isobutyrate | fruity |
| Limonene | citrus, orange |
| Linalool | floral, orange |
| Myrcene | green, resinous |
| Nerol | rose, citrus |
| Terpineol | woody |

Except the four terpenes Myrcene, Humulene, Caryophyllene, and Farnesene, it may turn out to be somewhat of a surprise to see what different aromas the GC-MS may detect during testing.

### 2.1.3 Hopping methods

There are a number of different hopping methods, all of which give a somewhat different flavor profile and to some extent also the same result. All hops that are boiled, whether added in the mash or during boiling will give a bitter taste due to the alpha-iso acids that

form from alpha acids in the hop (Hieronymus, 2012). The most volatile aromas will evaporate during boiling.

Mash hopping is a technique which has had its renaissance along with the craft brewer revolution. It consists in adding either a part of the bittering hops, or the late addition hops, directly in the mash. This method of hopping will not add a substantial amount of bitterness to the brew since the temperatures in the mash tun are generally too low to isomerize much of the alpha-acids in the hops. Some of the hop flavors will however remain in the wort (Hieronymus, 2012).

Another early hopping method is first wort hopping. This method means that hops are added during lautering in the fresh new wort. These hops will remain in the wort throughout boiling, thus adding bitterness. When first wort hopping is done then traditional hopping is omitted (Hieronymus, 2012). The traditional hopping method is also known as bitter hopping. The purpose of this is mainly to add bitterness. Bittering hops are usually boiled for 60 – 90 minutes.

Finish hopping, or late addition hopping is when more hops are added in addition to the bittering hops to the boil. These hops are added for less than 30 minutes. Finishing hops are used to add aroma to the beer. These hops still contribute somewhat to extra bitterness and some aroma compounds.

Aroma hops may also be added to the wort after boiling. A typical method in the breweries is whirlpool hopping. These are hops added to the wort as it starts to cool slightly and as the wort is being circulated in the whirlpool. A little amount of the hops added during whirlpool will still isomerize and add some bitterness. When the wort has cooled down to less than 85 °C the isomerization will cease (Steele, 2012).

The hopback is a vessel that was previously used to separate wort from the hops and the hot break after boiling. The hopback may however also improve filtration and add hop aroma when a layer of hops are spread over the slotted bottom of the hop-back vessel.

The most popular aroma hopping method is perhaps the dry hopping. Dry hopping is simple and straight forward. After the primary fermentation is complete fresh hops are added to the fermentation vessel. This means that these hops have not been heat treated and no

isomerization will occur. At this stage the pH has dropped and the alcohol content has risen, making infection less likely than in the chilled wort (Hieronymus, 2012).

## 2.2 Yeast

Yeast is a microorganism belonging to the Fungi kingdom. One gram of yeast contains twenty billion yeast cells. The scientific name for a species of yeast used both in cooking and alcohol production is *Saccharomyces cerevisiae*. Yeast cells digest sugar to provide energy for growth. Yeast is also a big contributor to beer flavor, as the yeast ferments the wort into beer. A plethora of aroma compounds also is made from the yeast's metabolism, yeast itself, yeast products and byproducts. These aromas are fusel alcohols, esters, organic acids, phenolic compounds, diacetyl and sulfur compounds (White and Zainasheff, 2010).

Higher alcohols, or fusel alcohols, are important to beer flavor. Fusel alcohols are more complex forms of alcohol than ethanol. Ethanol accounts for most of the alcohol in beer (White and Zainasheff, 2010). Ethanol is a two-carbon molecule; $C_2H_5OH$. Fusel alcohols are alcohols with more carbon molecules. For example, n-propanol ($C_3H_7OH$) has three carbon molecules and isobutanol ($C_4H_9OH$) has four. Both n-propanol and isobutanol may be found in beer and are made during fermentation along with, isoamyl alcohol, amyl alcohol and 2-phenylethanol (Pires et al., 2014). These alcohols are volatile, and many are intensely flavored. At elevated levels, the fusel alcohols may impart solvent-like or fruity characters to beer (White and Zainasheff, 2010).

As yeast starts to ferment the sweet wort into beer, the yeast multiplies and grows. For the yeast to be able to grow it requires amino acids to build new proteins and enzymes within the cell. Yeast absorbs most of the amino acids they need directly from the wort. The yeast removes the amino group from an amino acid, the amino group then is attached to an organic acid inside the yeast cell, thus creating a new amino acid. The original amino acid is left without the amino group, and now is an oxo-acid or a keto-acid. This molecule can be converted into an aldehyde by the loss of a $CO_2$ molecule, and then reduced to a higher alcohol (White and Zainasheff, 2010).

Esters are the class of compounds responsible for fruity flavors and aromas in beer. Esters are a combination of alcohols and fatty acids, the most common of which is ethyl acetate. Under fermentation conditions, the simple combination of alcohols and fatty acids will occur very slowly. Some beers taste like bananas, apples, strawberries or pineapple and esters are largely responsible for that. They are a major component of the flavor profile of ales rather than lagers. Mainly esters are formed during the vigorous phase of primary fermentation. The formation of alcohol must be done first as the esters are made of organic acids and alcohols (White and Zainasheff, 2010). Many different esters can be found in beer, but there are six that are of importance as aromatic constituents: ethyl acetate (solvent-like aroma), isoamyl acetate (banana aroma), isobutyl acetate (fruity aroma), phenyl ethyl acetate (roses and honey aroma), ethyl hexanoate (sweet apple aroma) and ethyl octanoate (sour apple aroma) (Pires et al., 2014).

Diacetyl is a buttery tasting component and is considered a fault in beer. Although some types of beer may allow for some levels of diacetyl to achieve a butterscotch flavor in the beer. At high levels the diacetyl will smell and taste like butter, even rancid butter. Diacetyl is what is also called a vicinal diketone (VDK) (White and Zainasheff, 2010). Commonly diacetyl is not a problem. Yeast cells synthesize valine, leucine and isoleucine. Diacetyl's precursor is alpha-acetolactate, which is excreted from the cell as the requirement for valine and leucine diminishes. Alpha-acetolactate can be oxidized to diacetyl when it is outside the cell. This means that diacetyl will form during fermentation (Fix, 1999). Healthy yeast will during conditioning, after primary fermentation, be able to reabsorb diacetyl. The yeast will reduce the diacetyl to acetoin, and further to 2,3 butanediol. Acetoin has a musty flavor and 2,3 butanediol has a high flavor threshold (White and Zainasheff, 2010).

## 2.3 Storing Beer

Beer is a very humble and grateful beverage to store. The alcohol and hop content protects the beer from spoiling too fast, even the beer that has not been pasteurized have an overall long shelf life. Once the beer arrives at the grocery stores or liquor stores it is kept in the store's temperature. It is also possible to find refrigerated beer in the stores in Norway,

these are however the same as the ones which are not refrigerated. Beer is sold cold for the customers' convenience, and not for any prolonged shelf-life.

## 2.4 Summarizing

The **theories** in this thesis are:

**1.** Storage temperature has an impact on the aroma in IPA and thus a cold stored IPA should lose its aroma slower than a warm (i.e. Room temperature) stored IPA.

**2.** Finishing hops will give beer fewer aromas than beer where hops are added during whirlpool which again will have fewer aromas than beer that has been dry hopped. In addition, beer with hops that has not been boiled will have larger amounts of aromas than beer with heat treated hops.

The **questions** this thesis explores are:

**1.** Will a test panel notice any difference in the aroma comparing cold stored and the warm stored beer?

**2.** Will bitterness change during storage?

# 3 MATERIALS AND METHODS

The development and degradation of aroma in beer with three different hopping methods; from the time of bottling and carbonation throughout the following division into warm and cold storage, was measured with the aid of Gas Chromatography – Mass Spectrometry (GC-MS) and Head Space Gash Chromatography (HS-GC). In addition other analyses, such as Anton Paar for measuring $CO_2$, alcohol, color and haze, were used to determine the bitterness, and sensory triangle tests executed.

## 3.1 Project Design

The project was designed after conversations with Nøgne Ø, and spending time at the brewery talking to the different brewers. The project is summed up in table 2 and in the flow chart in figure 3. The decision was to brew the same three brews twice; brew 1 and 4 are the same, brew 2 and 5 are the same and brew 3 and 6 are the same. As for hops all the brews are hopped with the same bittering hop and the same combination of aroma hops. The base of the brews themselves should be identical and the only differences are the three hopping methods.

**Table 2: Project plan, summarizing what hops to use in which brew, how big the batch sizes should be and how many bottles are needed for the whole project including how many bottles are needed for each of the test days.**

| | Bittering Hop | Aroma Hop | Aroma Hop | Aroma Hop | Batch sizes [L] | | | Bottles [Amount] | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Hop | Cascade | Cascade, Vic Secret | Cascade, Vic Secret | Cascade, Vic Secret | Boil | Ferment | Bottling | Brown 0.33 L | Stored at 4 °C | Stored at Room Temp |
| Brew 1 | x | x | | | 28 | 25 | 21 | 63 | 31 | 31 |
| Brew 2 | x | | x | | 28 | 25 | 21 | 63 | 31 | 31 |
| Brew 3 | x | | | x | 28 | 25 | 21 | 63 | 31 | 31 |
| Brew 4 | x | x | | | 28 | 25 | 21 | 63 | 31 | 31 |
| Brew 5 | x | | x | | 28 | 25 | 21 | 63 | 31 | 31 |
| Brew 6 | x | | | x | 28 | 25 | 21 | 63 | 31 | 31 |
| | | | | | Total amount of bottles | | | 378 | | |

| Test Day | 1 | 5 | 10 | 20 | 30 | 45 | 60 |
|---|---|---|---|---|---|---|---|
| Bottles pr batch chemical | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| Bottles pr batch sensory | | | | | 2 | | 2 |

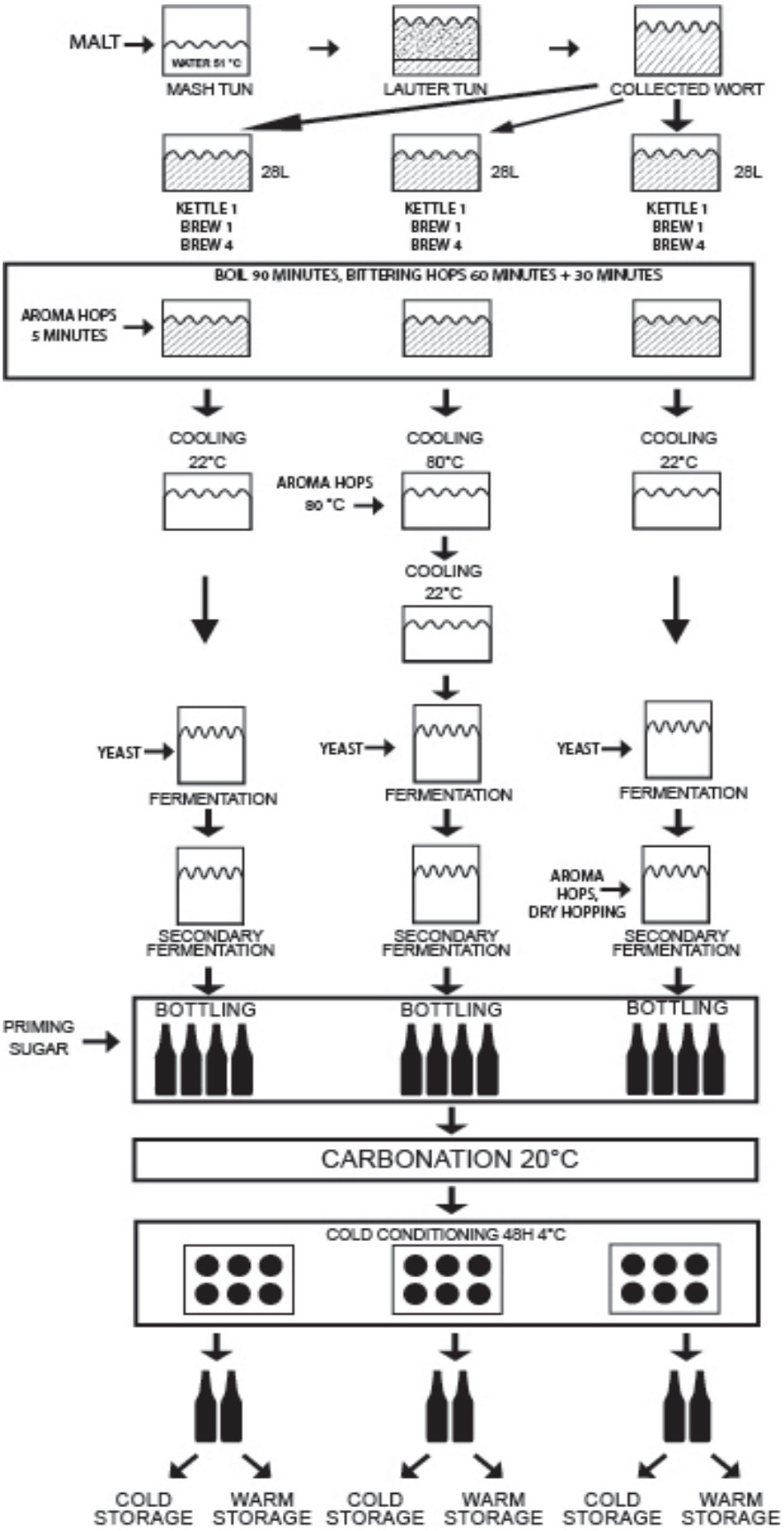



**Figure 3: Flow chart of the project.**

## 3.2 The Brewing Process

15 kg of pilsner malt (Weyerman, Bamberg, Germany) was weighed using a bucket and a scale (Avery Berkel DX 342, UK). 100% pilsner malt was chosen to eliminate disturbances from aromas and flavors that will occur from using other malts; this was also suggested by Nøgne Ø. The malt was milled in a two roller malt mill (Monster Mill MM2), with a base and funnel, using an electrical drill. The milled malt was inspected visually to ensure that the malt was mainly cracked and not milled to powder. Furthermore the milled malt was inspected for large amounts of whole grains as whole grains will not give off a substantial amount of sugar and thus lower the mash efficiency. To predict how much sugar that will come from the malt, pre trials to determine the extraction efficiency was executed. Extraction efficiency is the ratio of the amount of sugars that is actually obtained, to the theoretical maximum amount of sugars available. The assumed extraction efficiency of 75% was confirmed by the pre trials. 15 kg of pilsner malt for a batch of 84 L, which divided in three give 28 L per brew, gives a calculated alcohol by volume (ABV) at 5 %.

The brewery used for the mashing and lautering was a 60 L microbrewery from CoEnCo, delivered from Oostkamp, Belgium.

The milled malt was added to the mash tun which contained 45 L water at a temperature of 51 °C, the mashing-in temperature was chosen based on the instructions given during training, and learning how to operate the brewery. This temperature is also considered the dough-in rest for this mash. The agitator and heater were turned on as the mashing program was started. The mashing program was designed for the project and is depicted in table 3. The conversion temperature of 64 °C was chosen based on the optimized activity temperature of alpha- and beta-amylase. The mashout temperature of 78 °C is a commonly used mashout temperature, this will denaturize the enzymes and thus terminate their activity during lautering.

Table 3: The mash program with process, time and temperature.

| Process | Time [Minutes] | Degrees [°C] |
|---|---|---|
| **Mash in** | 10 | 51 |
| **Mash** | 60 | 64 |
| **Mashout** | 10 | 78 |

By the end of the mashing program the mash was transferred to the lauter tun where it was let to rest for 15 minutes. Subsequently the mash was set to recirculate in the lauter tun. Recirculation ended when the liquid was clear and free for particles.

