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Growth, metabolism and beer brewing with kveik

Nadia Marlen Aasen
Food Science

Foreword

This master thesis was written as a finishing part of a master's degree in Food Science, and it was delivered at the Faculty of Chemistry, Biotechnology and Food Science at the Norwegian University of Life Sciences in June 2020. The research that was performed took place in the time period September 2019 to April 2020. The thesis consists of 60 ECTs.

I chose an assignment with the topic of brewing and fermentation as these processes are very interesting and complex. I have been very lucky to be able to use a broad specter of methods and analyses, which has made the assignment fun, varied and extremely interesting. The work has been time-demanding, but the experience and knowledge that has come as a result of the work has made it more than worth it in the end.

I would like to thank everyone who made this assignment possible. My supervisors Trude Wicklund and Hilde Marit Østlie have been truly inspirational, and it has been an honor and a pleasure to work with them on this project. A special thanks goes out to May Aalberg, Ahmed Abdelghani, Kari Olsen and Davide Porcellato, who were a great help and inspiration throughout the entire process, and to the wonderful people at the Writing Centre at NMBU. My deepest thanks to everyone who shared their kveik, their knowledge and their expertise with me at the 2019 Norwegian Farmhouse Ale Festival. A sincere thank you to Lars Marius Garshol, who shared generously of his vast knowledge on kveik and traditional Norwegian brewing.

To my family, friends and classmates; thank you for your support and encouragement. I could not have done this without you.

Norwegian University of Life Sciences

Faculty of Chemistry, Biotechnology and Food Science

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Nadia Marlen Aasen

Sammendrag

I denne oppgaven ble den mikrobiologiske sammensetningen av ti ulike typer kveik studert. Stammer av bakterier og gjær fra de fire kveikene Ørjasæter, Gausemel, Otterdal og Gamlegrua er blitt isolert, sekvensert og identifisert. Eukaryote og prokaryote mikrobiota analyser ble utført på disse, i tillegg til kveikene Espe, Tormodgarden, Midtbust, Ebbegarden, Sigmund og Stalljen. De antibakterielle egenskapene hos einer ble studert mot melkesyrebakteriene (MSB) *Lactobacillus plantarum*, *Lactobacillus buchneri* og *Lactobacillus brevis*. Kveik typene Ørjasæter, Gausemel og Gamlegrua er blitt studert i vekst- og metabolisme studier, ved temperaturene 22 °C, 30 °C og 37 °C. Vekst- og metabolisme studier ble utført for å analysere kveikene ved flere inkubasjonstemperaturer og parametre enn det som var mulig å utføre i pilot-skala bryggingen. Målinger av pH, cellevekst, sukkerinnhold og aroma komponenter ble tatt ved 0, 2 og 7 dagers inkubering. Disse kveik typene ble deretter brukt i pilot-skala brygging. Hensikten med bryggingen var å studere forskjeller i metabolitter og sensoriske karakteristikker mellom de ulike kulturene når fermenteringen ble utført ved ulike temperaturer. Triplikat (n = 3) av 9 øltyper, kvadruplikat (n = 4) av 1 øltype og duplikat (n = 2) av 1 øltype, der de tre ulike fermenteringstemperaturene 22 °C, 30 °C og 37 °C ble benyttet, ble analysert ved hjelp av «headspace» gasskromatografi (HSGC).

Gjæren *Saccharomyces cerevisiae* ble isolert og identifisert ved Sangersekvensering i Ørjasæter, Gausemel, Otterdal og Gamlegrua. Bakteriene *Lb. plantarum* og *Acetobacter malorum* ble isolert og identifisert ved Sangersekvensering i Gausemel og Gamlegrua. Det kunne observeres sekvensvarianter av gjæren *S. cerevisiae* i kveikene fra eukaryot mikrobiota analyse. Den prokaryote mikrobiota analysen viste at alle ti kveiker i denne studien inneholdt bakterier. Antibakterielle egenskaper av einerekstrakt ble observert mot MSB. Forskjeller i innhold av flyktige komponenter og totalinnhold av fenoler mellom de ferdige ølene ble observert, både som resultat av ulik fermenteringstemperatur og kultur benyttet til å utføre fermenteringen.

Konklusjonen ved studien er at einerlog laget av kvist og nåler fra einer har en antibakteriell effekt mot MSB. Noe effekt ble observert av umodne bær. Alle ti kveik typene som er inkludert i denne studien består av sekvensvariasjoner av *S. cerevisiae* og sekvensvariasjoner av flere bakterier, derav *Acetobacter* spp., *Lb. brevis*, *Lb. backii* og *Lactococcus lactis*. Lavere fermenteringstemperaturer resulterer i høyere innhold av fenoler og estere i øl fermentert med kveik. Bruk av einerlog i ølbryggingen fører til et høyere totalinnhold av fenoler og et lavere innhold av diacetyl og estere i det ferdige ølet. Øl fermentert med kveik inneholder høyere nivå av estere, og lavere nivå av høyere alkoholer og diacetyl, sammenlignet med øl fermentert med US-05.

Abstract

In this thesis, the microbiological diversity of ten different types of kveik were studied. Strains of bacteria and yeast from the four kveiks Ørjasæter, Gausemel, Otterdal and Gamlegrua were isolated, sequenced and identified. Eukaryotic and prokaryotic microbiota assays were performed on these kveiks, in addition to the kveiks Espe, Tormodgarden, Midtbust, Ebbegarden, Sigmund and Stalljen. The antibacterial effects of juniper were studied against the lactic acid bacteria (LAB) *Lactobacillus plantarum*, *Lb. buchneri* and *Lb. brevis*. The kveiks Ørjasæter, Gausemel and Gamlegrua were studied in growth and metabolism studies, at the temperatures 22 °C, 30 °C and 37 °C. The purpose of the growth and metabolism studies were to test the kveiks using additional temperatures and parameters than what was possible to do in the pilot-scale brewing. Measurements of pH, cell count, sugar content and aroma components were done after 0, 2 and 7 days of incubation. These kveiks were also used in pilot-scale brewing. The purpose of the brewing was to determine differences in both metabolites and sensory characteristics between the cultures when the fermentation was done at different temperatures. Triplicate (n = 3) of 9 beers, a quadruplicate (n = 4) of one beer and a duplicate (n=2) of one beer, using the three fermentation temperatures 22 °C, 30 °C and 37 °C, were analyzed using Headspace gas chromatography (HSGC).

The yeast *Saccharomyces cerevisiae* was isolated and identified by Sanger sequencing in Ørjasæter, Gausemel, Otterdal and Gamlegrua. The bacteria *Lb. plantarum* and *Acetobacter malorum* were isolated and identified by Sanger sequencing in Gausemel and Gamlegrua. It was observed sequence variants of the yeast *S. cerevisiae* in the kveiks from the eukaryotic microbiota assay. The prokaryotic microbiota assay showed that all ten kveiks analyzed in this study contained bacteria. There were observed antibacterial effects of juniper extracts against LAB. Differences in content of volatile components and total phenol content between the finished beers were observed, both as results of differences in fermentation temperature and culture used to perform the fermentation.

The conclusion to this study is that einerlog prepared from juniper twigs and needles have an antibacterial effect on LAB. Some effect was observed by unripe berries. All ten kveiks included in this study consists of sequence variants of *S. cerevisiae* and sequence variants of several bacteria, such as *Acetobacter* spp., *Lb. brevis*, *Lb. backii* and *Lactococcus lactis*. Lower fermentation temperatures result in higher total phenolic content and ester content in beer fermented with kveik. The addition of einerlog in the brewing process resulted in a higher total phenol content and a lower content of diacetyl and esters in the finished beer. Beers fermented with kveik contains higher levels of esters and lower levels of higher alcohols and diacetyl than beers fermented with US-05.

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1 Introduction

The history of beer brewing can be traced back 8000 years to ancient Babylonia. The main raw materials used to brew beer are water, malted barley, hops and yeast (Willaert, 2012). Unmalted cereals (adjuncts) can be used in addition to malted barley (Belitz et al., 2009). The underlying science of brewing has been uncovered in the past 150 years, where the techniques and methods that are now used in the modern brewing industry were developed. Three of the forces that drives technological change in the brewing industry is cost savings, quality enhancement and safety (Bamforth, 2000). Carefully selected and preserved stains of *Saccharomyces cerevisiae* have been used in industrial brewing for centuries (Holt et al., 2019).

The Norwegian home brewing traditions were weakened in the late 19th and early 20th century, at the same time as industrial brewing was developed, which was not built on Norwegian brewing traditions. In some areas of Norway the traditions survived, and this ensured the survival of the traditional yeast: kveik (Bråtå, 2017).