This started the process of sparging and sieving the wort back into the mash tun. The sparging was executed as batch sparging and was repeated 4 times. Towards the end of the sparging the gravity of the wort was constantly measured to achieve the boil gravity of 10.5 °Plato, this would give an original gravity (OG) of 12 °Plato. Gravity was measured by sampling wort, pouring it into a cylindrical container attached to the brewery, which was cooled by cold water to 23 °C and measured with a hydrometer. Once the gravity was right the wort was transferred to three boiling kettles (Brewferm, Beer Brew 30, generation 3, Brouwland, Belgia). These were given the names Brew 1 (B1), Brew 2 (B2), and Brew 3 (B3). The wort was divided evenly so each contained 28 L wort. Since the malt was 100 % pilsner malt, it was decided to use 90 minutes boiling time to boil off as much dimethyl sulfide as possible. The wort was let to boil for 30 minutes before adding the bittering hops. The addition of hops is an important step in this thesis. IPAs are bitter beers and the need for a bittering hop was present. The Cascade is considered a popular all-round hop and was recommended by Nøgne-Ø as the bittering hop. For bittering hops it was therefore used 40 grams of Cascade hop pellets (Type 90 Hop Pellets, Alpha 7.6%, US Hops, USA) for 60 minutes and 30 grams of Cascade for 30 minutes. Since all three brews had the same amount of wort this gives a calculated bitterness of 52 IBU for brew 2 and 3. Brew 1 was added 35 g Cascade hop pellets and 35 g Vic Secret hop pellets (Type 90 Pellets, Alpha 15.7%, Simply Hops, Australia) at 5 minutes left of the boil, this addition raised the calculated IBU in brew 1 to 62. The wort chiller (Spiral cooler, 15M X 12MM, stainless steel)

was placed in the boiling kettles at 10 minutes left of boiling time to be sterilized before chilling the wort, then connected to the cold water tap. At the end of the boil the cold water was turned on and left on for brew 1 and 3 to completely chill the brews to 22 °C. Brew 2 was chilled to 80 °C and added hops. Brew 3 was added aroma hops during fermentation. The aroma hops additions can be viewed in table 1.

Once all brews were chilled to 22 °C they were transferred to sanitized fermentation vessels (Fermentation Bucket w/Spigot 30 L, white) and the yeast was pitched. The yeast chosen for this project was the Safale US-05 (Saccharomyces cerevisiae) 11.5 g. This yeast is a ready-to-pitch dry American ale yeast. The Safale US-05 was chosen because it is an easy and grateful yeast. It is often used for brewing modern IPA. The yeast produces beers which are balanced and with low diacetyl. The yeast was rehydrated with 110 ml distilled water that held a temperature of 25 °C as per instructed by Fermentis (Fermentis, n.a.). The fermentation vessels were sealed with an airtight lid and an airlock was attached to the lid. The brews were covered and placed in the brewery, holding a temperature of 20-22 °C to ferment. The whole process was repeated, the new brews were given the names Brew 4 (B4), Brew 5 (B5) and Brew 6 (B6). Where B1 = B4, B2 = B5 and B3 = B6.

### 3.2.1 Fermentation and re-racking

Brew 1, 2 and 3 was set to ferment; they fermented for 8 days and were then racked to a secondary fermenter. Brew 4, 5 and 6 was brewed one week later and got the same treatment as brews 1, 2 and 3. The primary fermentation stage is over when the bubbling rate in the airlock drops off dramatically to about 1-5 per minute. At this point the krausen[3] will have started to sink to the bottom of the fermentation vessel. The lid was removed from the vessels and a sanitized siphon (Fermtech, Regular 5/16" Auto-Siphon) was carefully inserted into the beer, making sure not to stir up the bottom layer. The beer was racked off

[3] Krausen is the creamy, foamy head that forms on top of the beer as it ferments; the krausen consists of yeast and wort proteins. PALMER, J. J. 2006. *How to brew - Everything you need to know to brew beer right the first time,* Boulder, Colo., Brewers Publications.

the trub[4] and into a sanitized fermentation vessel. The vessel was resealed with a sanitized lid and an airlock was affixed. After re-racking into secondary fermentation vessels the aroma hops; 35 g Cascade hop pellets and 35 g Vic Secret hop pellets (Type 90 Pellets, Alpha 15.7%, Simply Hops, Australia) were added to the brews 3 and 6. The brews were then covered set in the brewery at 20 – 22 °C for 14 days before bottling.

### 3.2.2 Cleaning the bottles

Brown beer bottles, size 0.33 L were cleaned with hot water and a bottle brush, then they were soaked in a solution of water and 2% sanitizer (Climax SU 388, Lilleborg, Norway) for 20 minutes and were rinsed thoroughly with 75 °C water. New bottle caps (Crown caps, gold, 26 mm) were sprayed with 70 % ethanol.

### 3.2.3 Carbonation

To make the beer carbonated the use of priming sugar was essential. With the use of the "Carbonation Calculator" from The Beer Recipator – A beer Calculator (Riley, 1998), the amount of priming sugar was determined. Batch 1, 2 and 3 each had 20 L trub free beer which held 20 °C, according to the Carbonation calculator, for an IPA; to obtain a $CO_2$ level of 2.3 g/L the priming sugar content should be 5.5 g/L beer which gives 110 g sugar per batch. The calculator also says that the residual $CO_2$ in the beer should be 0.9 g/L at the point of priming with the given temperature and size of the batch. Batch 4, 5 and 6 each had 20 L of trub free beer which held 20 °C, according to the Carbonation calculator, to obtain a $CO_2$ level of 2.3 g/L the priming sugar content should be 5.5 g/L beer which gives 110 g sugar per batch. The residual $CO_2$ should be 0.9 g/L in these brews as well. The suggested $CO_2$ content for IPAs are 1.5 – 2.3 g/L.

The sugar was dissolved in 0.5 L boiling water and allowed to cool. Then the syrup was added to a clean fermentation vessel (Fermentation Bucket w/Spigot 30 L, white). The brews

[4] Trub is the layer of sediment that appears at the bottom of the fermenter after yeast has completed the bulk of the fermentation. It is composed mainly of heavy fats, proteins and inactive yeast. JANSON, L. W. 1996. *Brew chem 101 : the basics of homebrewing chemistry,* Pownal, Vt., Storey Communications.

were gently siphoned into the fermentation vessels thus to mix in the priming sugar syrup without mixing in any air. The fermentation vessels were equipped with a spigot (Italian Bottling Spigot) for easy draining. A hose (Vinyl Tubing - 10 feet 5/16 ID - 7/16 OD - Food Grade) was attached to the spigot and a bottle filler (Fermtech Plastic Bottle Filler) was attached to the other end. Brown beer bottles, 0,33 L were filled to the neck of the bottle and capped with crown caps (Crown caps, gold, 26 mm). The beer bottles were set in room temperature for 14 days to carbonate, followed by two days cold crashing in the refrigerator to retard the yeast activity and thus stop the carbonation process.

## 3.3 Visual Assessment of beer; color and haze

On the day of brewing the chilled wort was visually assessed for haze and degree of haze was noted with -, +, ++ or +++; very little or no haze, some haze, haze, very hazy. As beer was being poured into the blue top bottles, they were left to sit for 30 minutes. These bottles are clear glass and a visual assessment could be done. The bottles were held up to the light and assessed for haze. Thereafter the bottles were placed on a white surface in a room with good daylight and the colors were compared.

## 3.4 Anton Paar

Beer bottles with caps were collected for analysis with the Anton Paar Alcolyzer (PBA-B Generation M, Alcolyzer Beer ME, density meter (DMA 5000 M or DMA 4500 M), CarboQC ME, HazeQC ME, and PFD filling device) (AntonPaar, 2015c). Cold stored beer bottles were set in room temperature an hour before analyzing when the Alcolyzer is sensitive to cold temperatures. The Alcolyzer was first checked with distilled water. A 0.5 L water bottle with a screw cap on was placed in the Anton Paar and the “Water Check” was performed. After the Water Check was passed the program “Beer Analysis” was loaded and a beer bottle with crown cap on was placed in the machine and the program was started. The results were noted for each beer bottle. The parameters that were analyzed were: Alcohol content (ABV) [% v/v], $CO_2$ [g/L], Density [g/cm$^{3]}$, Color [EBC] and Haze [EBC] (color and haze were only analyzed for T30, T45 and T60).

A bottle was placed in the vacuum chamber (PDF filling device), the crown cap was perforated and a 120 to 150 mL sample was automatically drawn up from the bottle and into the measuring system. The sample went through a filter and into the density meter (DMA4500). The density meter contains a density measurer which is an oscillating U-shaped tube. During the analysis the sample is passed through the tube which oscillates at a frequency that is determined by the density of the sample.

The instrument measures dissolved $CO_2$ (CarboQE ME). This is done with a delivery of the sample without any loss of $CO_2$ into the measuring cells of PBA-B. The PFD is equipped with a seal to close off a small area of the bottle cap or bottom of a can. The bubble-free sample delivery is achieved by applying a pressure that is significantly higher than the package pressure (AntonPaar, 2015c). CarboQC utilizes the patented Multiple Volume Expansion Method (AT 409673; GB 2373584; US 6,874,351), this method eliminates the influence of other dissolved gases in the samples and identifies the carbon dioxide content selectively(AntonPaar, 2015b) .

The alcohol content (ABV) is determined by near infrared measurements (NIR) NIR-radiation (800-2500 nm) from a light source that separates specific wavelengths in the instrument. The alcohol content is measured spectrophotometric by determining the absorbance in a specific range of alcohol in the NIR-spectrum. The Instrument measures the content alcohol in the sample by percent of total masse from the measured absorbance. The calibration curve, to which the sample is compared to, has been determined by standard solutions of alcohol concentration (EBC, 2008).

The turbidity meter (HazeQC ME) uses the approved ratio method with measurement at three angles (transmission 0°, scattered light at 25° and 90°) to calculate the turbidity value [EBC]. This instrument also measures the color [EBC]. The measurements are carried out at a wavelength of 650 nm (AntonPaar, 2015d).

Anton Paar is an all-in one measuring system which means no further calculations needs to be done. The instruments shows all the results based on the direct measurements described. The repeatability (r95) for the measurements in the Anton Paar Alcolyzer is as follows: alcohol 0.01 % v/v, $CO_2$ 0.01 g/L, color 0.1 EBC and turbidity 0.02 EBC (AntonPaar, 2015a).

## 3.5 Volatile aroma components (GC-MS)

The volatile components were analyzed using Thermal Desorption Gas Chromatography Mass Spectrometry (TDGCMS).

In aluminum cups (volume 106 ml, Plus Pack As, Odense DK), 5.00 g of the samples were weighed in. The plates were placed in a micro emission chamber (Micro-Chamber / Thermal Extractor M-CTE250, Markers International Ltd. Llantrisant, UK). The volatiles were concentrated onto adsorbent tubes (Tenax TA/Carbograph 1TD, Markers International). The adsorbent tubes were further placed in an automatic thermal desorption instrument (Thermal Desorber, TD-100, Markers International), with a 7890B GC System (Agilent Technologies Inc. Wilmington, De, USA) and connected to MS Systems, 5975 inert XL Mass Selective Detector (Agilent Technologies). The carrier gas used was helium degree 6.0 (AGA) with a flow of 1 ml/min. The software used was Mass Hunter GC / MS Acquisition B.07.00.1413 (Agilent Technologies).

Concentration of the volatile components of the sample over the adsorbent tube was made in the micro emission chamber, at 50 °C for 10 min, and an $N_2$-flow of 50 ml/min. The adsorbent tube was transferred to an automated thermal desorption instrument. Here, the sample was desorbed from the tube at 280 °C, 10 min, $N_2$-flow 30 ml/min onto an electric cooling trap that held minus 10 °C. The sample was further desorbed from the cooling trap (Peltier cell), where the temperature rise was 100 °C/s to 280 °C, holding time 3 min., split 10 ml/min. to the column. The volatile components were separated on a DB-WAXETR GC column (Agilent Technologies). The column had a length of 30 meters, with an internal diameter of 0.25 mm and film thickness of 0.5 microns. The GC temperature program was as follows: 35 °C, 3 min; increase by 5 °C min-1 to 40 °C, 2 min; increase by 15 °C min-1 to 70 °C, 2 min; increase by 10 °C min-1 to 130 °C; increase by 10 °C min-1 to 160 °C, 3 min; increase by 30 °C min-1 to 200 °C, 15 min. The components were detected with a 5975 inert XL Mass Selective Detector (Agilent Technologies). The mass spectrometer parameters were electronic ionization mode (70 eV), ion source temperature of 230 °C and continuously scanning in the mass range m/z 33-400. The software program used was Mass Hunter

GC/MS Acquisition B.07.00.1413. Identification of the volatile components was made using NIST 11 database (Agilent Technologies).

### 3.5.1 Sample preparation

The brown beer bottles were opened and each of them was gently poured into two blue top bottles, 40 ml. The bottles were marked with sample number and date then put in the freezer (-20 °C). The rest of the beer bottles were recapped and, marked with date, put in the freezer and saved as a backup. The day before analysis the samples were retrieved from the freezer and put in the refrigerator and thawed at 4 °C, overnight. On the day of analyses samples were gently shaken and the contents were weighed into sterile 50 mL Nunc tubes, all made to achieve the same weight. The Nunc tubes where spun at 3000 rpm for 20 minutes at 4 °C in the centrifuge (Thermo Scientific, Heraeus Multifuge X3R, Germany). The centrifuged beer samples were then ready for further processing.

### 3.5.2 Hop teas

To have some point of reference with what aroma compounds could be found in the hops, there were made 3 hop teas, for the two aroma hops that were used. The first hop tea was made of 5 dL distilled water and 5 g Cascade hop pellets that were boiled for 5 minutes. The second hop tea was made with 5 dL distilled water and 5 g Cascade hop pellets that were added when the water had a temperature of 80 °C. The third hop tea was made with 5 dL distilled water and added 5 g Cascade hop pellets when the water had a temperature of 20 °C. The same three hop teas were made with 5 dL distilled water and 5 g Vic Secret hop pellets. The hot teas were chilled to room temperature, measured into Nunc tubes and centrifuged for 20 minutes at 3000 rpm at 4 °C in the Thermo Scientific centrifuge (Heraeus Multifuge X3R, Germany). The centrifuged hop teas were then prepared for the GC-MS.

## 3.6 Bitterness

The content of the beer's bitter units (IBU) was measured with a spectrophotometer of the type Shimadzu, (UV-1601, Bergman, Oslo) and performed by a standard method disclosed by the European Brewery Convention (EBC) Analytica, method 9.8 (EBC, 2004).

The beer samples were stirred slowly in a glass measuring cup, with a magnet stirrer for 2 minutes. 2.5 ml of each sample was transferred to glass acid washed 50 mL Pyrex tubes with a screw top. The sample was also diluted with 2.5 ml distilled water. The samples was diluted because the calculated IBU was high and pretests gave too high absorptions readings, >1.000 at $A_{275nm}$. The samples were acidified with 250 µl 6 M HCl. 10 ml isooctane was added in the ventilation chamber. The glass tubes were secured tightly with screw cap lids and placed to be shook in the turner for 15 minutes. After 15 minutes of shaking the tubes were removed from the turner and placed in the centrifuge for 10 minutes. The samples came out of the centrifuge with two liquid phases, separated by a gel layer. The clear liquid in the top phase was drawn from the Pyrex tube and placed in a quartz cuvette (Micro Quartz Cuvette, White Wall, 1.4ml, 10 mm). The absorption chamber was zeroed out with isooctane. The absorption was measured at 275 nm ($A_{275}$) against a reference of pure isooctane (EBC, 2004). The results were recorded and processed in Excel (Microsoft Office Home and Student 2010) and MiniTab (Minitab® 17.2.1).

## 3.7 Sensory – triangle test

The sensory test was performed by a semi trained panel consisting of 10 panelists. The panelists performed a triangle test to decide whether there was a difference in the cold stored and the room temperature stored beer at day 30 and at day 60. The panel consisted of 70 % males and 30 % females, who all volunteered to join the test. The panelists were also used to drinking beer. The triangle test was performed in a white room where the panelists were sitting around a table and instructed not to discuss the beers during tasting. The beers were tempered to 8 °C. 20 ml of sample was served in plastic cups. Each cup was numbered with a three digit code. The samples were randomized. The panelists were presented with three cups where two were the same and one was different, they also got ballots to circle the odd one.