Before laboratory-grown, isolated yeast was invented, all professional brewers and homebrewers alike reused their yeast. If yeast is reused too many times, it can mutate and deteriorate. Some strains are more stable than others, but eventually even the most stable yeast stains will become contaminated or mutate. People have reused the yeast for brewing in Norway for a long time, and there are sources that clearly states that yeast was reused in the 1500s and 1600s (Garshol, 2017). There is a farmhouse brewing culture in Norway, where yeasts referred to as kveik has been passed down for generations. Typical traits of kveik are yeasts with high fermentation rates, who tolerates high fermentation temperatures and are phenolic off-flavor negative. The studies performed on kveik thus far indicates that kveik is a domesticated beer yeast with properties relevant to brewing (Preiss et al., 2018). These days, kveik receives a lot of attention, but there is a lack of knowledge regarding the Norwegian farmhouse yeasts (Preiss & Netto, 2019a).

Juniper (*Juniperus communis*) can be found in large parts of the world and it has a larger prevalence than any other tree species. There are many varieties of the specie, and the most common varieties in Norway are highland juniper (*J. c.* var. *saxatilis*) and lowland juniper. There are two main types of lowland juniper; up-right, single-stemmed juniper (*J. c.* var. *suecica*) and bush juniper. It has been traditional in Norway to use an extract of juniper, made by boiling

branches of juniper in water, to clean wooden appliances in the household. This extract goes by the name “einerlog”. Juniper has traditionally been used to brew beer in Norway, both by using einerlog instead of water in the brewing, and by straining the wort through branches of juniper in the brewing process. The juniper was added to increase the nutritional content, shelf-life and body of the beer, and to make the beer healthier. Traditionally, juniper berries have been used as medicine, supposedly protecting against infections, healing colds and stomach aches, and guarding against evil powers. Einerlog is thought to have antibacterial qualities (Høeg, 1981).

The purpose of the master’s thesis was to analyze ten traditional Norwegian Farmhouse Ale Yeasts, known as kveik, where four kveiks were analyzed in detailed studies. Microbiota studies were performed and microorganisms from kveik were isolated. A series of experiments were conducted to investigate several aspects: the effect of the fermentation temperature of the ale on the finished product, the antibacterial effect of einerlog, the sensory aspects of beer brewed with kveik compared to beer brewed with commercial ale yeast, and the effect on the phenol content of the finished beer when juniper extract and kveik is used in the brewing.

2 Theory

2.1 History and characteristics of the kveiks used in this study

The following information about the kveiks used in this study has been given by the respective owners of the kveiks or found in the sources listed in the text. The kveiks are organized in subchapters relative to their origin. Figure 2.1 shows a map of the places of origin for the kveiks in this study.

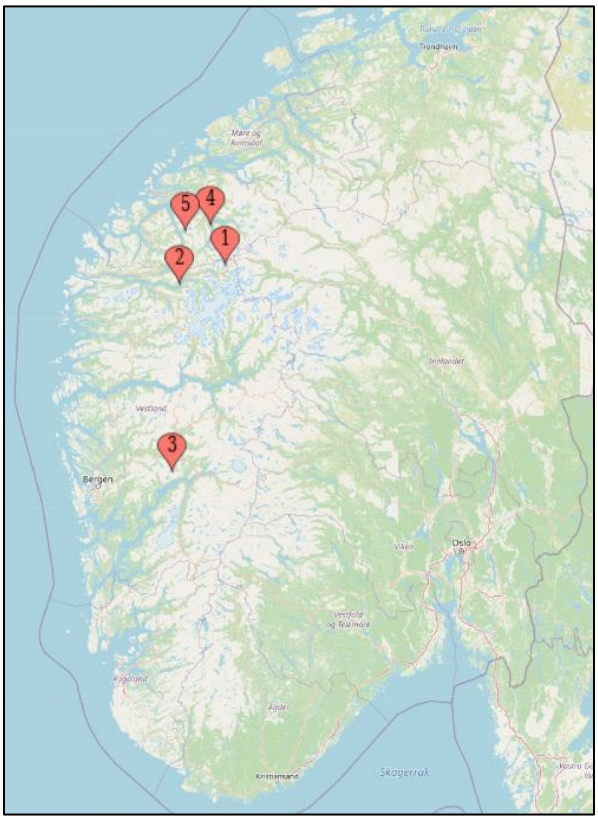

Figure 2.1. *Map of Norway showing the places of origin of the kveiks in this study. The numbers indicate the following places; 1: Geiranger, 2: Hornindal, 3: Voss, 4: Stordal and 5: Sykkylven (Kaeding, 2014-2020).*

2.1.1 Geiranger

One of the kveik types in this study, Ørjasæter, was donated from a man named Ivar Flydal in Geiranger. The owner of this kveik is Leiv Øystein Ørjasæter, who started using it in brewing back in the 1960s when the kveik was circulating among the locals. He has not received kveik from anyone else for a very long time. According to Flydal, it should normally be harvested from the top of the ale as soon as it rises up and creates a lid, which takes approximately 12-24 hours. Traditionally the yeast was dried after harvest and added in a dry state to the next brew.

2.1.2 Hornindal

Idar Nygård is the owner of Gamlegrua Brewery, located at Gausemel in Grodås, and he is the owner of Gamlegrua kveik. Gamlegrua kveik is a mixture of several types of kveik. Idar grew up at the farm Nygård in Hornindal, where he learned how to brew by his father. When Idar was approximately 30 years old, his interest in brewing had grown and he started to brew himself. Sadly, the kveik his father had used was no longer viable. After some time, Idar took the brewing more seriously. He contacted traditional brewers in Hornindal who he knew produced good beer and received kveik from them. He mixed 3-4 of the kveik types together, which became the genesis of the kveik he uses today. He has used this kveik for the last 25 years. This kveik goes by the name Nygård in the farmhouse yeast registry (Garshol, 2020), but it is referred to as Gamlegrua in this study.

The Espe kveik is owned by Arve Espe from Grodås, who got it from his father. He has never mixed it together with other types of kveik, but nothing is known of the history of this kveik before he received it. Espe kveik has got a taste of cognac and plums. It consists of 4 strains of *S. cerevisiae*, and it is typically harvested from the top of the fermentation vessel after 10 days (Garshol, 2020).

The kveik known as Gausemel is owned by Olav Sverre Gausemel from Grodås. The yeast originates from his home farm, Gausemel, and he started using it in 1977. He has mixed it with other yeasts several times since then, e.g. with Stalljen. The kveik consists of both bacteria and six yeast strains, and it is harvested from the top of the fermentation vessel after 48 hours (Garshol, 2020).

Stig Jarle Seljeseth in Grodås owns the kveik Stalljen, which has its name from the brewery that is in the house that used to be the stable on the farm. He got the kveik from his father in the 1970s and he has never mixed it with any other kveik. The kveik consists of 5 strains of *S. cerevisiae* and no bacteria. The fermentation time is 72 hours, but the kveik is harvested from either the top or the bottom of the fermentation vessel after 24 hours (Garshol, 2020).

The owner of the Otterdal kveik is John Arnfinn Grodås, from Otterdal in Hornindal, who got it from Rasmus O. Otterdal in the 1980s. Before this, the kveik is believed to have been shared between the farms in this area (Garshol, 2020).

2.1.3 Voss

Sigmund Gjernes is the owner of the kveik Sigmund, which originates from Vestbygdi. The kveik has been used by people from the Gjernes farm, who used to get kveik from Veka in Dyrvedalen. Sigmund has mixed it with the kveik from a neighbor, who had kveik from Bordalen. The kveik consists of 3 strains of *S. cerevisiae* and no bacteria, and it is harvested from the bottom of the fermentation vessel after 84 hours. Sigmund kveik has a taste of orange peel, earth and Christmas spice (Garshol, 2020).

2.1.4 Stordal

The Midtbust kveik originates from Stordalen. The owner of this kveik is Odd H. Midtbust. This kveik is harvested from the top of the fermentation vessel after 72 hours, and it consists of 6 strains of *S. cerevisiae* (Garshol, 2020).

The kveik Ebbegarden is owned by Jens Aage Øvrebust from Stordal. This kveik can make a tropical fruit aroma and it can emphasize the bitterness of boiled hops. Ebbegarden consists of both bacteria and *S. cerevisiae* yeast. It is harvested from the top of the fermentation vessel after 120 hours (Garshol, 2020).

2.1.5 Sykkylven

Tormodgarden kveik is owned by Sigurd Johan Saure in Sykkylven, and the grandfather of the owner claimed that the kveik had been on the farm since the 16th century. It has been mixed with other types of kveik, at least once in the 1950s with a kveik from Straumsdalen. The kveik consists of 10 strains of *S. cerevisiae* and no bacteria, and it is harvested from the bottom of the fermentation vessel after 84 hours (Garshol, 2020).

2.2 The modern brewing process

The modern brewing process consists of seven main steps: malting, mashing, boiling, fermentation, bottling, carbonation and maturation. A brief overview of these processes are given in this chapter.

2.2.1 Malting

The malting process consists of steeping, germination and kilning (Bamforth, 2017).

2.2.1.1 Steeping

The grain is soaked in water during the steeping, and the grain absorbs water, resulting in the hydration of the embryo of the grain. Air-rests during steeping is important to prevent the conditions from becoming anaerobic. A steeping program lasts for approximately 40-68 hours, depending on the amount and time of the air-rests. The moisture content of the grain increases from 10% to approximately 45%. The steeping results in the awakening and distribution of enzymes throughout the kernel, and it stimulates the start of germination (Lalor & Goode, 2010).

2.2.1.2 Germination

The germination of the barley grains is done in shallow vessels. The humidity and the temperature are controlled to prevent moisture loss from the grain. Due to the growing rootlets that develops as the grain germinates, the barley is turned regularly to prevent it from growing together. Enzymes breaks down parts of the cell wall, as well as proteins, lipids and starch during germination. The hormones known as gibberellins are important for the germination of seeds (Lalor & Goode, 2010). The germination time of barley is 3-7 days at 16-20 °C (Bamforth, 2017). A root sheath protrudes from the base of the grain when the germination starts, which turns into rootlets during germination. Acrospires grow along the sides of the grains, and the germination is usually terminated when they have reached 3/4 or 7/8 of the grain length (Briggs et al., 2004).

2.2.1.3 Kilning

The kilning involves roasting the germinated malt grain. This dries the grain, stops the germination and inactivates the enzymes (Humia et al., 2019). The kilning temperature decides the color and enzyme activity of the malt. Darker colored malts kilned at higher temperatures have lower enzyme activity. The malt after kilning have a low moisture content to make it stable and storable (Lalor & Goode, 2010). During kilning, Maillard reactions produce melanoidins, which imparts color to the beer (Bamforth, 2000). The germinated malt grain is kilned for 24 hours at 50-100 °C. The malt is stored for several weeks before it can be used, to decrease the level of enzymes in the malt (Bamforth, 2017).

2.2.2 Mashing

To initiate the mashing, the malt is milled before it is added to the mashing vessel, along with hot water. The mashing temperature decides which enzymes are active. The starch granules swell, and the starch are converted to fermentable sugars by the enzymes present in the malt (Humia et al.,

2019). The pH value, salt content, and the mashing time and temperature are all parameters that affect the mashing process. During mashing, the proteins are degraded into peptides and free amino acids, and polysaccharides are degraded into sugars (Lalor & Goode, 2010). A typical mashing program is approximately 1 hour at 45-72 °C (Bamforth, 2017).

The pH and temperatures where enzyme groups are active during mashing are illustrated in Figure 2.2.

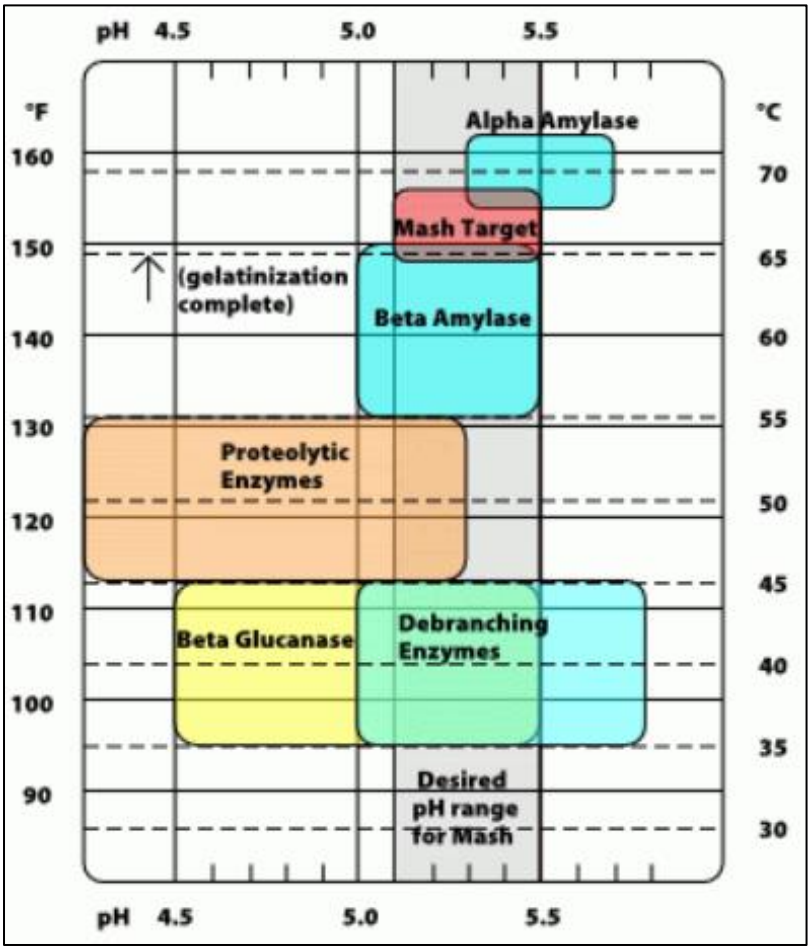

Figure 2.2*. Enzyme groups, pH values and temperatures during mashing (Palmer, 2000).*

The optimal pH during mashing is 5.1-5.5. The mashing temperature target is 64-68 °C, which is a compromise between the ideal temperature for β- and α amylase activity. The mashing temperature can be modified depending on the desired composition of the wort and the finished product. Mashing at higher temperatures that favors α amylase activity results in a sweeter, less fermentable beer, and mashing at lower temperatures that favors β amylase activity results in a drier, thinner bodied beer (Palmer, 2000).

Lautering is done at the end of the mashing process to separate the solids from the liquid fraction, i.e. the wort (Humia et al., 2019). The wort is strained through the grain hulls and other residues. When the solids are separated from the wort, the lautering residue is sparged with water to acquire more wort and adjust the sugar content (Belitz et al., 2009).

2.2.3 Hop addition and wort boiling

Boiling is required to sterilize the wort, precipitate proteinaceous complexes, remove unwanted volatile substances, concentrate the wort and to extract bitterness from hops. The precipitate generates an insoluble complex, known as trub, which is removed before cooling the wort. The common way to remove the trub is to use a whirlpool (Bamforth, 2017). The amount and type of hops added to the wort depends on the type of beer being made. Hops contain bitter substances, known as α-acids. After the boiling and hop addition, the wort is cooled down to the pitching temperature, filtered, aerated and pitched with yeast (Belitz et al., 2009). Oxygen is needed for the synthesis of sterols and unsaturated fatty acids, which makes the aeration of the wort prior to fermentation important. These components are building blocks of the cell membrane of the yeast and are required for the yeast cells to grow (Willaert, 2012).

2.2.4 Fermentation

The aroma of beer is mainly derived from the malt, the hops and the yeast metabolism (Humia et al., 2019). The fermentation of beer is primarily done by the yeasts *Saccharomyces cerevisiae* and *Saccharomyces pastorianus*. The former is a top-fermenting yeast used to ferment ales, and the latter is a bottom-fermenting yeast used to ferment lagers. The character of the beer will be affected by which yeast type is selected to conduct the fermentation, as the different strains can produce varying amounts and types of metabolites. A controlled fermentation depends on the growth media for the yeast (the nutritional content of the wort), the yeast (viability and quantity) and the fermentation conditions (temperature, type of fermentation vessel and oxygen-addition) (Bamforth, 2017). During fermentation, the sugars in the wort are metabolized by the yeast, resulting in the production of alcohol and other metabolites (Humia et al., 2019). An accelerated fermentation has a negative impact on the aroma of beer, as it increases the diacetyl and higher alcohol content at the same time as it decreases the ester and acid content (Belitz et al., 2009).

When the yeast clump together and fall to the bottom of the fermentor, the fermentation is finished. This is called flocculation. The degree of attenuation, i.e. the amount of sugars fermented by the

yeast, is another way to determine when the fermentation is complete. The attenuation typically varies between 65-85% for brewer's yeast (White Labs, 2020).

The quality of beer is affected by the pH in the product. The pH level of wort during fermentation decreases quicker in the early stages of the process compared to the later stages. If the yeast remains in contact with the wort after the sugar is depleted, a slight increase in the pH may occur. There are several theories as to why the pH in the wort decreases during fermentation that involves the yeast metabolism, such as direct excretion of hydrogen ions, absorption of bases and the removal of buffering substances (Coote & Kirsop, 1976).

2.2.5 Bottling, carbonation and maturation

After the fermentation, the beer is either transferred to bottles or casks. The maturation time can vary, depending on the style of the beer. The beer can be filtered to remove yeast and create a stable product, but this is optional (Humia et al., 2019). Cold conditioning is used to precipitate, settle and remove solids in beers, where the beer is stored at -1 °C for three days or more before filtration (Bamforth, 2000).