The materials used in the test were:

- Room temperature stored beer: brew 1, brew 2, brew 3, brew 4, brew 5, brew 6
- Cold stored beer: brew 1, brew 2, brew 3, brew 4, brew 5, brew 6
- Plastic sample cups
- Water for rinsing the mouth between samples
- Triangle Test Ballot (appendix)
- Pens
- Permanent markers for marking the sample cups with three digit codes

Each panelist was given two sets of three samples and asked to circle the one that was different from the other. There were six ways of presenting the three samples; either with two room temperature samples and one cold stored sample or two cold stored samples and one room temperature sample. Table 4 and 5 show the order the samples were given. The room temperature stored beer was given the letter "A" and the cold stored beer was given the letter "B". The combinations were as following:

1. AAB
2. ABA
3. BAA
4. ABB
5. BAB
6. BBA

**Table 4: The three digit identification numbers that were given the beer samples, and the order the beer samples were served each panelist for the brews 1, 2 and 3.**

| **Brew 1** | **Room temp** | **Brew 2** | **Room temp** | **Brew 3** | **Room temp** |
|---|---|---|---|---|---|
| | **767** | | **458** | | **625** |
| | **189** | | **962** | | **837** |
| | **Cold** | | **Cold** | | **Cold** |
| | 312 | | 243 | | 364 |
| | 570 | | 168 | | 472 |
| **Assessor** | **Order** | **Assessor** | **Order** | **Assessor** | **Order** |
| 1 | **767**, 312, **189** | 1 | 243, **962**, 168 | 1 | **837**, 472, **625** |
| 2 | **767**, 312, 570 | 2 | 243, **962, 458** | 2 | **837,** 472, 364 |
| 3 | **767, 189**, 570 | 3 | 168, 243, 458 | 3 | **837, 625,** 364 |
| 4 | 312, **189**, 570 | 4 | 962, 168, **458** | 4 | 472, **625,** 364 |
| 5 | 312, **189, 767** | 5 | **962,** 168, 243 | 5 | 472, **625, 837** |
| 6 | 570, 312, **767** | 6 | **962, 458,** 243 | 6 | 364, 472, **625** |
| 7 | **189**, 570, **767** | 7 | 168, **458,** 243 | 7 | **625**, 364, **837** |
| 8 | **189,** 570, 312 | 8 | 168, **458, 962** | 8 | **625**, 364, 472 |
| 9 | **189, 767,** 312 | 9 | 243, 168, **458** | 9 | **625, 837**, 472 |
| 10 | 570, **767,** 312 | 10 | **458**, 243, **962** | 10 | 364, **837**, 472 |

**Table 5: The three digit identification numbers that were given the beer samples, and the order the beer samples were served each panelist for the brews 4, 5 and 6.**

| **Brew 4** | **Øl 18°C** | **Brew 5** | **Øl 18°C** | **Brew 6** | **Øl 18°C** |
|---|---|---|---|---|---|
| | **126** | | **027** | | **501** |
| | **391** | | **156** | | **049** |
| | **Øl 4°C** | | **Øl 4°C** | | **Øl 4°C** |
| | 281 | | 632 | | 583 |
| | 884 | | 454 | | 796 |
| **Assessor** | **Order** | **Assessor** | **Order** | **Assessor** | **Order** |
| 1 | 281, 884, **126** | 1 | **156,** 454, 632 | 1 | 796, 583, **501** |
| 2 | 884, **126, 391** | 2 | 156, 454, **027** | 2 | **501,** 583, 049 |
| 3 | 884, **126,** 281 | 3 | 454, 632, 027 | 3 | **049,** 796, **501** |
| 4 | **391, 126,** 281 | 4 | 632, 156, 027 | 4 | **501,** 583, 796 |
| 5 | **391,** 884, 281 | 5 | 632, **156,** 454 | 5 | **049,** 796, 583 |
| 6 | **391**, 884, **126** | 6 | **027, 156,** 454 | 6 | **501, 049,** 796 |
| 7 | 884, 281, **126** | 7 | **027,** 632, 454 | 7 | **049, 501, 583** |
| 8 | 281, **391, 126** | 8 | **027**, 632, **156** | 8 | 583, **049,** 796 |
| 9 | 281, **391**, 884 | 9 | 632, 454, **027** | 9 | 796, **501,** 583 |
| 10 | **126, 391**, 884 | 10 | 454, **027, 156** | 10 | 583, **049, 501** |

# 4 RESULTS

Analyses of all the brews were performed at 1, 5, 10, 20, 30 and 60 days after bottling and storage. The storage was either cold storage; 4 °C, or room temperature; ~20 °C. The analyses were carried out in the brewery or laboratory.

The results of the main study are presented as mean values of two brews, table 6 shows which brews are the same and analyzed as one except for the results for color and haze. Color and haze results are for brews 4, 5 and 6 only.

Table 6: Similar brews in the trials are represented as one.

| Brews | Hop addition |
|---|---|
| **1 and 4** | 5 minutes finishing, aroma hops |
| **2 and 5** | 80 C, post boil, aroma hops |
| **3 and 6** | Dry hop, aroma hops |

## 4.1 Anton Paar

The Anton Paar results are as follows; alcohol content [%], carbon dioxide [g/l] and color and haze [EBC]. EBC stands for the European Brewing Convention; EBC is the unit for measuring beer and wort color, as well as the EBC units for quantifying turbidity, haze, in beer.

### 4.1.1 Alcohol

Alcohol measurements, using the Anton Paar Alcolyzer, show an increase of alcohol the first 10 days after priming and bottling, and that the alcohol content moves towards stabilizing after these 10 days. Both the cold and the room temperature stored beers have this increase. The differences between brew 1, 2, 3 and 4, 5, 6 were tested for significance in MiniTab. The MiniTab tests show that brew 1 and 4, brew 2 and 5, and brew 3 and 6 are not significantly different. The brews were tested against the null hypothesis $H_0$: The brews are equal. The p-values for the tests are greater than 0.05 (see table 7) which means the samples from the similar brews can both be represented as one with the average of the two brews.

Table 7: Two-sample t-test for alcohol content, results for brews 1, 2, 3, 4, 5 and 6, p-values are greater than 0,05 which means the null hypothesis cannot be rejected.

| Brews | Hop addition | Storage | p-value | Significantly different |
|---|---|---|---|---|
| **1 and 4** | 5 minutes finishing, aroma hops | Room Temperature | 0,860 | No |
| **2 and 5** | 80 C, post boil, aroma hops | Room Temperature | 0,619 | No |
| **3 and 6** | Dry hop, aroma hops | Room Temperature | 0,189 | No |
| **1 and 4** | 5 minutes finishing, aroma hops | Cold | 0,631 | No |
| **2 and 5** | 80 C, post boil, aroma hops | Cold | 0,783 | No |
| **3 and 6** | Dry hop, aroma hops | Cold | 0,134 | No |

For the beers with the 5 minute finishing hop addition (figure 4), the alcohol increases rapidly from day 1, after priming and bottling, to day 5 and till day 10. The increase continues more slowly from day 20 and stops increasing at day 45. Cold and room temperature stored beer follow each other well developing alcohol after bottling.

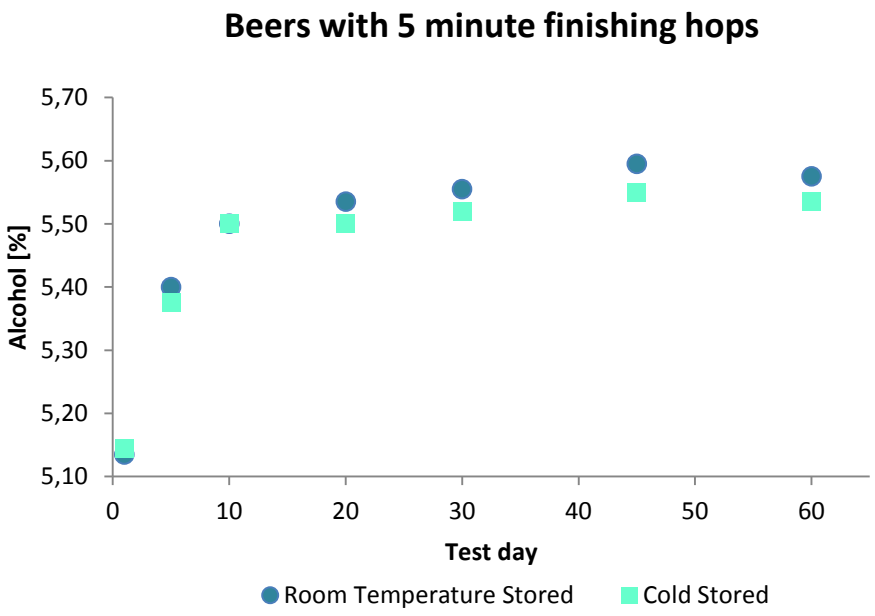



Figure 4: The alcohol content [%] in beers hopped with 5 minute finishing aroma hops increased for both cold stored and room temperature stored beer.

Beers that were hopped at 80 °C post boil (figure 5) follows the same path as the 5 minute finishing hops beers. The alcohol content increases rapidly from test day 1 after priming and bottling to test day 5 and to test day 10. The increase continues all the way to test day 60,

but more slowly from test day 10. Cold- and room temperature stored beer follow each other developing more alcohol throughout the test period.

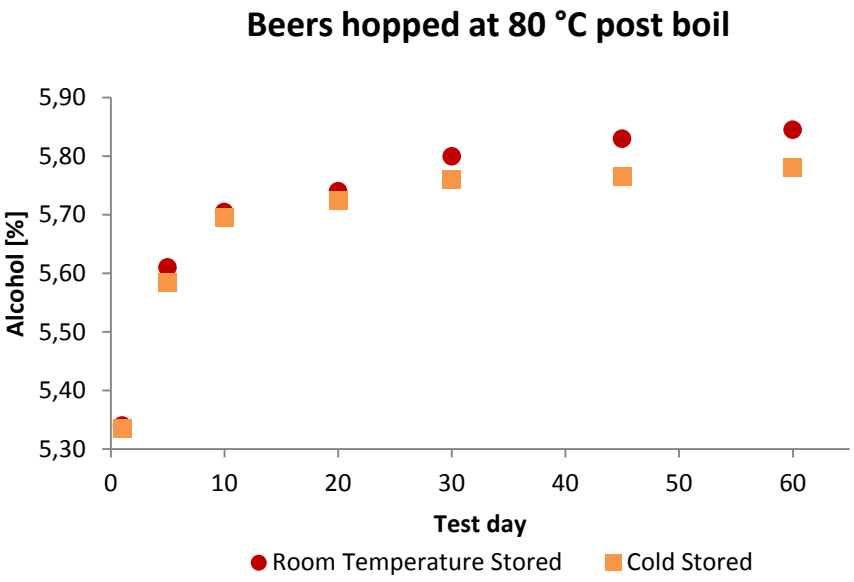



**Figure 5: The alcohol content [%] in brew 2 on each test day. Dots represent the room temperature stored beers and squares represent the cold stored beers.**

The alcohol content in the dry hopped beers (figure 6) also increases rapidly from day 1 after priming and bottling to day 5 and till day 10. The increase seems to stabilize well after day 30. The dry hopped beers start out with higher alcohol content than the beers with 5 minute finishing hops and the beers that were hopped at 80 C post boil. The dry hopped beers also end up with higher alcohol content than the other two hopping methods.

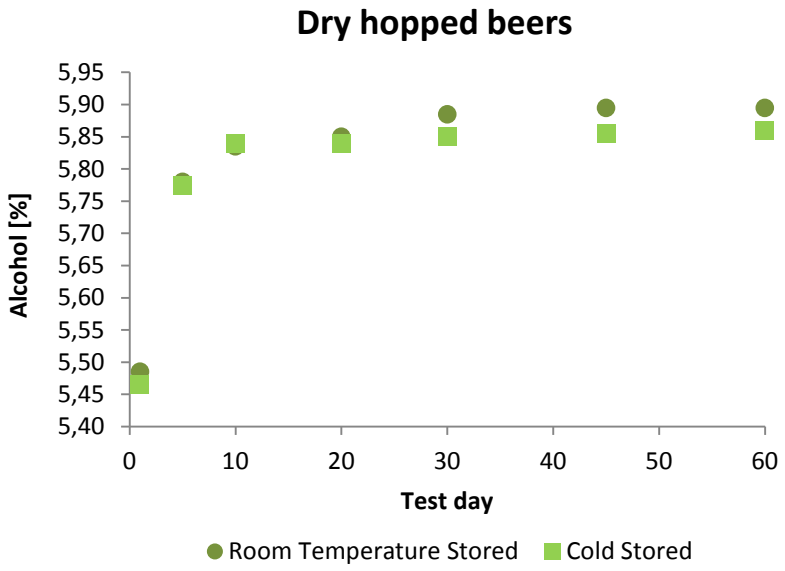



Figure 6: The alcohol content [%] in dry hopped beer increases for both cold stored and room temperature stored beer. Dots represent the room temperature stored beers and squares represent the cold stored beers.

### 4.1.2 Carbon dioxide

The carbon dioxide ($CO_2$) measurements with the Anton Paar Alcolyzer show an increase of $CO_2$ the first 10 days after priming and bottling as it did for the alcohol content. Further it also seems that the $CO_2$ content starts to stabilize after 10 days. Both the cold and the room temperature stored beers follow this pattern. The figures 5 – 7 show that the $CO_2$ is very similar for both the cold stored (squares) and the room temperature stored (dots) beers.

Again the differences between brew 1, 2, 3 and 4, 5, 6 were tested for significance in MiniTab. The MiniTab tests show that brew 1 and 4, brew 2 and 5, and brew 3 and 6 are not significantly different. The brews were tested against the null hypothesis $H_0$: The brews are equal. The p-values for the tests are greater than 0.05 (see table 8) which means the samples from the similar brews can both be represented as one with the average of the two brews.

Table 8: Two-sample t-test for $CO_2$ content, results for brews 1, 2, 3, 4, 5 and 6, p-values are greater than 0,05 which means the null hypothesis cannot be rejected.

| Brews | Hop addition | Storage | p-value | Significantly different |
|---|---|---|---|---|
| **1 and 4** | 5 minutes finishing, aroma hops | Room Temperature | 0,239 | No |
| **2 and 5** | 80 C, post boil, aroma hops | Room Temperature | 0,093 | No |
| **3 and 6** | Dryhop, aroma hops | Room Temperature | 0,315 | No |
| **1 and 4** | 5 minutes finishing, aroma hops | Cold | 0,687 | No |
| **2 and 5** | 80 C, post boil, aroma hops | Cold | 0,118 | No |
| **3 and 6** | Dryhop, aroma hops | Cold | 0,294 | No |

For the beers with the 5 minute finishing hop addition (figure 7), the $CO_2$ content increases rapidly from day 1, after priming and bottling, to day 5 and till day 10. The increase continues all the way till day 60, but more slowly from day 10. Cold and room temperature stored beer follow each other, although the cold stored beers seem to develop less $CO_2$ than the room temperature stored beers.

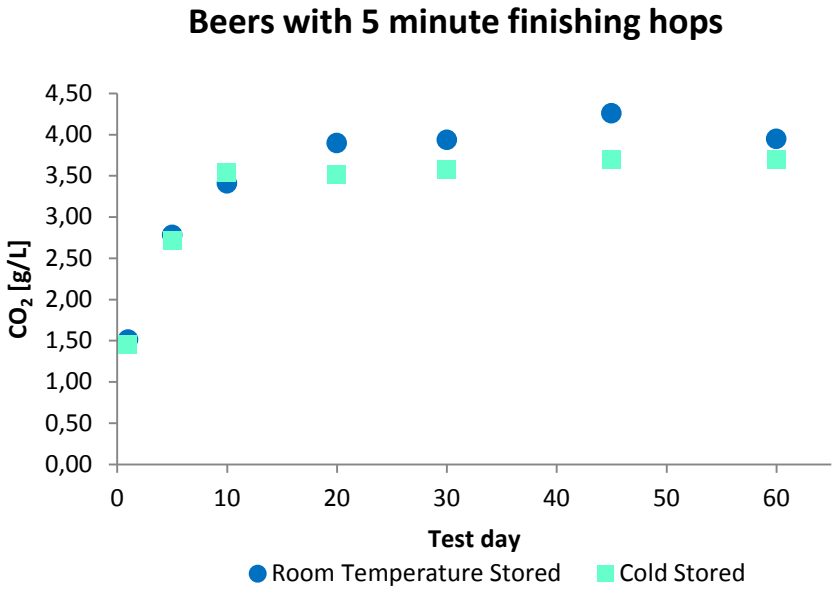



Figure 7: The $CO_2$ [g/l] content in bottled beers increases in both cold stored and room temperature stored beers. Dots represent the room temperature stored beers and squares represent the cold stored beers.

In the beers that were hopped at 80 °C post boil (figure 8) the $CO_2$ content increases rapidly from test day 1 after priming and bottling to test day 5. The increase continues, but seems to stabilize at test day 30. The $CO_2$ contend in cold stored beers is slightly less than for the room temperature stored beers from test day 30 and out.

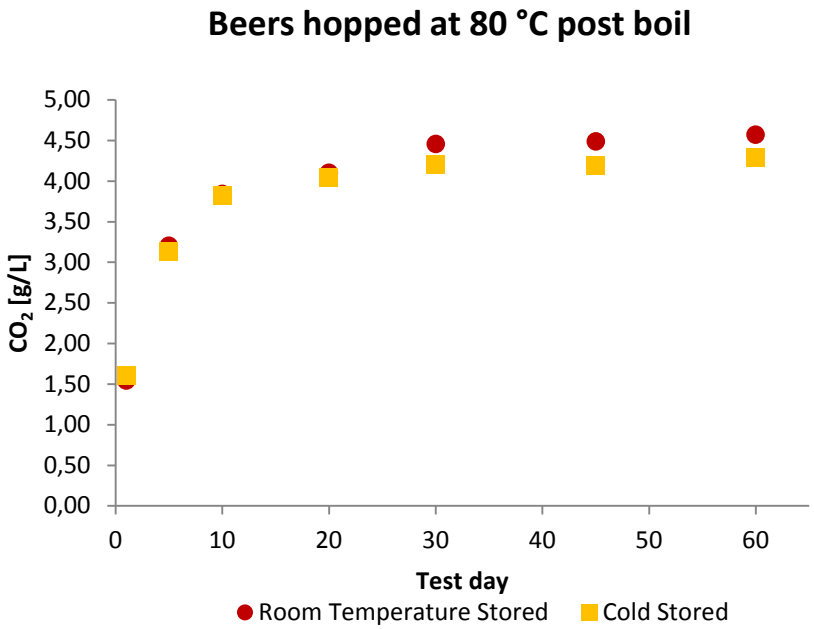



**Figure 8: The $CO_2$ [g/l] content in bottled beers increases in both cold stored and room temperature stored beers. Dots represent the room temperature stored beers and squares represent the cold stored beers.**

The $CO_2$ in dry hopped beers (figure 9) also increases rapidly from day 1 after priming and bottling to test day 5 and to test day 10. The increase seems to stabilize well after day 20. The $CO_2$ content seems to be very similar in both cold stored and room temperature stored beers. The development of $CO_2$ follows that of alcohol development after bottling.

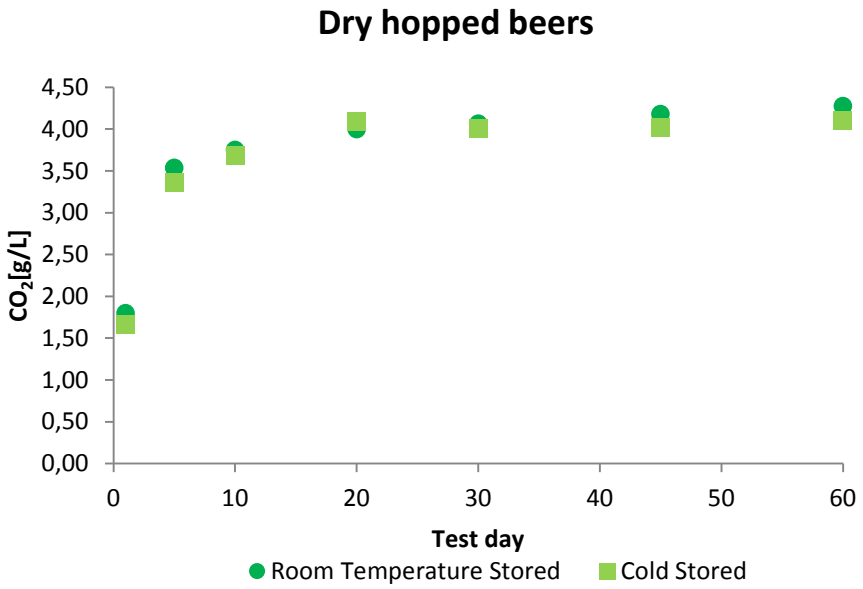



**Figure 9: $CO_2$ [g/l] content in brew 3 on each test day. Dots represent the room temperature stored beers and squares represent the cold stored beers.**

### 4.1.3 Color and Haze

Due to the lack of equipment in the beginning of the project the color and haze results are for brew 4, 5 and 6 for test day 30, 45 and 60 only.

For brew 4 (5 minutes finishing hops) the color and haze readings (figure 11) decrease with time both for the room temperature stored and for the cold stored beers. For room temperature stored brew 4 the color seems to darken slightly between day 45 and day 60 while the color in cold stored beer seems to lighten in the same period. An EBC of 20 – 25 is according to the approximate beer color chart (figure 10), on the orange side.


Figure 10: A color chart for beer showing both EBC and SRM.

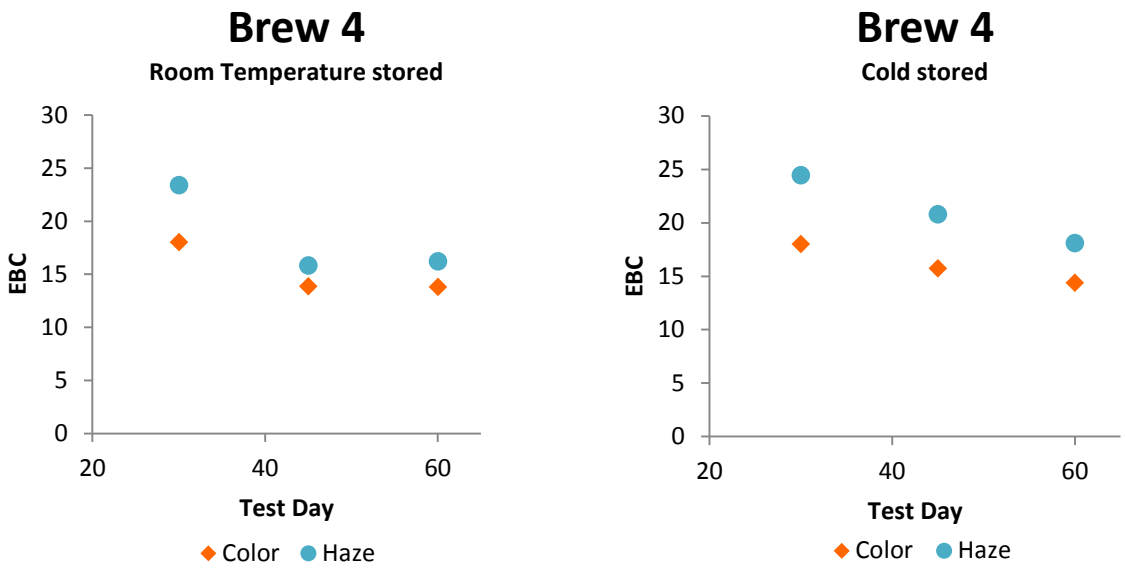



Figure 11: The color and haze both decreased in the beers that were hopped with 5 minute finishing aroma hops. The figure on the left shows the decrease in the room temperature stored beer and the figure to the right shows the decrease in the cold stored beers.

The color and haze readings (figure 12) decrease slightly for brew 5 (beer hopped with aroma hops at 80 °C post boil) with time both for the room temperature stored and for the cold stored beers. The decrease is less dramatic than for brew 4. For the room temperature stored beers the color seems to darken slightly between day 45 and day 60 while the color in cold stored beer seems to lighten or stabilize in the same period. An EBC of 6-8 is yellow according to the approximate beer color chart (figure 9). The haze in brew 5 was very low for both cold stored and room temperature stored beer.

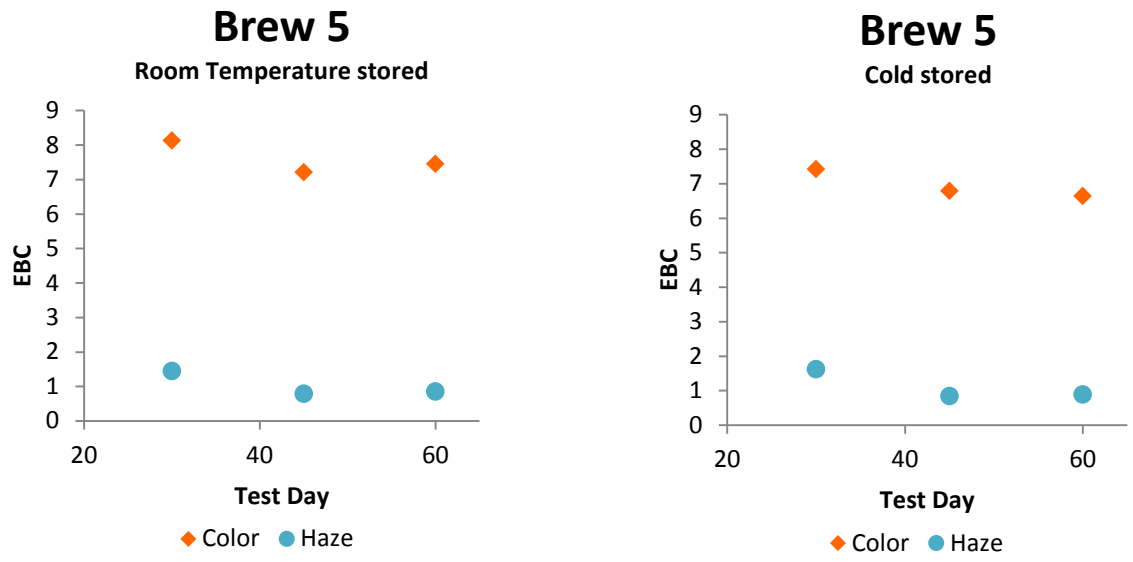



**Figure 12: The color and haze both decreased in the beers that were hopped with aroma hops at 80 °C post boil. The figure on the left shows the decrease in the room temperature stored beer and the figure to the right shows the decrease in the cold stored beers.**

The color and haze readings, in figure 13, for brew 6 (dry hopped beers) decrease only slightly with time both for the room temperature stored and for the cold stored beers. The decrease is also here less dramatic than for brew 4. An EBC of 7-9 is yellow according to the approximate beer color chart (figure 9). The haze in brew 6 was fairly low in both cold stored and room temperature stored beer. The haze decreased more in the cold stored beer than in the room temperature stored beer.

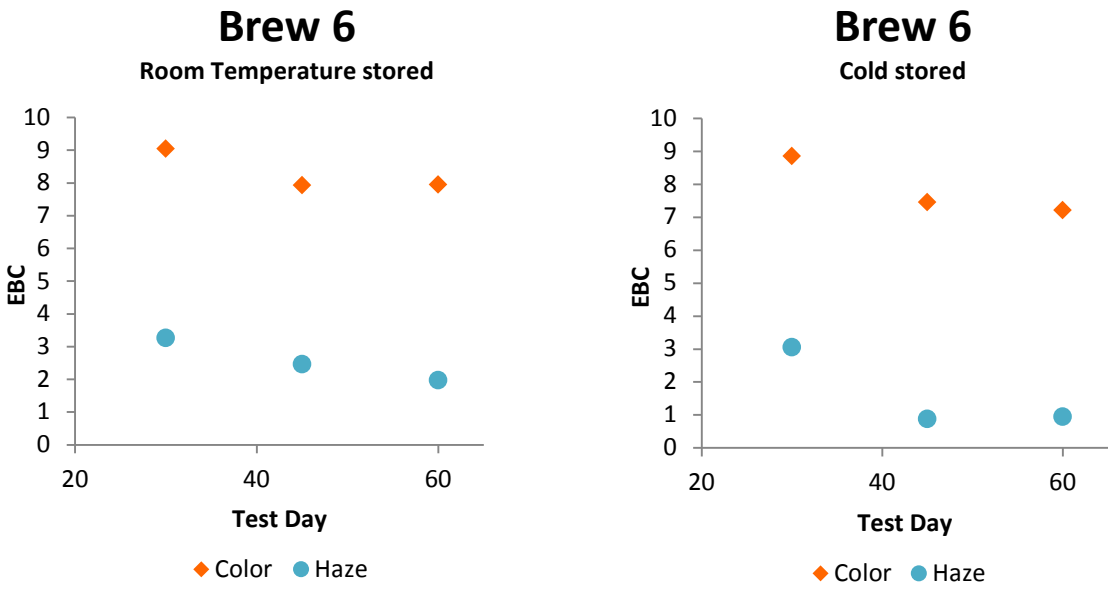



**Figure 13: The color and haze both decreased in the beers that were dry hopped. The figure on the left shows the decrease in the room temperature stored beer and the figure to the right shows the decrease in the cold stored beers.**

## 4.2 Visual assessment of color and haze

The beer was brewed using 100% pilsner malt and therefore obtained a light yellow color, however beer that was dry hopped gained a slight peach-colored appearance. By visual assessment the color remained stable throughout storing for the hot hopped beers (i.e. Beer 1, 2, 4 and 5), while the color in the dry hopped beers (i.e. Beer 3 and 6) seemed to change to a darker, almost brownish color for cold stored beer and remain unchanged for room temperature stored beer.

Temperature is important when beer is brewed to avoid haze. Cooling the wort is a vulnerable step when it comes to making sure the beer is clear or hazy. Also, stirring during or after cooling the wort may result in hazing. Haze is most often caused by proteins. Haze was present in wort and in beer after storage, but not after being frozen. Samples were assed visually and judged with -; very little or no haze, +; some haze – the wort or beer is transluscent, but not clear, ++; mostly hazy – the wort or beer is slightly opaque, but it is possible to see contours when the sample is held up against a light source, +++; very hazy – the wort or beer is completely opaque, it is not possible to see through. Table 9 sums up the visual assesment of the beers for chilled wort, test day 1, 30 and 60 and for a frozen sample.

**Table 9: Haze in wort and in beer after storage and freezing. Samples were assed visually and judged with -, very little or no haze, +; some haze ++; hazy and +++; very hazy.**

| | | | Haze | | | | |
|---|---|---|---|---|---|---|---|
| **Brew** | **Hop addition** | **Storage** | **Chilled Wort** | **Test day 1** | **Test day 30** | **Test day 60** | **Frozen Sample** |
| 1 | 5 min | Warm | ++ | + | – | – | – |
| 1 | 5 min | Cold | | + | – | – | – |
| 2 | 80 °C | Warm | +++ | ++ | ++ | + | – |
| 2 | 80 °C | Cold | | ++ | ++ | + | – |
| 3 | Dry | Warm | – | – | – | – | – |
| 3 | Dry | Cold | | – | – | – | – |
| 4 | 5 min | Warm | +++ | ++ | ++ | ++ | – |
| 4 | 5 min | Cold | | ++ | ++ | ++ | – |
| 5 | 80 °C | Warm | – | – | – | – | – |
| 5 | 80 °C | Cold | | – | – | – | – |
| 6 | Dry | Warm | ++ | + | + | + | – |
| 6 | Dry | Cold | | + | – | – | – |

## 4.3 GC-MS

As presented in the theory chapter, yeast is a great contributor to many of the volatile components in the finished beer other than ethyl alcohol and carbon dioxide. These results have been arranged so that it is only the volatile compounds that arise from the use of hops and different hopping methods that will be presented. However, the compounds limonene and citronellol are compounds that do not come directly from hops, but rather from the interaction between hop compounds and yeast.

### 4.3.1 Beta-Pinene

Beta-pinene, or β-pinene, is one of the first hop derived volatile compounds that were detected by the GC-MS. As the name suggests the flavor and odor of this compound is somewhat of piney and turpentine like (Furia and Chemical Rubber Company., 1980). Beta-pinene is a bicyclic monoterpene, and can be found in hops, cumin and pine.