Carbon dioxide is naturally produced during primary and secondary fermentation. The concentration of the gas in the beer after primary fermentation vary from 2 to 5 g/L. As an alternative to secondary fermentation, carbon dioxide can be added to the finished product. Carbonation is important for the mouthfeel of the beer, as beers lacking carbon dioxide are dull and lifeless. Temperature and pressure determines the amount of gas dissolved in the product (Briggs et al., 2004).

During maturation the composition of the beer changes. The concentration of e.g. acetaldehyde in the beer increases for about three weeks during storage, before the concentration decreases (Delcour et al., 1982).

2.3 Key components to traditional Norwegian farmhouse brewing

2.3.1 Einerlog

Einerlog is used instead of water in traditional Norwegian farmhouse brewing. There is no general agreement on the process to make einerlog, and the elements of the process, such as the boiling time, the amount of juniper, and which parts of the juniper are used, can differ significantly. Some brewers use all parts of the juniper to make the einerlog, but others only use the twigs. This results

in a varying amount of juniper flavor in the final product. Some brewers claim that using unripe berries along with twigs and needles in the einerlog results in a foul taste in the ale. In Hornindal, the color of the einerlog is a very subtle, golden brown. The color of the einerlog will vary depending on the ratio of juniper and water, the boiling time and the fractions of juniper used to make the einerlog.

The einerlog is thought to have antibacterial qualities. However, a small study in 1979 showed no antibacterial effect from einerlog against one strain of *Escherichia coli* and one strain of *Streptococcus lactis* (Høeg, 1981).

2.3.2 Kveik

The people who brew with kveik taste it to make sure that it has not "gone bad", and they therefore keep multiple copies of the kveik. Kveik generates unique aromas. The Norwegian kveiks are very distinct from each other. Whether the kveik should be collected from the top or the bottom during fermentation, and when it should be collected, depends on the type of kveik. The easiest way to store kveik is to keep it in a jar in the fridge, with the lid not tightened all the way to ensure that generated pressure can escape from the jar. The best way to store the kveik is to dry the slurry and freeze it, and this way it can be stored for 10-25 years. A traditional way to store kveik has been to dry it on a kveik ring ("gjærkrans") or kveik log ("gjærstokk"), which was done by dragging the wooden artifact through the foam at the top of the fermenting ale and hanging it up to dry. The large surface area of the wooden artifact ensures that a large amount of kveik is attached to it (Garshol, 2017).

2.4 The traditional Norwegian brewing process

The following information on traditional Norwegian brewing was collected from traditional brewers in the Hornindal municipality. This description of a traditional Norwegian brewing process is only one out of many different ways this process can been done.

2.4.1 Malt

Traditionally, the brewers would often prepare the malt themselves. Very few traditional brewers are still doing this and most of them buy their malt. Pilsner malt is frequently used, as well as pale ale malt. There is, however, a great deal of variation in the malt recipe that is used by the farmhouse brewers and it is not uncommon to include smoked malt in the recipe.

2.4.2 Mashing

One way to make the einerlog in Hornindal is to boil five logs of juniper in water for approximately 15 minutes. The juniper logs do not contain needles or berries. Before the mashing starts, parts of the einerlog is used to rinse and clean all of the equipment that is to be used in the brewing process. The einerlog is poured into milk pails to cool it down to approximately 70 °C before it is used to make the mash. The malt and einerlog is mixed together incrementally in the mashing vessel with a mashing shovel made out of wood. When all the malt is added to the mash and mixed together with the einerlog, the mashing shovel should stand upright in the mash without tilting. This is a measure of the correct ratio between einerlog and malt, and it decides the point when the mash is ready to rest. The mash rests for one hour.

2.4.3 Lautering and hop addition

Green branches of juniper, which has previously been washed with einerlog, is used as a filter in the lautering of the mash, along with a juniper log with many holes drilled into it (Figure 2.3). This juniper log needs to be weighed down to prevent it from floating up in the mash. If it floats up, the risk is high for the outlet of the lautering vessel clogging due to the particles in the mash. The lautering vessel is filled to the rim with mash and einerlog before the lautering is set in motion. The lautering is a lengthy process, with a thin stream of wort trickling from the tap at the bottom of the lautering vessel.

Figure 2.3. *The lautering log of juniper with holes drilled into it, and the lautering vessel.*

Aroma hops is used during the lautering of the mash. A bag of hops is placed in the container where the lautered wort is transferred from the mash. The hop used is a flour, not pellets. The reason for this is probably to extract as much as possible from the hops in a short time, as the hops is only kept in the vessel where the wort goes during the lautering, and the wort is transferred from this vessel to the fermentation vessel after a short while. The wort in traditional Norwegian brewing is in many cases not boiled, with temperatures not surpassing 80 °C, resulting in a raw ale as the end product. There

is therefore no extraction of hops during boiling, another reason to use hop flour instead of hop pellets.

2.4.4 Fermentation

The first wort that is lautered from the mash is poured in a bowl and cooled down to approximately 30 °C. The dried kveik is usually added after the wort has been tempered, making a starter culture of which they call “mariauge”. The starter comes to life and a bubble-formation starts as the kveik dissolves in the wort. It is said that the wort used to make the starter, and the temperature of the wort before adding the kveik, should be the same temperature as the teat of a cow, i.e. “spenevarm”. This was a common way to know that the temperature of the wort was just right in the time before it was common to use thermometers. Making the starter is not necessary, but it makes the fermentation process start quicker and it is therefore a common part of the brewing process. Most kveik types can handle direct pitching using dry yeast.

Traditional brewers use a low pitch rate for kveik fermentation (Preiss & Netto, 2019a).

The fermentation vessel is usually insulated in some way or other. One example is to place insulation in a large barrel, with a fermentation vessel placed in center, or to wrap the fermentation vessel in a duvet or blanket. In this way the brewer can control the temperature in a better way and make sure that the temperature doesn’t drop before the fermentation initiates. The fermentation takes about 2-5 days, depending on the temperature used. A common temperature to use in Hornindal is 28-32 °C.

When the foamy head of yeast at the top of the fermentation vessel is removed, known as “krausen”, and there is no new krausen formation within 1-2 hours, the ale is considered to be ready. If the kveik has not completed fermenting the wort at that time, there will be a new krausen forming very quickly after the old one has been removed.

2.4.5 Bottling, carbonation and shelf life

Due to the nature of the raw beer, the beer cannot be bottled in a regular way. The fermentation process is stopped at a place where the kveik is still active, and glass bottles would not be able to handle the pressure that is produced by the kveik in the secondary fermentation. The solution to this is to store the beer in Cornelius kegs or on plastic containers. The plastic can, however, create an undesired taste in the beer if it is stored for a longer period of time. The beer is naturally carbonated by the residual sugar in the wort without any addition of sugar after the primary fermentation, resulting in a beer with low carbonation. The optimal serving temperature is thought to be 10-15 °C, and the optimal tasting experience is thought to be after a few days. The shelf life can be up to six months, but it can be assumed to have a wide span, depending on the brewing process, the sanitary conditions of the brewing and the storage conditions.

2.5 Aromatic and sensory aspects

The flavor of beer is imparted by the malt (Maillard reaction products and dimethyl sulphide), hops (bitter and aroma compounds) and yeast (esters, higher alcohols, sulphur components and diacetyl) (Bamforth, 2000). The chemical classes and compounds, the sensory threshold values and the aromas of the volatile components that are emphasized in this study are listed in Table 2.1.

Table 2.1. *The volatile components commonly found in beer, including chemical class, threshold values and aroma characteristics (Briggs et al., 2004; Dong et al., 2014; Harrison, 1970; Holt et al., 2019; Humia et al., 2019; Olaniran et al., 2017; Preiss et al., 2018; Saison et al., 2009; Tan & Siebert, 2004; Viejo et al., 2019; Xu et al., 2017).*