The only hop tea that beta-pinene was detected in by the MS-GC was the tea where Vic Secret was boiled for 5 minutes. Beta-pinene was detected at RT 11.8 min.

Figure 14 shows a decrease in beta-pinene for the room temperature stored beers that were hopped with 5 minutes finishing hops. However, the starting points being so different for the beers with the different storage temperatures do create some controversy in the validity of the first results, i.e. day 1, 5 and 10. From test day 20 to test day 60 the fluctuations in the beta-pinene seem to start with the same amount and end with the same, lower amount.

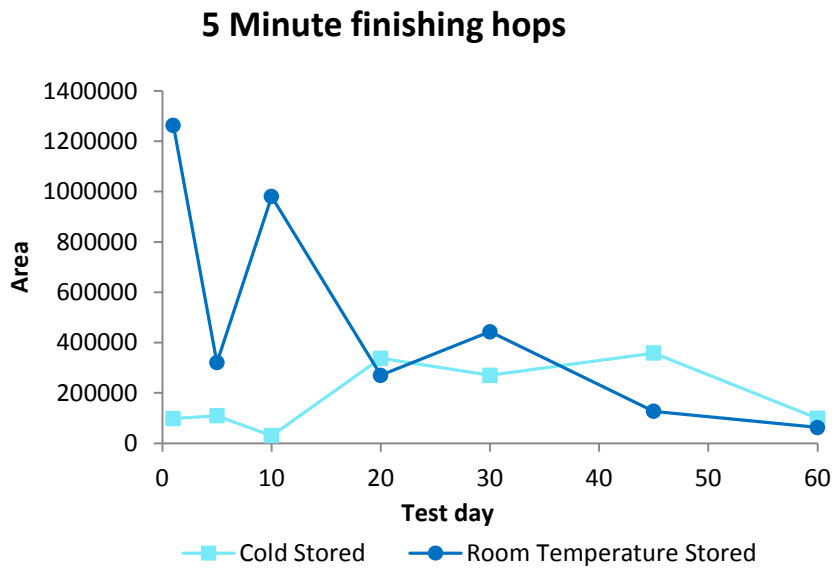



Figure 14: The development of beta-pinene in bottled beers, from day 1 to day 60 after bottling. The beers are aroma hopped with 5 minute finishing hops. Dots represent the room temperature stored beers and squares represent the cold stored beers.

The results for the brews hopped at 80 °C after the boil also show fluctuations that moves towards a synchronized decrease after test day 45. Figure 15 also show that the GC-MS did not detect any beta-pinene for test day 20 in the cold stored beers.

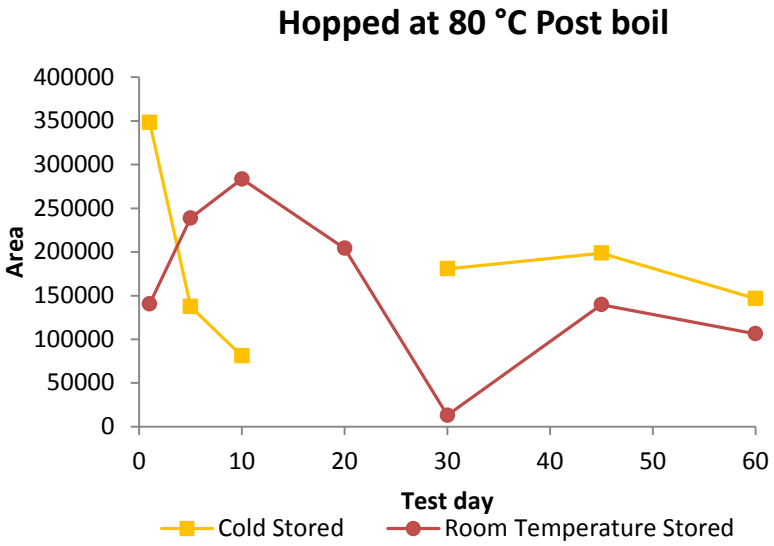



Figure 15: The development of beta-pinene in bottled beers, from day 1 to day 60 after bottling. The beers are aroma hopped at 80 °C post boil. Dots represent the room temperature stored beers and squares represent cold stored beers.

The dry hopped beers have the more stable results for beta-pinene. Both the cold stored and the room temperature stored beers show the same pattern in the fluctuations. Figure 16 suggests that in both storage methods there is more beta-pinene 10 days after bottling for room temperature stored beer. The least amount of beta-pinene is 20-30 days after bottling. On test day 60 both room temperature stored, and cold stored beers have a decreased amount of beta-pinene compared to test day 1.

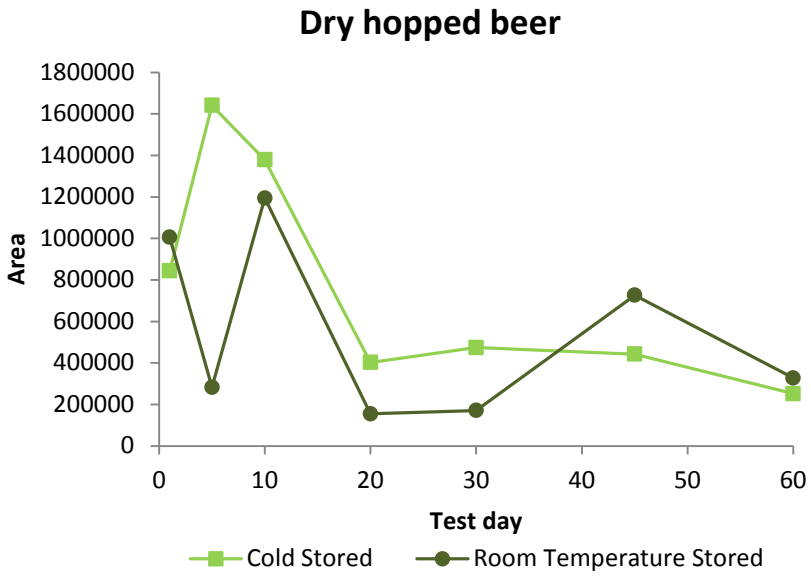



Figure 16: The development of beta-pinene in bottled beers, from day 1 to day 60 after bottling. The beers are dry hopped. Dots represent the room temperature stored beers and squares represent cold stored beers.

### 4.3.2 D-Limonene

Limonene is a cyclic terpene, and is mainly found in citrus fruits, hence the name. The flavor and odor of limonene is that of lemon- and citrus fruit rind. D-limonene was detected in all brews, but not for all samples, the figures show the development for D-limonene over the test period of 60 days. The cold stored samples lack several detections while the room temperature stored samples only lacks detection for day 10 in the dry hopped beers.

D-limonene was detected by the GC-MS for all the hop teas hopped with Cascade hops, the Vic Secret hop teas did not show any D-limonene. Limonene was detected in the 5 minute boiled Vic Secret tea. The D-limonene and limonene was detected at RT ~12.7 min.

In figure 17 the D-limonene starts out high on test day 1, for the room temperature stored beers, just like for beta-pinene, and then drops drastically on test day 5. The limonene fluctuates in the room temperature stored beers, while for the cold stored beers the limonene increases from test day 20. On test day 45 the amount of limonene is approximately the same for the brews that were hopped with 5 minute finishing hops.

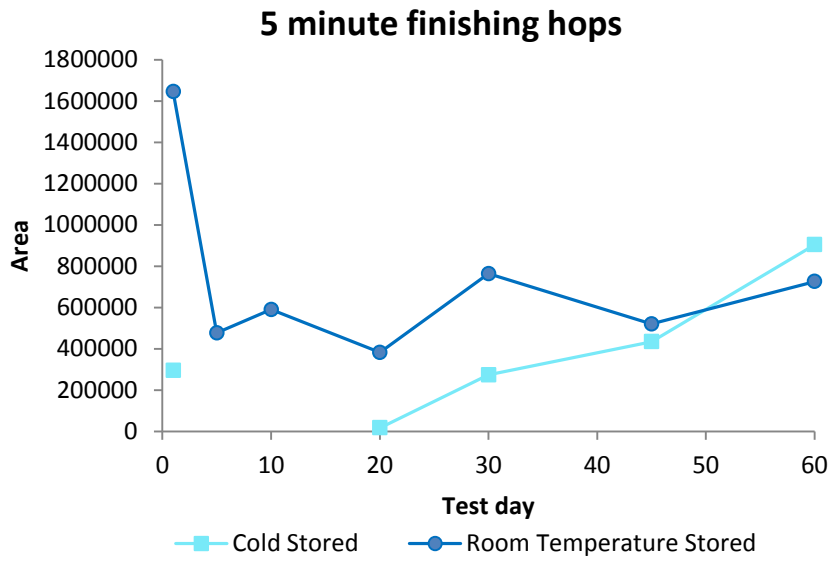



**Figure 17: The development of D-limonene in bottled beers, from day 1 to day 60 after bottling. The beers are aroma hopped with 5 minute finishing hops.**

The development of D-limonene in room temperature stored beer and cold stored beers, that was aroma hopped at 80 °C after the boil, was not synchronized, figure 18. The limonene in the room temperature stored beers increased from day 1 to day 5. While the limonene in the cold stored beers decreased. The fluctuations are opposites until day 45 after bottling, like for the 5 minute finishing hops brews the limonene is approximately the same in both the cold stored and room temperature stored beers.

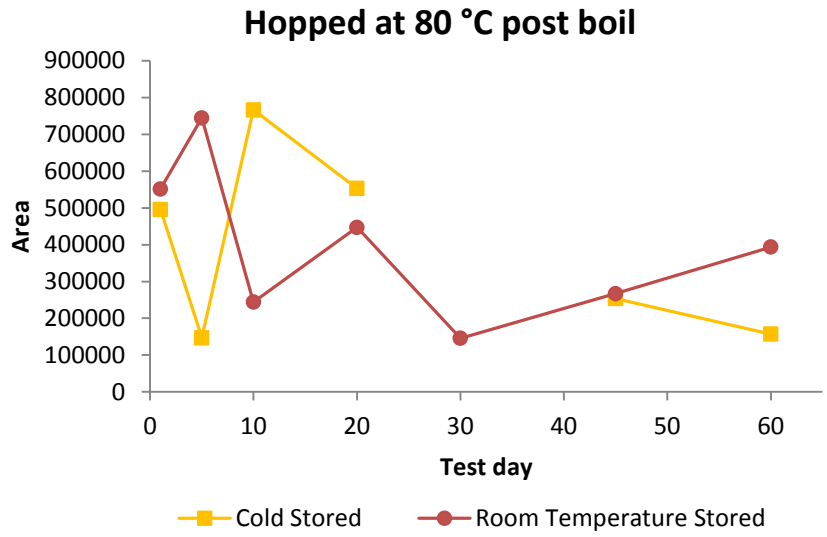



Figure 18: The development of D-limonene in bottled beers, from day 1 to day 60 after bottling. The beers are aroma hopped at 80 °C.

For the dry hopped beers, the GC-MS did not detect D-limonene for several of the cold stored beers. On day 45 after bottling again the limonene is approximately at the same level for both the room temperature stored beers and the cold stored beers, figure 19. For the room temperature stored beer the development of limonene follows the same pattern as for the beers that were aroma hopped at 80 °C after the boil, decreasing from day 20 to day 30 and increasing from day 30 to day 60.

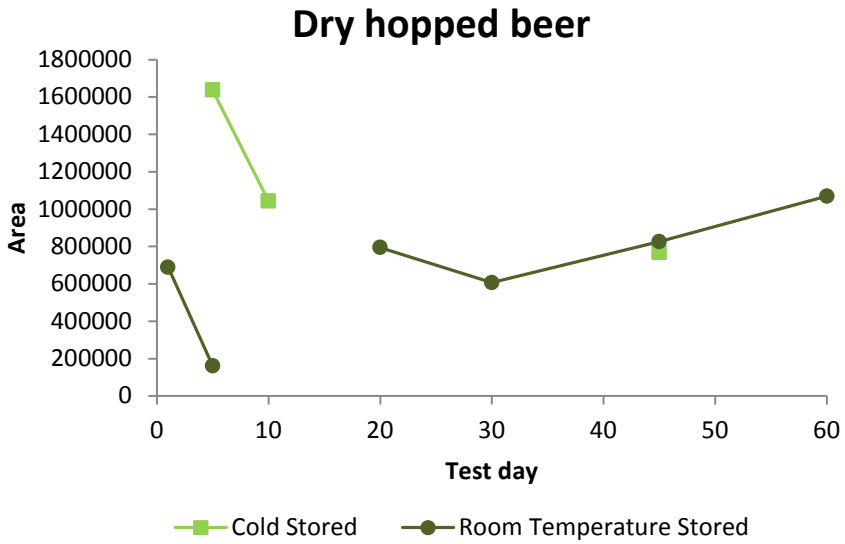



Figure 19: The development of D-limonene in bottled beers, from day 1 to day 60 after bottling. The beers are dry hopped.

### 4.3.3 Gamma-terpinene

Gamma-terpinene, or γ-terpinene, is a natural terpene that can be found in several plants. The odor and flavor of this compound is described as turpentine, citrus, lime, oily, green and a tropical fruity nuance (Furia and Chemical Rubber Company., 1980).

Gamma-terpinene was detected in all the hop teas that were hopped with Vic Secret, and detected in the 80 °C hop tea with Cascade hops. The gamma-terpinene was detected at RT ~12.9 min.

As for D-Limonene, also the gamma-terpinene lacked a few detections in the MS-GC. For the 80 °C post hopped brews gamma-terpinene did not get detected for any of the day 30 samples.

The development for gamma-terpinene was synchronized for the cold- and room temperature stored beers from the 5 minute finishing hops brews. This synchronized development is visible from day 10, in figure 20, and throughout the testing period. From day 10 the gamma-terpinene decreases and stays stable till day 45. Between day 45 and day 60 the gamma-terpinene increases for both cold- and room temperature stored beers.

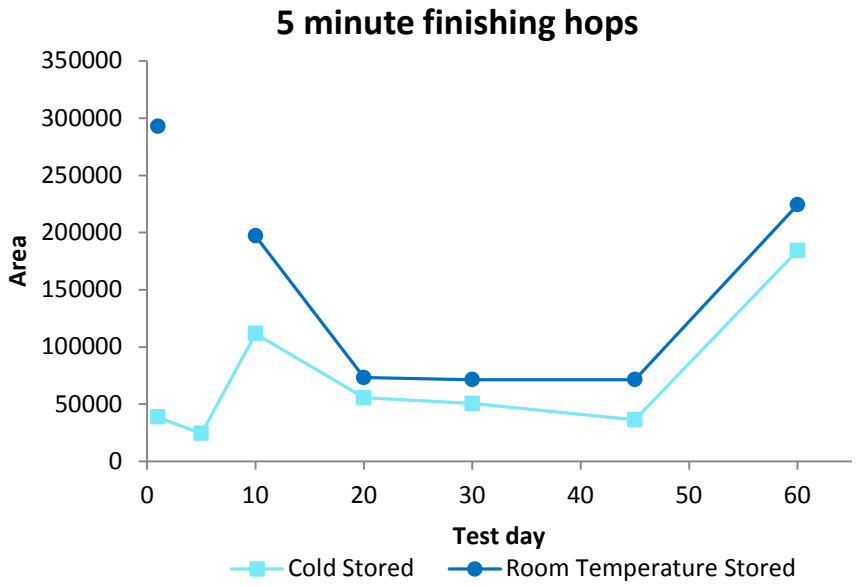



Figure 20: The development of gamma-terpinene in bottled beers, from day 1 to day 60 after bottling. The beers are aroma hopped with 5 minute finishing hops.

The GC-MS did not detect any gamma-terpinene for day 30, in figure 21, for the beers that were hopped with aroma hops at 80 °C after the boil. However the amount of gamma-terpinene starts at a lower point than what it started at for the 5 minute finishing hops brews. The pattern still seems to be the same as for the previous brews.