Chemical class	Chemical compound	Threshold value (ppm)	Aroma
Ester	Ethyl acetate	5-10[1]; 25-50[1]; 30[2,10]	Solvent[1, 2], butter[1], fruity[2], sweet[2]
Ester	Isoamyl acetate	0.03[1]; 1-2.5[1]; 1.2[2,5,6,10]; 0.51[8]	Fruit[1,2,6], banana[1,2,6,8], apple[2,6], solvent[2,6], estery[2,6,8], tropical fruit[6], berry[6]
Ester	Isobutyl acetate	0.67[7]; 1.6[10]	Fruit[7], solvent[7]
Ester	Ethyl hexanoate	0.014-0.2[1]; 0.2-0.3[1]; 0.005[2]; 0.17[6]; 0.21[6,10]	Apple[6], fruit[6], orange[6], aniseed[6], sweet[6], berry[6]
Ester	Ethyl heptanoate	0.4[10]	Pineapple[3], banana[3]
Ester	Ethyl octanoate	0.9[1,10]; 0.5[2]; 0.33[7]	Honey[1], fruit[1,6], rose[1], flower[1], Brandy[7], sweet[6.7], apple[6]
Higher alcohol	1-propanol	800[2,10]	Alcohol[2]
Higher alcohol	2-methyl-1-propanol	100-175[1]; 200[2]	Alcohol[2], apple[6]
Higher alcohol	3-methyl-1-butanol	70[2]	Tropical fruit[6], orange[6], Alcohol[2]
Higher alcohol	2-methyl-1-butanol	65[2,10]	Alcohol[2], banana[2], solvent[2], medicinal[2]
Higher alcohol	2-hexanol	4[10]	
Sulphur components	Dimethyl sulphide	0.06[9]	Cooked sweet corn[11]
Aldehyde	Acetaldehyde	1.114-5[1]; 10-25[2]; 1.114[8]; 25[10]	Green apple[2], green leaves[2], fruity[2], sweat[4], pungent[4]
Aldehyde	2-methyl-propanal	0.0023[4]; 0.086[8]	Wine[4], solvent[4], malty[4]
Aldehyde	3-methyl-butanal	0.032[4]; 0.056[8]	Malt[4]
Aldehyde	2-methyl-butanal	0.032[4]; 0.045[8]; 0.157[8]	Cocoa[4], candy[8], flowery[8]
Aldehyde	Hexanal	0.030[4]; 0.088[8]; 0.35[10]	Grass[4], tallow[4], fat[4]
Ketone	Acetone	100[9]; 200[10]	
Ketone	Diacetyl	0.1-0.2[1]; 0.1-0.15[2]; 0.017[8]; 0.15[10]	Stale milk[1], butter[1,8], solvent[1], toffee[1, 2]

[1] Humia et al., 2019; [2] Olaniran et al., 2017; [3] ; Viejo et al., 2019; [4] Dong et al., 2014; [5] Preiss et al., 2018; [6] Holt et al., 2019; [7] Xu et al., 2017; [8] Saison et al., 2009; [9] Harrison, 1970; [10] Tan & Siebert, 2004; [11] Briggs et al., 2004.

Where several threshold values are listed for a singular chemical compound, this study considers the lowest value to be the sensory threshold for that compound.

The relationship between the aromatic components produced in metabolic pathways in beer are illustrated in Figure 2.4.

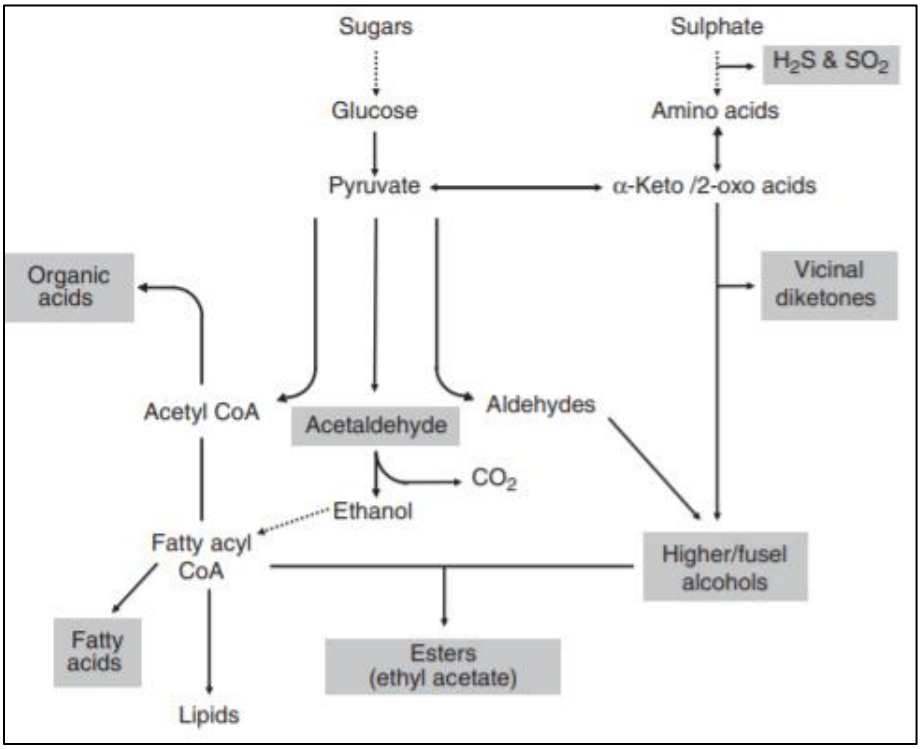

Figure 2.4. *Relationships between the aromatic components in beer (Lodolo et al., 2008).*

This chapter includes information about phenols and the components highlighted in the illustration.

2.5.1 Esters

Esters are made from higher alcohols and acetyl-coenzyme A (acetyl-coA). A high-gravity wort results in an overproduction of acetate esters. Various esters can have synergistic effects and affect the profile of the beer, even in concentrations that are lower than the threshold values. The use of dark malts decreases the amount of esters found in beer, likely due to Maillard reaction products such as melanoidins. This can reduce the availability of important cofactors, such as magnesium. Another thing that can affect ester production is fermentation temperature, as the temperature has got an impact on the amount of acetyl-coA in the wort (Humia et al., 2019). Higher temperatures during fermentation encourage ester production (Palmer, 2000). Ester production is optimal at a certain oxygen level, and an ideal aeration of the wort is therefore important for the production of esters (Verstrepen et al., 2003).

2.5.2 Higher alcohols

The biosynthesis of higher alcohols is directly related to amino acid metabolism. The amino acids leucine, isoleucine and valine are the most important substrates for the flavor of beer (Holt et al., 2019). The main group of volatile components found in beer is higher alcohols. These compounds can be divided in two groups: aliphatic (alcoholic or solvent aroma) and aromatic (rose aroma) higher alcohols (Willaert, 2012).

2.5.3 Sulphur components

Sulphur compounds may be formed during wort boiling, produced by Maillard reactions in the malt. They can also be produced through the metabolic activity of yeast. Traces of sulphur-containing compounds, many with low sensory thresholds, are found in hop oil. Dimethyl sulphide (DMS) is a major flavor compound in beer and an important component of lager beers at moderate concentrations (Briggs et al., 2004).

2.5.4 Aldehydes

Acetaldehyde is an intermediate in ethanol production (Humia et al., 2019). Off-flavors in wort is mainly caused by aldehydes, such as 2- and 3-methyl-butanal. These aldehydes are reduced to the corresponding alcohols and intracellular aldehydes are reduced to fusel alcohols, such as 1-propanol. The yeast *S. cerevisiae* reduces aldehydes by alcohol dehydrogenase. Metabolic activity is necessary for aldehyde reduction, as the reduction is coupled to oxidation of cofactors NADH and NADPH, which have to be continuously regenerated for aldehyde reduction to take place (van Iersel et al., 2000). Large volumes of air in the neck of the beer bottles increases the content of acetaldehyde during maturation, and the formation of acetaldehyde is promoted in the presence of polyphenols and ethanol (Delcour et al., 1982).

2.5.5 Ketones

Diacetyl is a natural by-product of primary fermentation that affects the quality of beer. The concentration of diacetyl should not surpass 0.1 ppm (Humia et al., 2019). An intermediate in one of the pathways in yeast, α-acetolactate, leaks from the cell and decarboxylates, producing diacetyl. The yeast slowly reduces diacetyl to acetoin (Bamforth, 2000).

2.5.6 Phenols

Malted grains and hops impart simple, plant-derived phenolic compounds to the beer during the brewing process. Another source of phenols is extraction from wood through barrel aging. Phenols

can impart rather pleasant aromas and flavors (spice, clove and black pepper), as well as strong and offensive aromas and flavors (smoke, burnt, medicinal, stable and band-aid). The yeast strain used in the fermentation can be the cause of these flavors, or it could be the use of a fermentation temperature above the recommended range. A source of volatile phenols is the metabolites of contaminating organisms that should not be in the beer. Several species of LAB within the genera *Lactobacillus* and *Pediococcus* can produce phenols. Wild strains of *S. cerevisiae*, as well as other yeast genera, have been characterized to produce volatile phenols (Lentz, 2018). Phenolic acids are antioxidants that enhance the stability of beer (Vanbeneden et al., 2008).

2.5.7 Organic acids

Organic acids are excreted by yeast cells during beer fermentation. Beer contains more than a hundred types of organic acids, such as acetate, lactate and pyruvate. The pH and taste of beer is influenced by organic acids. The amount of organic acids in the beer is affected by the fermentation rate and the yeast strain used to ferment the beer (Willaert, 2012).

2.5.8 Fatty acids

Medium-chain fatty acids, which is approximately 90% of the fatty acids in beer, contributes flavors of sweat, yeast and goat to beer. These components are produced by yeast during anaerobic fermentation. The yeast growth rate affects the amount of medium-chain fatty acids in the beer, hence increasing the fermentation temperature, the oxygenation of the wort and the pitching rates results in elevated concentration of these components in the beer. Elevated levels of medium-chain fatty acids are also associated with yeast autolysis caused by e.g. prolonged contact between the wort and the yeast during fermentation (Willaert, 2012).