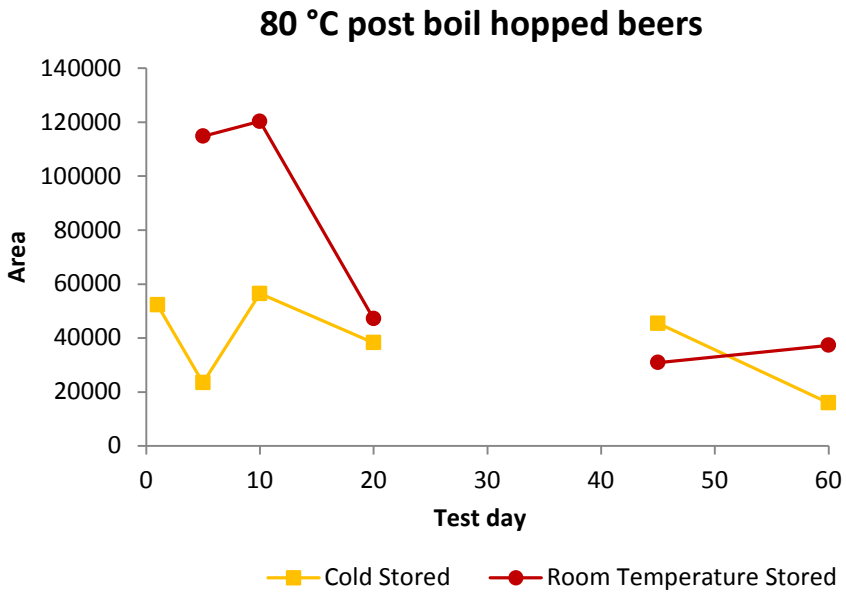



**Figure 21: The development of gamma-terpinene in bottled beers, from day 1 to day 60 after bottling. The beers are aroma hopped at 80 °C.**

Figure 22 shows somewhat of the same tendencies for both the cold stored and the room temperature stored beers for the dry hopping method. Gamma-terpinene was detected for all the test days in the dry hopped beers. From day 10 the development of the cold- and room temperature stored beer is fairly synchronized. The gamma-terpinene decreases from day 10 and increases to day 45. Both also decrease from day 45 to day 60. However, in the room temperature stored beers the gamma-terpinene content ends up at a higher level than in the cold stored beers.

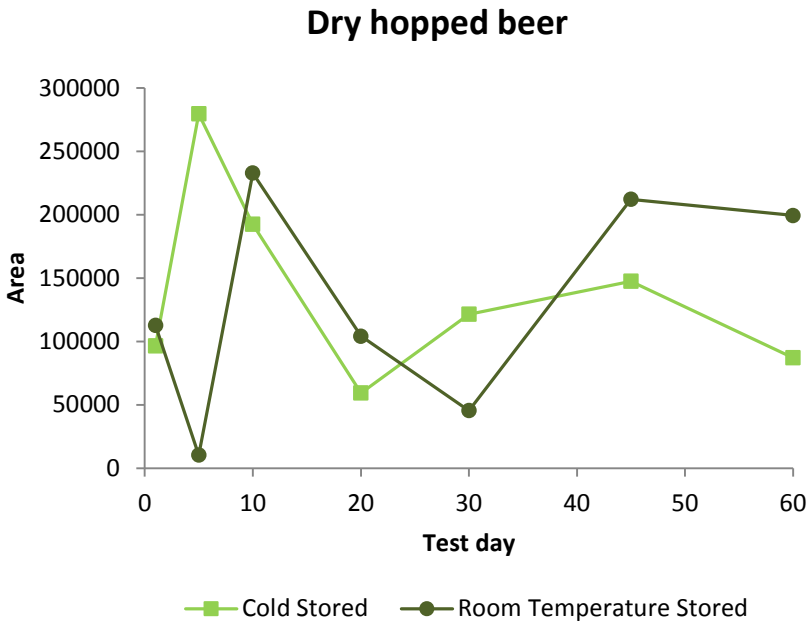



Figure 22: The development of gamma-terpinene in bottled beers, from day 1 to day 60 after bottling. The beers are dry hopped.

### 4.3.4 6-methyl-5-hepten-2-one

6-methyl-5-hepten-2-one is a heptenone, with a methyl group at position 6. It is a volatile oil component of citronella oil, lemon-grass oil and palmarosa oil. The flavors and odors are fruity, apple, musty, ketonic and creamy with slight cheesy and banana nuances.

This compound was well detected in all the beers for each test day. The development for 6-methyl-5-hepten-2-one seems to have some of the same fluctuation patterns for all the different hopping methods and both storage temperatures. The amount of 6-Methyl-5-hepten-2-one is almost doubled in the dry hopped beers than in the hot hopped beers.

6-methyl-5-hepten-2-one was detected by the GC-MS in all the hop teas at RT 15.7 min.

Figure 23 suggests that cold stored beer hopped with 5 minutes finishing hops keeps the 6-methyl-5-hepten-2-one better than room temperature stored beer. The fluctuations in these brews are not particularly synchronized. It seems that the cold stored beers have an increase of the 6-Methyl-5-hepten-2-one while the room temperature stored beers have a decrease between day 1 and day 60.

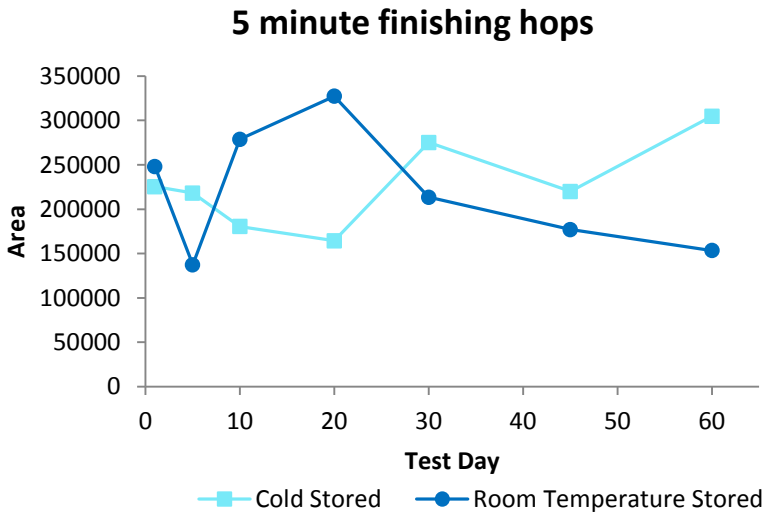



Figure 23: Development of 6-methyl-5-hepten-2-one in beer that has been aroma hopped with 5 minutes finishing hops.

For both storage temperatures the development of the 6-methyl-5-hepten-2-one starts with a decrease, figure 24, and increases into a peak from day 10 to day 20. The peak is followed by a decrease for both storage temperatures. The room temperature stored beers seem to end up on day 60 with the same amount of 6-methyl-5-hepten-2-one as on day 1. The cold stored beers end up on day 60 with a slightly lesser amount than the start on day 1.

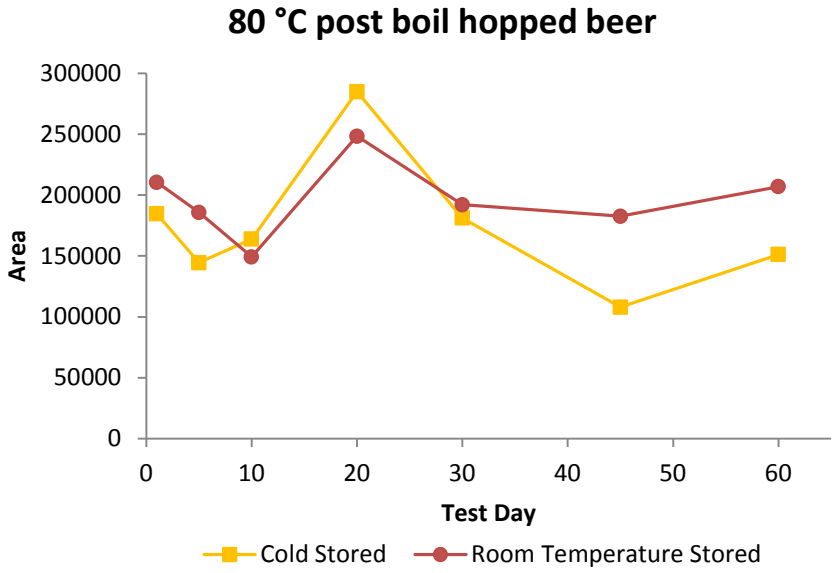



Figure 24: Development of 6-methyl-5-hepten-2-one in beer that has been aroma hopped with post boil hops at 80 °C.

Figure 25 shows there is more 6-methyl-5-hepten-2-one in the dry hopped beer than in the hot hopped beer. The measurements for the dry hopped beers show that the development of 6-methyl-5-hepten-2-one room temperature stored beers fluctuate more than for the cold stored beers. For both storage temperatures; between test day 1 and test day 60 the content of 6-methyl-5-hepten-2-one has decreased.

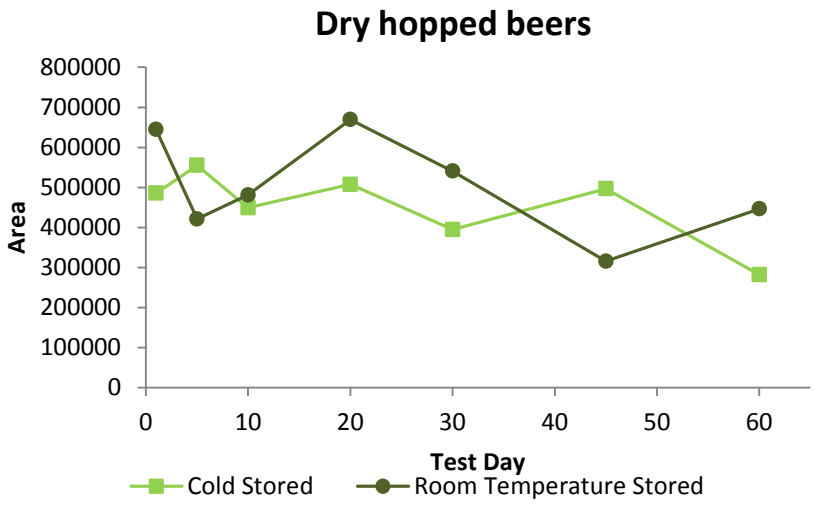



Figure 25: Development of 6-methyl-5-hepten-2-one in beer that has been dry hopped.

### 4.3.5 Citronellol

Citronellol, or dihydrogeraniol, is a natural acyclic monoterpenoid. This compound is made by hydrogenation of geraniol or nerol. Geraniol is also a monoterpenoid which is found in among others rose oil, citronella oil and palmarosa oil. Citronellol has rosy, floral, citrusy, woody and spicy flavors and odors.

Neither geraniol, nerol nor citronellol was detected in the hop teas.

The citronellol content of the beers seems to start out on approximately the same amount for the different hopping methods. During the 60 days of testing the citronellol develops.

Beers stored at both temperatures show a decrease in the citronellol content the first five days after bottling, for the brews that were aroma hopped with 5 minute finishing hops, figure 26. This is followed by an increase on day 10. On day 20 the citronellol content is the

same for both the cold stored and the room temperature stored beers, the content stays low and the same until day 45. The citronellol in the room temperature stored beers has increased on test day 60, while the citronellol in the cold stored beers stays low.

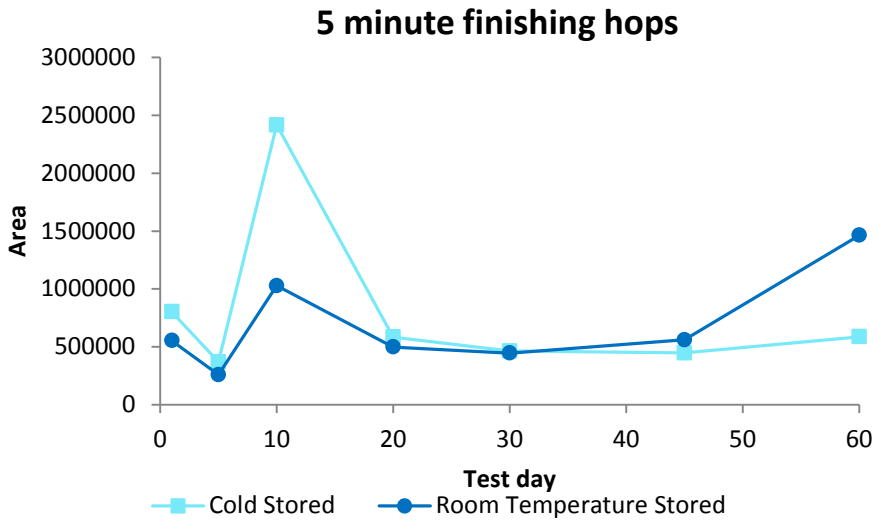



**Figure 26: Development of citronellol in brews that were hopped with aroma hops for 5 minutes of the boil, for cold stored and room temperature stored beers over the period of 60 days, after bottling.**

The citronellol content of the beers hopped at 80 °C after boiling is slightly lower than for beers hopped with 5 minute finishing hops. In these brews, figures 27 shows that the development of the citronellol starts with an increase. The cold stored beers show more dramatic fluctuations than the room temperature stored beer. On day 60 the content of citronellol has increased in the cold stored beers compared to day 1. For the room temperature stored beers the content of citronellol stays on the same level.

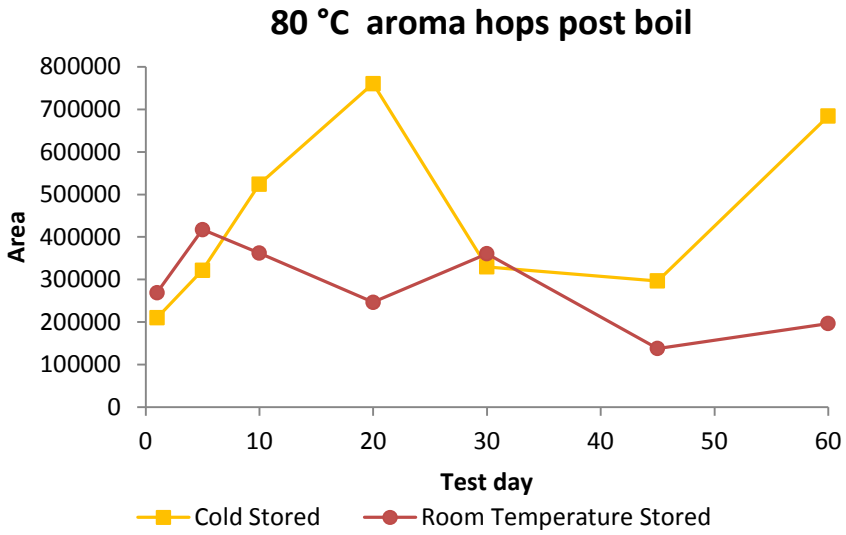



**Figure 27: Development of citronellol in brews that were hopped at 80 °C after the boil, for cold stored and room temperature stored beers over the period of 60 days, after bottling.**

The citronellol content in the dry hopped beers starts out low and increases during the 60 day period of testing, figure 28. The room temperature stored beers seem to have an overall greater increase than the cold stored beers. The citronellol content stays on approximately the same level for both temperatures until day 30. Between day 30 and 45 there is an increase, a large increase in the room temperature stored beers and a moderate increase in the cold temperature stored beers. Between day 45 and 60 the citronellol content in room temperature stored beers stays the same and the cold stored beers citronellol decreases.

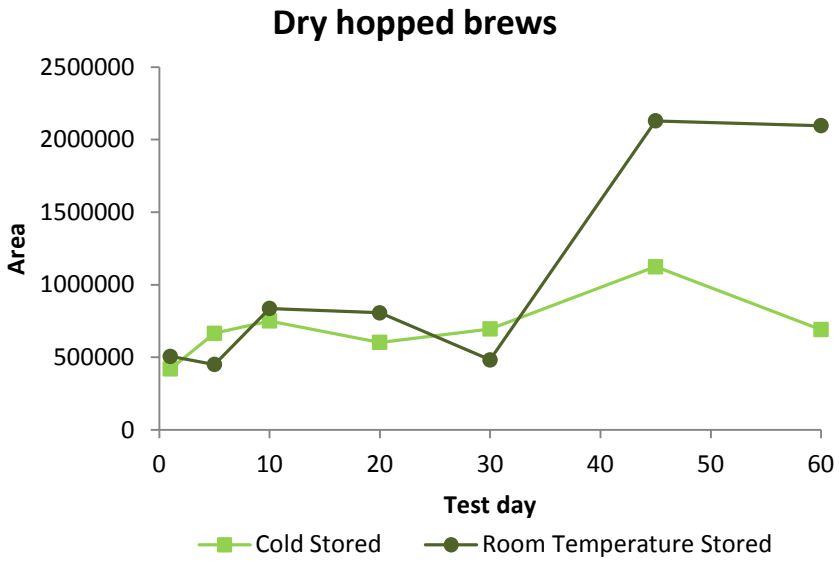



Figure 28: Development of citronellol in dry hopped brews for cold stored and room temperature stored beers over the period of 60 days, after bottling.

### 4.3.6 Nonanal

Nonanal is one of the common aroma compounds in hops, it is also one of the compounds that contribute to the citrusy scent and flavor of beer. The odor is described to be of rose and orange (Hawley and Lewis, 2002). Nonanal is found in many essential oil derived from plants, such as rose, citrus and pine.

Nonanal was detected in all brews, and all hop teas, both for the Vic Secret and the Cascade hops at RT ~16.6 minutes.

The nonanal readings show developments in the different beers that is somewhat synchronized both for the different hopping methods and the two storage temperatures. All the brews seem to have approximately the same amount of nonanal.

In the 5 minute finishing hops beers, the nonanal has the same fluctuations for both cold stored and room temperature stored beer from day 10. Further the nonanal decreases for day 20 and increases on day 30, followed by a new decrease on day 45, figure 29.

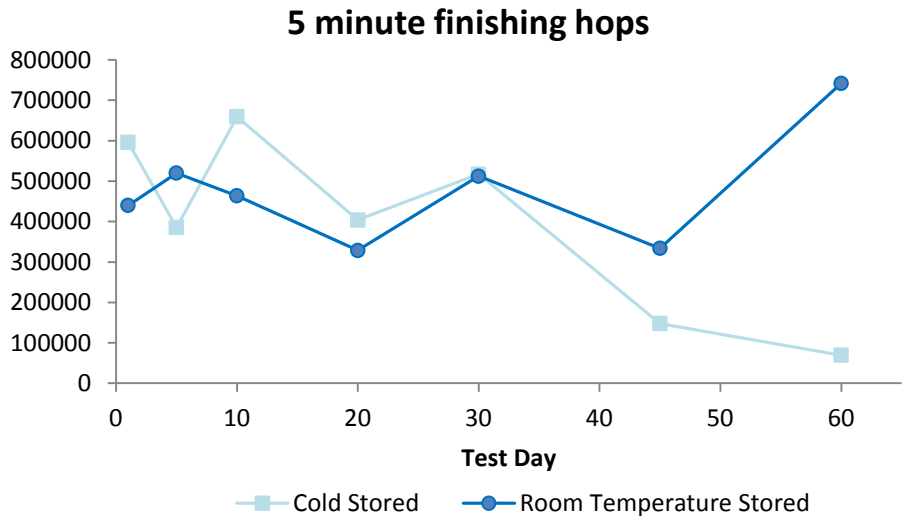



Figure 29: Development of nonanal, day 1 to day 60 after bottling and storing in beers hopped with 5 minute finishing hops.

To a certain extent the nonanal, in the 80 °C post boil hopped beers, has the same fluctuations for both cold stored and room temperature stored beer also from day 10, as can be seen in figure 30. The nonanal decreases for day 20 and increases on day 30, followed by a new decrease on day 45.

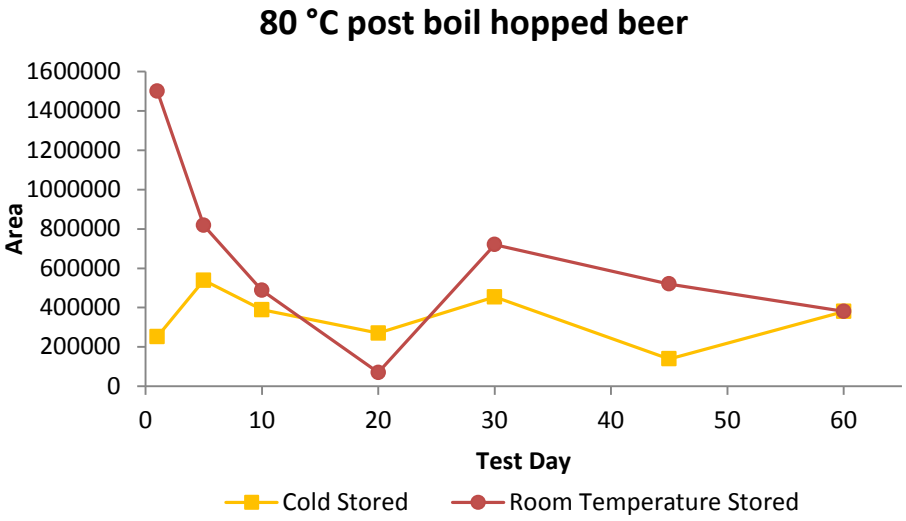



Figure 30: Development of nonanal, day 1 to day 60 after bottling and storing in beers that were aroma hopped at 80 C after the boil.

The nonanal content in the dry hopped beers follows the same pattern as the other brews. In figure 31 the content of the nonanal, seem to be the most synchronized of the three hopping methods.

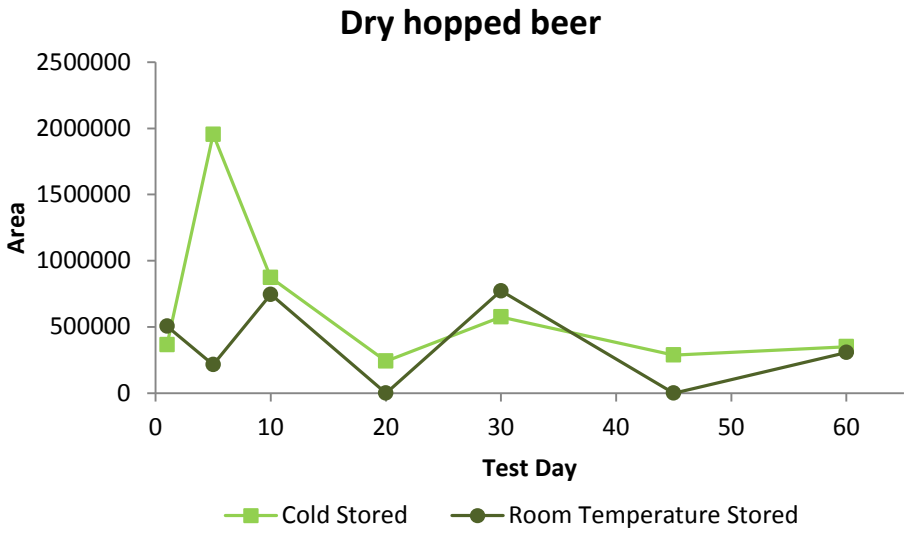



Figure 31: Development of nonanal, day 1 to day 60 after bottling and storing in beers that were dry hopped.

## 4.4 Bitterness

The bitterness that was measured using the EBC standard method resulted in similar trends in the bitterness development in the different brews. The bitterness developments are presented as the average for the brews with the same hopping methods. The brews seem to show some of the same pattern; a decrease in the bitterness on test day 20 followed by an increase again at day 30, followed by a new decrease on day 45.

The similar brews were tested with a two-sample t-test in MiniTab. The bitterness in room temperature stored beers from brew 2 and 5 was significantly different, however, the average of these two will still be presented together because the trends in the fluctuations are similar. The p-values for the two-sample t-tests are presented in table 10.

Table 10: Two-sample t-test for alcohol content, the similar brews were tested against each other in a two-sample t-test for significance. The p-values must be greater than 0.05 for the brews not to be considered different.

| Brews | Hop addition | Storage | p-value | Significantly different |
|---|---|---|---|---|
| **1 and 4** | 5 minutes finishing, aroma hops | Room Temperature | 0,726 | No |
| **2 and 5** | 80 C, post boil, aroma hops | Room Temperature | 0,026 | Yes |
| **3 and 6** | Dryhop, aroma hops | Room Temperature | 0,351 | No |
| **1 and 4** | 5 minutes finishing, aroma hops | Cold | 0,848 | No |
| **2 and 5** | 80 C, post boil, aroma hops | Cold | 0,051 | No |
| **3 and 6** | Dryhop, aroma hops | Cold | 0,133 | No |

Figure 32 shows the decrease in bitterness from day 10 to day 20, and the increase from day 20 to day 30 clearly. Both the cold stored beers and the room temperature beers with the 5 minute finishing hop addition follow the same pattern. Both the cold stored and the room temperature stored beers start out at a bitterness of ~60 IBU on test day 1. The beers then end up with a bitterness of ~50 IBU on test day 60.

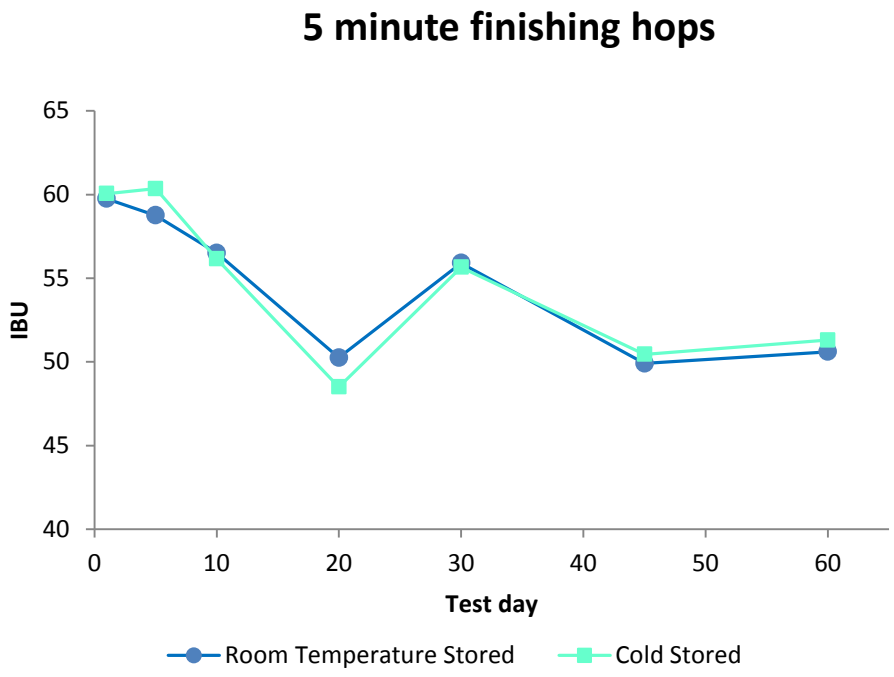



Figure 32: Development of bitterness in bitter hopped and 5 minute aroma hopped beer from day 1 to day 60 after bottling.

The bitterness in the beers where the brews were hopped at 80 °C after the boil also decreases from day 10 to day 20, and increases from day 20 to day 30. Both the cold stored

beers and the room temperature stored beers follow the same pattern. Figure 33 shows that despite the fluctuations during storage the bitterness starts and ends up in the same area; at ~51 IBU.

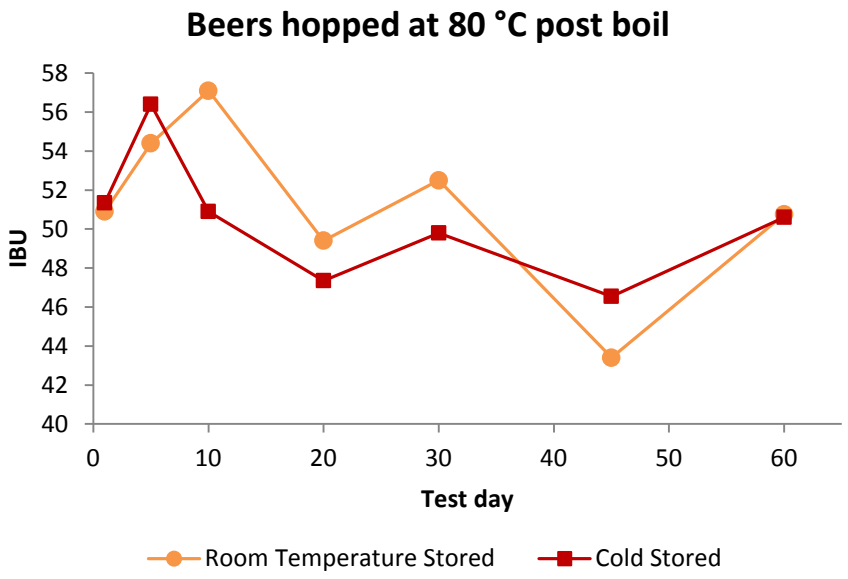



Figure 33: Development of bitterness in bitter hopped and dry hopped beer from day 1 to day 60 after bottling.

The dry hopped brews show that the bitterness in the cold stored beers fluctuated less than the room temperature stored beers. For the room temperature stored beer there is the decrease in bitterness from day 10 to day 20, and increase from day 20 to day 30. This is true for the cold stored beers too, but the fluctuations are less dramatic. Figure 34 shows the fluctuations for the room temperature stored beers during storage where the bitterness starts at ~56 IBU and ends up at ~53 IBU on test day 60. The cold stored beers also have the same decrease from ~55 on test day 1 to ~51 on test day 60.

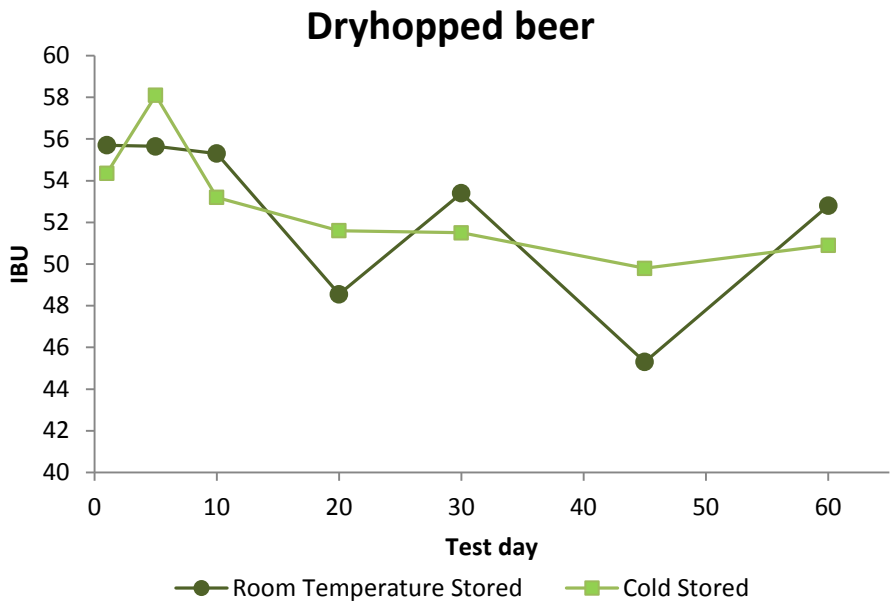



Figure 34: Development of bitterness in bitter hopped and dry hopped beer from day 1 to day 60 after bottling.

## 4.5 Sensory

- 10 panelists with two sets of samples give 20 assessments.
- n = number of trials = 20
- $E_c$= Expected number of correct responses = n (1/3) = (20)(1/3) = 6,67 ≈ 7
- $E_i$ = Expected number of incorrect responses = n(2/3) = (20)(2/3) = 13,33 ≈ 13
- α = risk of a Type I error = 0.05
- Amount of correct responses needed to reject $H_0$ (Appendix) = 11
- The room temperature stored beer was given the letter “A” and the cold stored beer was given the letter “B”.
- The Triangle Test was executed against the null hypothesis, $H_0$: A=B

The amount of correct answers needed in the triangle test was 11, the most correct answers were in the triangle test on day 30 for brew 3 and brew 4 with 10 correct as seen in table 4.