2.6 Genotypic identification by sequencing

The 16S rRNA gene sequences are commonly used to study bacterial phylogeny and taxonomy. These gene sequences are present in all bacteria, and sequence changes are a measure of evolution due to a lack of change over time in the function of the gene. The 16S rRNA gene consists of 1,500 base pairs (Janda & Abbott, 2007).

The internal transcribed spacer (ITS) region of yeasts and fungi has got a size of approximately 450-800 bases. This region evolves faster than the large subunit and the small subunit of the ribosomes, and it therefore has a higher degree of variation (Raja et al., 2017). The 5.8S-ITS region

of the ribosomal RNA (rRNA) gene and the D1/D2 region of the 26S rRNA gene are useful to identify yeast to the species level (Hesham et al., 2014).

2.6.1 Polymerase chain reaction

Polymerase chain reaction (PCR) is a technique used to generate many copies of entire sequences of DNA. This process requires template DNA, nucleotides (dNTP), DNA primers and DNA polymerase (Merck KGaA, 2020). PCR consists of three steps: double-stranded DNA (dsDNA) denaturation to single-stranded DNA (ssDNA), primer annealing to the resulting ssDNA, and production of dsDNA by polymerase extension. These three steps are repeated 20-30 times (Marimuthu et al., 2014). A set of primers, where one primer anneals the sense strand (5' → 3') and the other primer anneals the antisense strand (3' → 5'), are chosen based on the specific region of DNA that is to be amplified (Lorenz, 2012).

2.6.2 Sanger sequencing

Sanger sequencing is a method used to determine the nucleotide sequence of DNA. It generates every thinkable length of DNA up to the length of the target DNA. In addition to the materials needed to perform a PCR (DNA template, DNA polymerase, dNTP), the process requires dideoxynucleotides (ddNTP) and only one primer is used in the PCR reaction (Merck KGaA, 2020). Sanger sequencing can sequence 1000-1200 base pairs (Zhang et al., 2011). Chain-terminating ddNTPs are selectively integrated by DNA polymerase (Malla et al., 2019).

2.6.3 High-throughput next generation sequencing

High-throughput next-generation sequencing (NGS), often called "massively parallel sequencing", can sequence DNA very rapidly. The DNA genome is broken down into pieces, who are ligated to adapters for random read during DNA synthesis. Many different DNA templates are read in parallel, and the read length is short (50-500 bp) compared to Sanger sequencing (Zhang et al., 2011). NGS can be used to analyze the composition of whole microbiomes and to study properties of microbial communities (Malla et al., 2019).

2.6.4 Online database sequence search tool

A Basic Local Alignment Search Tool (BLAST) search in an online database of the nucleotide sequence acquired in the sequencing can be used to verify the identity of bacteria and fungi. To be considered the same species of for example fungi, the ITS should have 97% or more similarity with ≥80% query coverage (Raja et al., 2017).

2.7 Chemical analysis

2.7.1 Phenolic content

Phenolic compounds, such as flavonoids, phenolic acids, tannins and proanthocyanidins, are secondary metabolites of plants (Humia et al., 2019). Phenolic compounds can be found in beverages of plant origin, such as beer and wine. The phenolic compounds are associated with the nutritional value and antioxidant capability of the product (*Functional food ingredients and nutraceuticals - processing technologies*, 2007).

2.7.2 Beer alcolyzer – Anton Paar

The Anton Paar beer alcolyzer calculates several parameters, such as alcohol and original extract content, in the beer. The relationship of absorbance at near infrared (NIR) wavelengths are used to determine the alcohol (% v/v) content of beer. A density meter is used to determine the density of the sample. The remainder of the parameters (e.g. real degree of fermentation and apparent degree of fermentation) that can be determined using a beer alcolyzer is calculated from these two values (Evaluation of the Anton Paar alcolyzer for measurement of alcohol and original gravity, 2004). More detailed information regarding parameters and technical specification can be found on the websites of Anton Paar (www.anton-paar.com).

2.7.3 Headspace Gas Chromatography

Headspace gas chromatography (HSGC) is a common method to use to determine and quantify the presence of volatile components in beer (Viejo et al., 2019). It is a sampling technique that determines the concentration of volatile components of a sample by analyzing the vapor phase, which is in thermodynamic equilibrium with the sample, in a closed system. The sampling is usually done at elevated temperatures to increase the vapor pressure of the sample. The equipment used for HSGC analysis varies. A simple way is to place the sample in a glass bottle, which is sealed with a rubber septum cap and placed in a thermostat. When thermodynamic equilibrium is reached, a syringe is used to remove some of the vapor and inject it into a gas chromatograph (Robards et al., 2004).

2.8 Purity testing by Nanodrop

Detection and purity-assessment of DNA can be done by analyzing the absorption, i.e. the optical density, at certain wavelengths. A NanoDrop™ spectrophotometer can be used for this analysis. Nucleic acids absorb ultraviolet (UV) light due to the bases in the nucleotides. Using a low salt

buffer as a solvent for the DNA gives reproducible readings, whilst using water results in more variation in the readings. Values equal or greater than 1.8 for A_{260}/A_{280} and values between 1.5 and 1.8 for A_{260}/A_{230} are suitable for analysis. Low A_{260}/A_{280} values may indicate protein contamination, and low A_{260}/A_{230} values may indicate contamination with salts, carbohydrates or solvents. An A_{260}/A_{280} ratio of >1.8 could indicate RNA contamination, as the A_{260}/A_{280} ratio of pure RNA is 2.0. Phenols and other contaminants can absorb light at 280 nm and can affect the calculated ratio. RNA contamination would, on the other hand, be detected easily on an agarose gel, where a separate band would form on the gel (*Understanding and measuring variations in DNA sample quality*, 2011).

2.9 Statistical analysis

A type I error is when a null (H_0) hypothesis is rejected when the H_0 hypothesis is true: a false positive result. The significance level is therefore often set to 5% to avoid making a type I error. A type II error is when there is a significant difference and the H_0 is not rejected: a false negative result (Smith et al., 2002).

When multiple comparisons are made, the chances of making a type I error is greater than the error rate of one comparison alone. Tukey post hoc method contains a lot of groups, multiple comparisons are made, and the p-values are adjusted to minimize the probability to make a type I error. This is done by adjusting the confidence levels for each individual interval. Due to this, the indications of the initial analysis of variance (ANOVA) analysis can be altered when Tukey's method is used to analyze the same data (Support Minitab 18, 2019).

A two-way ANOVA test evaluates two factors on a response variable. There are two designs, depending on if the sample sizes are equal (balanced design) or inequal (unbalanced design). For unbalanced designs, type III ANOVA is used. All ANOVA tests assume that the observations within the groups have been obtained independently, that the observations are normally distributed and have equal variances (Larson, 2008).

2.10 Sensory analysis

Descriptive sensory tests involve the detection and description by trained judges of both quantitative and qualitative characteristics of products. The characteristics include e.g. aroma, appearance, flavor and texture. There are many different descriptive analysis methods, such as quantitative descriptive analysis and free-choice profiling. The training of the panel of judges starts with developing and agreeing on a common language for the product attributes, then the panel is exposed to a frame of reference of which the analyzed products are compared to (Murray et al., 2001).

3 Materials and methods

3.1 Design of experiments

This study consists of small preliminary experiments, in addition to beer brewing in the pilot plant. These small projects were executed to decide the parameters of the pilot-scale brewing, and to achieve a greater knowledge of what kveik really is and how it can best be used in brewing. The four different parts of the project is listed below.

- A **growth experiment** with 3 kveik types: Gamlegrua, Ørjasæter and Gausemel. SafAle US-05 yeast was used as a control.
- **Microbiota assays** were used to determine the microbiological diversity and differences between all ten types of kveik used in this study. Colonies from four types of kveik were **isolated** and **sequenced**, the kveik selected for this were Gausemel, Ørjasæter, Gamlegrua and Otterdal.
- Lab trials were used to test the **antibacterial properties in different fractions of juniper**; unripe and ripe berries, twigs and needles. They were tested against the lactic acid bacteria *Lb. buchneri*, *Lb. plantarum* and *Lb. brevis*.
- **Pilot-scale brewing** based on preliminary studies were completed using only selected types of kveik: Ørjasæter, Gausemel and Gamlegrua, along with SafAle US-05 as a control. **Sensory analysis** was performed on the finished products.

3.2 Kveik and juniper

An overview of the different types of kveik used in this study can be found in Table 3.1 below. This is information that was either publicly available before the preliminary trials with the different kveiks, or the information was collected from the owners of the kveiks.