Table 11: Total amount of correct answers during triangle testing of beer.

| Test Day | Brew 1 Correct Answers | Brew 2 Correct Answers | Brew 3 Correct Answers | Brew 4 Correct Answers | Brew 5 Correct Answers | Brew 6 Correct Answers |
|---|---|---|---|---|---|---|
| **30** | 5 | 6 | 10 | 10 | 7 | 6 |
| **60** | 5 | 8 | 9 | 8 | 6 | 9 |

# 5 DISCUSSION

The calculated alcohol for the beers should be 5 %, the Anton Paar measurements show that day 1 after bottling all brews are higher than 5 %. The brews with 5 minute finishing hops had an alcohol percent of 5.14, the brews that were added aroma hops at 80 °C had an alcohol percent of 5.34 and the dry hopped beers had an alcohol percent of 5.49. After 60 days the alcohol percent had raised with 0.4 – 0.5 % for all the brews, both cold stored and room temperature stored. For home brewers this raise in alcohol after bottling bodes not problems, however for microbreweries that bottle carbonate their beers this can become an issue. Beers that are sold in the grocery stores may have a maximum ABV of 4.7 %, a raise of alcohol by 0.5 % gives ABV of 5.2 % which should be sold in the liquor stores.

After bottling the beer still contains some live yeast, it is this yeast that will carbonate the beer in the bottles and raise the alcohol level. To achieve carbonation the beer is added sugar; priming sugar. The anaerobic respiration of the yeast will again develop alcohol and $CO_2$. According to the calculator, the residual $CO_2$ in the beers were 0.9 g/L and to achieve a carbonation volume of 2.3 g/L the amount of sugar added in the brews should be 5.5 g per 1 L brew. On day 1 after bottling the $CO_2$ started at ~1.5 g/L for all the brews, and on day 60 the $CO_2$ were 3.95 g/L for room temperature stored beers with the 5 minute hopping, and 3.69 g/L for the cold stored beers. For the room temperature stored beers that had been hopped at 80 °C the $CO_2$ was at 4.57 g/L and for the cold stored it was 4.28 g/L. The dry hopped beers, room temperature stored beer had a $CO_2$ of 4.29 g/L and the cold stored had 4.10 g/L. This shows that the cold stored beers developed slightly less $CO_2$ than the room temperature stored beers. However the $CO_2$ levels are higher than calculated. This suggests primarily that too much priming sugar was added, or the residual $CO_2$ was higher than 0.9 g/L, or the beer was left to carbonate for too long before cold conditioning. When the $CO_2$ increases too much, this can cause exploding bottles, or fountains when the beers are opened.

Both the alcohol and the $CO_2$ in the beers increased after bottling, possibly too much because of too much priming sugar. Even with calculations and following instructions the $CO_2$ ended up almost double of what it should have been. This suggests that the beer should

be primed with less sugar, i.e. at the low end of the suggested $CO_2$ for IPA; 1.5 g/L which would have been 2.5 g sugar/L wort (Riley, 1998).

Some of the brews showed haze (brews 1, 2, 4 and 6), which means the hot break and cold break proteins didn't precipitate well enough. While some of the brews were clear from the day they were brewed (brews 3 and 5). The clear brews did not get hazy during cold conditioning which means the chilling of the wort made the chill haze proteins precipitate well. When it comes to the Anton Paar results compared to the visual assessment the color readings from the Anton Paar becomes wrong for very hazy beer. The Anton Paar color option uses light to decide both the color and the haze. This means if the beer is very hazy, the light that goes through the sample will read as darker than what it actually is. This is due to the haze not letting light through to the color detector. Looking at brew 4, the EBC measurement for color said 20 – 25 which suggest this should have been an orange to brown looking beer, while in fact this beer was very light, around 4 – 6 EBC, but hazy. The brews 5 and 6 gave better readings in the Anton Paar, even brew 6 with some haze did not read much higher than brew 5 with no haze. Brew 5 read 6 – 8 EBC for color and 1 – 2 EBC for haze, brew 6 read 7 – 9 EBC for color and 3 – 1 EBC for haze. The decreased haze was more evident for the cold stored beer than for the room temperature stored beer. This can be because the chill haze precipitates forms in the colder temperature. According to the visual assessment of the beers, most of the brews, both the cold stored and the room temperature stored becomes less hazy with storage. This project the beers were brewed without the addition of Irish moss[5], which means that the beers that turned out hazy may have a fair bit of proteins left in suspension. In the studies of Steiner et al. (2010) look at methods for identifying different forms of haze in beer. Although the most likely reason for the haze in these beers is the presence of proteins, there may also be some haze due to calcium oxalate or glucans. Glucans may be present in the beer if the lautering water was too hot or if large starch kernels, due to poor milling, survived the boiling (Steiner et al., 2010).

[5] Irish Moss is a seaweed derived fining agent used by many brewers to help make a clear beer without the need for a filter, and to prevent chill haze. Irish Moss accelerates protein coagulation during the end of the boil which helps prevent chill haze. PALMER, J. J. 2006. *How to brew - Everything you need to know to brew beer right the first time,* Boulder, Colo., Brewers Publications.

In 2013 Van Opstaele et al. found 91 different volatile compounds in hops. These compounds were classified as esters, terpenes, ketones, aldehydes and furans. Several aroma compounds were detected with the GC-MS in this thesis, and six of these were chosen to represent some of the aromas in beer. These compounds are beta-pinene, D-limonene, gamma-terpinene, 6-methyl-5-hepten-2-one, citronellol and nonanal. Five of these are present in hops as they are. Citronellol on the other hand is converted from geraniol (Takoi et al., 2010) with the help of the enzyme Old Yellow Enzyme (OYE2) that is present in yeast (Steyer et al., 2013). Although these compounds are present in the hops, and later in the beer, it does not necessarily mean they can be tasted. All aroma compounds have a certain flavor threshold (Schönberger and Kostelecky, 2011). The six chosen compounds are described to have similar flavors and odors; citrusy, woody, piney. Generally green and grassy flavors are attributed to aldehydes, citrus flavors stems from esters, linalool and nerol, while floral and fruity flavors appears from, among others, citronellol, geraniol, ketones and esters (Schönberger and Kostelecky, 2011).

Beta-pinene was only detected in hop tea that was boiled for 5 minutes with the Vic Secret hop pellets. However beta-pinene was detected in all the beers during testing. This is because beta-pinene only slightly soluble in water, and more soluble is in alcohol (PubChem, 2015). Beta-pinene should give the beer a woody and piney aroma. With the results that came from the GC-MS it is not directly possible to say which of the hopping methods resulted in more beta-pinene in the beers. The beta-pinene developed during the 60 days in the bottles.

D-Limonene and gamma-terpinene lacked a few detections in the MS-GC. For the 80 degree post hopped brews gamma-terpinene did not get detected for any of the test day 30 samples. Even though the dry hopped beers seems to show some kind of the same tendencies for both the cold stored and the room temperature stored samples it is possible these results are coincidental. However, what is interesting about the D-limonene in this trial is that on day 45 after bottling the amount of D-limonene is the same for both cold stored and room temperature stored beers, for all brews. It is surprising that the amount of D-limonene is equal in the different brews on day 45. The dry hopped beer received the hops a week later than the other brews.

Gamma-terpinene showed synchronized and similar amounts in the 5 minute finishing hops brews and in the dry hopped beers. While the 80 °C post boil hopped beers lacked the detection of gamma-terpinene on day 30.

6-methyl-5-hepten-2-one is described by Furia (1980) to be colorless, with a powerful, fatty, green citrus odor and of fruity flavor (Furia and Chemical Rubber Company., 1980). This suggests that the compound may come from the hops and not from the yeast. When looking at the distilled water and hop teas that were brewed fresh and analyzed this compound is also found in all the brews at the same retention time, ~15.7 minutes. Therefore it is safe to say that this compound derives from the hop addition. For the different brews there is a decrease in the bitterness on day 20 for most of the, at test day 20 there is simultaneously an increase of the 6-methyl-5-hepten-2-one. These comparisons would have been interesting to see in a bigger scale production if several samples from the same test days give the same results or if these are coincidences. However, for a home brewer, even for a microbrewery, to recreate the exact same beer is difficult. Although brew 1, 2 and 3 were brewed several days before brews 4, 5 and 6 they showed the same tendencies. The presence of 6-methyl-5-hepten-2-one, indicate that there may be linalool present in the beers as 6-methyl-5-hepten-2-one can be converted into linalool (Bauer et al., 2001).

The most even of the aroma compounds were nonanal, this aroma had a synchronized and level development for all the brews. Only for the 5 minute hopped brew, which was room temperature stored did it seem like the nonanal got a drastic increase from day 45 to day 60. The other brews ended on approximately the same amount of nonanal in the beers on day 60. When both the cold stored and the room temperature stored beers show synchronized development for several of the aroma compounds, it is reason to believe that there may not be all coincidences.

Unfortunately this thesis is lacking in detection of several of the major volatile compounds that should have been present, garniol, farsene, humulene, myrcene, caryophyllene among others. These could possibly have been detected by using Head Space Gas Chromatopgraphy (HS-GC). This machine had a mal function and needed replacing; the machine did not get in operating condition in time to add results for this thesis. On the other hand, the studies of King and Dickinson (2003) shows that a substantial part of the geraniol is biotransformed by

*Saccharomyces cerevisiae* into citronellol, and some nerol and linalool. Citronellol was detected in all the brews in decent quantities. The presence of citronellol also confirms the presence of geraniol. Their studies also showed that after 15 days of fermentation none of the beta-caryophyllene, alpha-humulene and beta-myrcene was detected (King and Dickinson, 2003). The loss of beta-myrcene during fermentation is also interesting considering that beta-myrcene is the dominant compound of the monoterpene hydrocarbons. While beta-myrcene content in fresh hops may be up to 95 % of the monoterpene hydrocarbons, the last 5 % is beta-pinene and alpha-pinene (Van Opstaele et al., 2013). This may indicate that some of the compounds in hops are so volatile that they will not make it through fermentation and into the finished beer.

When it comes to the bitterness some interesting results came about, as the samples were not tested on the same day, nor were they tested in the order the samples were collected (due to freezing the samples). To begin with the results looked like the bitterness in the different beers was ranging over 40 – 60 IBU randomly. After putting the results in order however, the brews seem to show some of the same pattern; a decrease in the bitterness on test day 20 followed by an increase again at day 30, followed by a new decrease on day 45. This phenomenon suggests that the bitterness is not set definitely at the end of the boil. When it comes to the bitterness, samples should have been collected and measured at the end of the boil as well. Taniguchi et al. (2013) found that during storage the unisomerized alpha- and beta-acids becomes oxidized to humuliones and hulupones, respectively. It is a fact that neither alpha-acids nor beta-acids from the hops are particularly bitter, but their oxidized forms are. Beta-acids are more heat stable than the alpha-acids and will therefore remain in the wort as beta-acids until they begin to be oxidiced (Verzele and De Keukeleire, 1991). During storage these two components may therefore add to the bitterness in beer. For storage at 20 °C, the development of the oxidizing beta-acids were measured over a period of 40 weeks, the development went from close to 0 µmol/L to ~20 µ/L (Taniguchi et al., 2013). Their study also suggested that the hulupone absorbed better at a wavelength of 330 nm. This further may indicate that the even if the beta-acids contribute to bitter taste in the beer; they may not contribute to higher readings of the bitterness that was measured at 275 nm in this thesis. The spectrophotometric method will measure any compound that

absorbs at 275 nm, like for instance the alpha-acids that have not been isomerized (Benitez, 1997). The fact that the different brews show the similar pattern is compelling.

It seems that the beers with 5 minutes finishing hops have the largest drop in bitterness, from ~60 to ~50 IBU. However the start out IBU, all the brews seem to end up at ~50 IBU, which is also very close to the calculated IBU of 52 for brews 2, 3, 5 and 6, but less than for brew 1 and 4 which had a calculated IBU of 62. Staling of beer is linked to the loss and alterations of the iso-alpha-acids (De Cooman et al., 2000). Mikyška et al. found that beer that was hopped with aroma hops developed more polyphenols stabilizes the flavor of the beer (Mikyška et al., 2002). Only 60 days of storage on these brews, with such high IBU (50 – 60) would not be affected by staling.

The sensory panel was not able to distinguish between the cold stored and the room temperature stored beers. When comparing to the MS-GS results, this may not be surprising. The decrease in aromas, in especially the dry hopped beer, is not as large as assumed. A sensory panel consisting of fine-tuned tasters may have given a different result. During discussions with the panelists after the sensory tests the panelists all agreed that the most of the test had been guesswork and they could not find a difference in the cold stored and room temperature stored beers, they did however mention that there was a difference between some of the beers that had been hopped differently. This should also have been a triangle test to see if this was confirmed.

When it comes to the MS-GC results, a pattern was not clearly detected for the measured volatiles. The true patterns would also require larger amounts of samples for each testing day as well as a longer aging period.

If this project had been done in a bigger scale, as in completely brewing all the batches in the brewery equipment, then the brews number 2 and 5 would’ve had the hops added during whirlpooling, which is more common for microbreweries, this could’ve resulted in a different aroma profile than the 80 °C hopped brews got. Another possibility would’ve been to add the hot brew to a wider kettle or a fermenter bucket and make a whirlpool using a drill with a propeller blade connected; this would’ve had to be bought or made specially and was not opted as a possibility this time.

In this thesis the measurements were done on bottled beer only, however loss of aroma may also happens in the boil, during cooling, during fermentation and so forth. To see if this occurred, and how much was lost during cooling and fermenting then measurements could have been executed in this time period too.

One of the biggest challenges in this project was temperature regulations. During mashing the set temperature for mash-in, mash and mash-out worked flawlessly due to the professional equipment. Cooling of the wort were also considered good enough with cold water running through the coil and cooling the wort fairly fast, as the kettle volumes were small (~25 L). The fermentation temperature was more challenging to keep at a fixed temperature. As the brewery was situated in direct sunlight the daytime temperatures for fermenting rose a few degrees, good aeration of the brewery luckily did not elevate ambient temperatures. During fermentation the temperature in the fermentation vessel will increase. The yeast strain that was used in this thesis, Safale US-05, is a yeast that can grow well in temperatures from 12 to 25 °C. Torija et al. found that with increased temperatures during fermentation, the secondary products, such as fusel alcohols would increase at the expense of the ethanol. The increased temperature that caused noticeable changes was however 35 °C (Torija et al., 2003). Although it is important to keep a flat fermentation temperature, Torija et al., did not find drastic differences for the fermentation temperatures under 35 °C. With the fermentation temperature rising during the vigorous first part of the fermentation, the result may be that the fermentation goes too fast. This can also result in very fruity ester flavors such as old bananas, from isoamyl acetate. The formation of esters is not only based on raised temperatures, it may also depend on the sugar content. Ultra-high gravity worts, 20 °Plato, can give up to 75 % more ester formation when diluted to a normal gravity 12 °Plato wort (Verstrepen et al., 2003). The brew in this thesis was of normal gravity, and the temperatures did not reach 35 °C, even without proper temperature control of the fermentation. The temperature may have rose to 25 °C during the primary fermentation, but the beer did not seem to have suffered noticeably.

# 6 CONCLUSION

Of the hop aromas that were detected with the GC-MS and further investigated in this thesis. The GC-MS results for the five chosen aroma compounds that come from hops in this thesis were not alone enough to say if the aromas degraded more or less based on storage temperature. The sensory triangle test confirmed the analyses, the differences in the aroma for beers stored at different temperatures was not detectable. Further for the chosen aroma compounds there is a slight difference in the amounts when it comes to hot hopped and dry hopped beers; the dry hopped beers generally had more of the volatile aroma compounds.

What is important to know about homebrewed, or even microbrewery brewed beer is that these beers are living products, and it is not as black and white as theorized. The overall theory is that volatile aroma compounds will degrade in the end. There was a tendency for some of the compounds to be at a lower point on day 60 compared to day 1. However, during this period there were fluctuations up and down, some compounds increased and some decreased. To be able to find a true pattern for the development of the volatile aroma compounds, bigger projects must be done with several more samples per test day.

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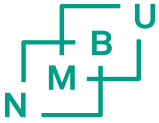
# APPENDIX

See attached CD for raw data from the project.



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