Table 3.1. *Overview of the kveiks used in this study, along with place of origin, owner and pitching temperature (Garshol, 2020).*

Kveik	Origin	Owner	Pitching temperature (°C)
Ørjasæter	Geiranger	Leiv Øystein Ørjasæter	31-33
Gamlegrua	Gausemel	Idar Nygård	30
Espe	Grodås	Arve Espe	20
Gausemel	Grodås	Olav Sverre Gausemel	30
Sigmund	Vestbygdi	Sigmund Gjernes	39
Midtbust	Stordalen	Odd H. Midtbust	33
Ebbegarden	Stordalen	Jens Aage Øvrebust	28
Tormodgarden	Sykkylven	Sigurd Johan Saure	30
Stalljen	Grodås	Stig Jarle Seljeseth	31
Otterdal	Otterdal	John Arnfinn Grodås	29

The kveiks that were used in this study were collected from brewers at Norsk Kornølfestival in Hornindal in October 2019. The exceptions to this are the Ebbegarden, Sigmund and Midtbust kveik samples, which were bought in the craft- and hobby-store Vinbua located in Ålesund in October 2019. The Otterdal kveik used in this research was donated by Pellabakkjen Låvebryggeri. The Ørjasæter kveik used in this study was harvested in September 2019 from the bottom of the fermentation tank.

The juniper used in this study was lowland juniper collected at Dimnøya in the municipality Ulstein (Møre og Romsdal) in October 2019.

3.3 Storage of original kveik samples

The genetic material of the kveiks used in this research were secured by placing 750 µL of overnight culture of the sample, incubated for one day at 30 °C, into a freezing vial along with 250 µL of 60% glycerol. This resulted in 15% (vol/vol) glycerol, which was added to protect the organic material during freezing. The contents of the vials were mixed, and they were stored in a -80 °C freezer. Eight such samples were secured from the original sample of Ørjasæter kveik in October 2019.

The dried samples of Otterdal, Gausemel and Gamlegrua were resuspended in wort, in a 1:10 ratio, and incubated for 1 day at 30 °C before freezing with 15% glycerol. The samples were frozen in October 2019.

The remaining dry samples of kveik were prepared using the same method with resuspension before -80 °C freezing with glycerol. These samples were frozen in November 2019.

3.4 Microbiological analysis of kveik

3.4.1 Growth media

The growth media were made according to the manufacturers' recommendations. All growth media were autoclaved at 121 °C for 15 minutes, except Rose-Bengal Agar, which was autoclaved at 121 °C for 5 minutes. An overview of agars and broths used in this experiment can be seen in Table 3.2. All of the prepared growth media was stored at 4 °C and in absence of light in the time between the preparation and the use of the media. The broths were distributed in reagent tubes before autoclavation.

Table 3.2. *The agars and broths used to cultivate bacteria and yeast, along with the manufacturer, expected growth, incubation temperatures and incubation times.*

Agar / Broth	Manufacturer	Cultivation	Incubation temperature (°C)	Incubation time (days)
Plate Count agar (PCA)	Merck KGaA (Darmstadt, Germany)	Total viable cell count	30	3
Potato Dextrose agar (PDA)	Merck KGaA	Yeast	30	3
Glucose Yeast Extract Peptone (GYP) broth [a]	Oxoid LTD (Basingstoke, Hampshire, England) & Merck	Yeast	30	1 - 3
Yeast and Mold (YM) agar	Oxoid LTD	Yeast and mold	30	3
Rose-Bengal Chloramphenicol agar	Oxoid LTD	Yeast and mold	30	3
De Man, Rogosa and Sharpe (MRS) broth	Oxoid LTD	Lactic acid bacteria (LAB)	30	1
MRS agar [b]	Oxoid LTD & VWR International BVBA (Leuven, Belgium)	LAB	30	3

[a] *GYP broth: 20 g/L anhydrous D(+)-Glucose (Merck), 1.5 g/L yeast extract (Oxoid LTD) and 10 g/L mycological peptone (Oxoid LTD).*
[b] *MRS agar: 15 g/L Agar powder (VWR) was added to MRS broth (Oxoid LTD).*

Cycloheximide solution from a stock of 100 mg/mL was added to MRS- and PCA agars to make a selective growth medium for wild yeast and bacteria, inhibiting the growth of domesticated yeast. 50 µL of solution was added per 200 mL of agar, resulting in a final concentration of 25 mg/L of cycloheximide in the growth media. The incubation time and temperature for these agars were 3 days at 30 °C.

The wort used for yeast propagation was made using the following mashing program: 65 °C for 45 minutes, 72 °C for 15 minutes and 78 °C for 2 minutes. The hop Spalt Select with an α acid content of 4.6 % was used as bitter hops, resulting in a calculated international bittering units (IBU) value of 20. Weyermann Extra Pale Pilsner Malt was used to make the wort. The sugar content in the wort was measured to be 9.4 °Plato using a digital refractometer. The wort was autoclaved at 121 °C for 15 minutes before use.

3.4.2 Isolation

The four different kveik samples were diluted and plated on MRS, PCA, Yeast and Mold (YM) agar, Rose-Bengal agar, MRS + cycloheximide agar and PCA + cycloheximide agar. The agar plates were incubated according to Table 3.2 and information given in this chapter.

The dilution series were made using 9 mL of Ringers solution and 1 mL of sample for each dilution step. All plates were incubated aerobically at 30 °C, and all broth cultures in tubes were incubated at 30 °C. The plates were plated in two parallels. The streaking and inoculation were done using 10 µl inoculating loops. After the isolates were pure, the cultures were inoculated (1%) in GYP broth before incubation overnight. 750 µL of each of the purified isolate cultures were secured and frozen in cryotubes at -80 °C along with 250 µL of 60% glycerol.

An overview of the agars, number of colonies picked from each type of agar, and broths and dilutions used in the isolation process are listed in Table 3.3.

Table 3.3*. An overview of the isolation of yeast and bacteria from four types of kveik. The table shows the kveiks, the agars used to plate the culture from the dilution series (primary agar), which dilutions were plated on the primary agar (dilutions plated), how many colonies were picked from each type of agar (colonies picked), which dilutions these colonies were picked from (dilution plate) with the number of the colonies, along with the broths and agars used to isolate the pure yeast/bacteria.*

Kveik	Primary agar	Dilutions plated	Colonies picked	Dilution plate (colony number)	Broth	Secondary agar
Ørjasæter	MRS	$10^{-2} – 10^{-6} + {}^{a}10^{0}$	10	10^{0} (1-4) + 10^{-6} (5-10)	MRS	MRS
	PCA	$10^{-2} – 10^{-6}$	0	-	-	-
	Yeast and mold (YM)	$10^{-3} – 10^{-7} + {}^{a}10^{0}$	10	10^{0} (1-3) + 10^{-7} (4-10)	GYP	PDA
	Rose-Bengal	$10^{-3} – 10^{-7} + {}^{a}10^{0}$	10	10^{0} (1-3) + 10^{-7} (4-10)	GYP	PDA
	MRS + cycloheximide [b]	$10^{-2} – 10^{-6}$	0	-	-	-
	PCA + cycloheximide [b]	$10^{-2} – 10^{-6}$	5	10^{-2} (1-3)	GYP	RB & YM
Gausemel	MRS	$10^{-5} – 10^{-7}$	10	10^{7} (1-10)	MRS	MRS
	PCA	$10^{-5} – 10^{-7}$	0	-	-	-
	YM	$10^{-5} – 10^{-7}$	10	10^{7} (1-10)	GYP	PDA
	Rose-Bengal	$10^{-5} – 10^{-7}$	10	10^{7} (1-10)	GYP	PDA
	MRS + cycloheximide [b]	$10^{-1} – 10^{-2}$	10	10^{-1} (1-10)	MRS	MRS
	PCA + cycloheximide [b]	10^{-2}	2	10^{-2} (1-2)	GYP	PDA
Gamlegrua	MRS	$10^{-5} – 10^{-7}$	10	10^{7} (1-10)	MRS	MRS
	PCA	$10^{-5} – 10^{-7}$	0	-	-	-
	YM	$10^{-5} – 10^{-7}$	10	10^{7} (1-10)	GYP	PDA
	Rose-Bengal	$10^{-5} – 10^{-7}$	10	10^{-6} (1 + 6-7) + 10^{-7} (2-5 + 8-10)	GYP	PDA
	MRS + cycloheximide [b]	$10^{-1} – 10^{-2}$	10	10^{-2} (1-10)	MRS	MRS
	PCA + cycloheximide [b]	10^{-2}	10	10^{-2} (1-10)	GYP	PDA
Otterdal	MRS	$10^{-5} – 10^{-7}$	10	10^{-6} (1-4) + 10^{-7} (5-10)	MRS	MRS
	PCA	$10^{-5} – 10^{-7}$	0	-	-	-
	YM	$10^{-5} – 10^{-7}$	10	10^{7} (1-10)	GYP	PDA
	Rose-Bengal	$10^{-5} – 10^{-7}$	10	10^{-6} (4 + 8-10) + 10^{-7} (1-3 + 5-7)	GYP	PDA
	MRS + cycloheximide [b]	$10^{-1} – 10^{-2}$	0	-	-	-
	PCA + cycloheximide [b]	10^{-2}	0	-	-	-

[a] singular plate.
[b] 25 mg/L.

All of the primary agar plates were incubated for 2-3 days. The GYP and MRS broths were incubated for 1-3 days. The secondary agar plates were incubated for 2-4 days. From MRS, YM and Rose-Bengal 10 colonies were randomly picked from each kveik. From MRS and PCA supplemented with cycloheximide, 2-10 colonies were picked. All colonies were sub-cultured to purity on MRS agar and PDA.

The original Ørjasæter liquid sample was used directly to make the dilution series before plating on the primary agars (MRS, PCA, Rose-Bengal, MRS + cycloheximide and PCA + cycloheximide) and incubation for 3 days. The original sample was stored at 4 °C in the time between harvesting and plating. In total, 28 PDA isolates were recovered from Ørjasæter kveik.

1.207 grams of dried Gausemel kveik was dissolved in 9 ml of wort before incubation overnight. A second inoculation (2%) and incubation overnight was done before dilution in Ringers and plating on the primary agars. In total, 24 PDA and 24 MRS isolates were recovered from Gausemel kveik.

1.193 grams of dried Gamlegrua kveik was dissolved in 9 ml of wort before incubation overnight. A second inoculation (2%) and incubation overnight was done before dilution in Ringers and plating on the primary agars. All of the primary plates were incubated for two days. In total, 30 PDA and 20 MRS isolates were recovered from Gamlegrua kveik.

1.198 grams of dried Otterdal kveik was dissolved in 9 ml of wort before incubation overnight. A second inoculation (2%) and incubation overnight was done before dilution in Ringers and plating on the primary agars. All of the primary plates were incubated for two days. In total, 20 PDA and 10 MRS isolates were recovered from Otterdal kveik.

3.4.3 Identification

3.4.3.1 Microscopy

The isolated strains of bacteria and yeast were identified by phase contrast microscopy, using a Leica DM750 microscope (Leica Microsystems, Wetzlar, Germany).

3.4.3.2 Sanger sequencing of isolated strains

The primers used to sequence strains of yeast and bacteria are listed in Table 3.4. The primers used in this study were made by Invitrogen (Thermo Fisher Scientific, Waltham, MA, USA).

Table 3.4. *Primers used to sequence strains of yeast and bacteria, along with the primer sequence, organism and target gene.*

Primer	Sequence	Organism	Target gene
NL1	5'-GCA TAT CAA TAA GCG GAG GAA AAG-3'	Eukaryote	26S rRNA
NL4	5'-GGT CCG TGT TTC AAG ACG G-3'	Eukaryote	26S rRNA
ITS1	5'-TCC GTA GGT GAA CCT GCG G-3'	Eukaryote	ITS1-5.8S rRNA-ITS2
ITS4	5'-TCC TCC GCT TAT TGA TAT GC-3'	Eukaryote	ITS1-5.8S rRNA-ITS2
5R	5'-ATG AAG AAT AGG GCG AAG GG-3'	Prokaryote	16S rRNA
1F	5'-CTC CTC AAA GCA ATA CAC TG-3'	Prokaryote	16S rRNA

All cultures were inoculated in MRS (1%) or GYP (1%) before incubation at 30 °C overnight. 1.5 mL of overnight culture was transferred to 1.5 mL Eppendorf tubes and centrifuged at 3600 rpm for 10 minutes. The supernatant was discarded, and the pellet was resuspended in 1 mL of Ringers solution. The sample was then centrifuged for 5 minutes at 3600 rpm. The supernatant was discarded, and the pellet was resuspended in 200 µL of an elution buffer (Sigma-Aldrich, Saint Louis, MO, USA) with pH 9, containing 10 mM TRIS and 0.5 mM EDTA. The Eppendorf tube containing the sample was placed on a 98 °C heating block for 10 minutes. Approximately 0,5 grams of acid-washed glass beads (Sigma-Aldrich) with a diameter of ≤106 µm were weighed into Fastprep tubes (Sarstedt, Nürnbrecht, Germany). The sample was transferred from the Eppendorf tube on the heating block to the Fastprep tube containing glass beads. The Fastprep tube was put in a shaker (Vortex-genie 2, Scientific Industries, INC, NY, USA) for 10 minutes at maximum speed, centrifuged at 3600 rpm for two minutes, and the supernatant was transferred to a new Eppendorf tube. The sample now contained template DNA and could be stored short-term at 4 °C or long-term at -20 °C.

The master mix was prepared, containing the reagents listed in Table 3.5. The DNA polymerase was added after reagents no. 1-5, and the template DNA was added at the end.

Table 3.5. *The reagents used to prepare PCR master mix.*

Reagent	Initial concentration	Final concentration	Volume per reaction (µL)
1. Iproof HF buffer (BioRad)	5x	1x	8
2. dNTPs (Sigma-Aldrich)	10 mM	200 µM	0.8
3. Forward primer	5 µM	0.25 µM	2
4. Reverse primer	5 µM	0.25 µM	2
5. PCR grade H_2O			24.8
6. Iproof DNA polymerase (BioRad)	2 U/µl	0.02 U/µl	0.4
7. Template DNA	5 – 100 ng/µl	7.5 – 150 ng	2
		Total amount	40

The Bio-Rad C1000 Thermal Cycler (Bio-Rad, Hercules, CA, USA) was used to perform the PCR reactions. The PCR programs used in this project are listed in Table 3.6, Table 3.7 and Table 3.8.

Table 3.6. *PCR program when using primers NL1 and NL4.*

PCR step	Time (minutes)	Temperature (°C)	Repeats
Initial denaturation	0:30	98.0	1
Denaturation	0:07	98.0	34
Annealing	0:30	52.0	
Elongation	0:30	72.0	
Final elongation	7:00	72.0	1
	Forever	4.0	1

Table 3.7. *PCR program when using primers ITS1 and ITS4.*

PCR step	Time (minutes)	Temperature (°C)	Repeats
Initial denaturation	0:30	98.0	1
Denaturation	0:10	98.0	35
Annealing	0:30	55.0	
Elongation	0:30	72.0	
Final elongation	10:00	72.0	1
	Forever	4.0	1

Table 3.8. *PCR program when using primers 5R and 1F.*

PCR step	Time (minutes)	Temperature (°C)	Repeats
Initial denaturation	0:30	98.0	1
Denaturation	0:10	98.0	35
Annealing	0:30	55.0	
Elongation	0:45	72.0	
Final elongation	10:00	72.0	1
	Forever	4.0	1

To make sure there was a PCR product after the PCR reaction, an agarose gel electrophoresis was run with the PCR products. 150 mL of 1% agarose gel (Lonza™ SeaKem™ LE Agarose, Fisher Scientific, Finland) was used to make a 0.5 cm thick gel, with 30 wells. 1x Tris-acetate EDTA (TAE, Merck) solution was utilized to make the gel and to put in the gel electrophoresis chamber. 2 µL of loading buffer, 6 µL of water and 2 µL of PCR product were mixed together and placed in wells. The loading buffer adds color and sugar, making the DNA samples easier to see on the gel and heavier to make sure that the DNA drops into the respective well. A 100 bp DNA ladder (New England Biolabs Inc., UK) was used. The gel electrophoresis was run at 80 V for 1-2 hours.

PCR products were purified with a GenElute™ PCR Clean-Up Kit (Sigma-Aldrich®). The wash solution concentrate was diluted with 48 mL of 100% ethanol prior to use. A GenElute plasmid mini spin column was inserted into a collection tube. 0,5 mL of the Column Preparation Solution was added to each mini spin column and centrifuged at 12,000 x *g* for 30 seconds. The eluate was discarded. The Column Preparation Solution maximizes binding of the DNA to the membrane and results in more consistent yields. 200 µL of Binding Solution was added and mixed with 40 µL PCR amplification product. The solution was transferred to the binding column, and the column was centrifuged at maximum speed for 1 minute. The eluate was discarded. The binding column was replaced into the collection tube and 500 µL of diluted Wash Solution was applied to the column. After centrifuging at maximum speed for one minute, the eluate was discarded, and the column was replaced into the collection tube. The column was centrifuged at maximum speed for an additional two minutes to remove excess ethanol. Any residual eluate, as well as the collection tube, was discarded. The column was transferred to an unused collection tube, and 50 µL of Elution

Solution was applied to the center of each column. After one minute incubation at room temperature, the column was centrifuged at maximum speed for 1 minute. The eluate, containing the purified PCR amplification product, was then stored at -20°C.

After purification, the DNA concentration was measured using Thermo Scientific NanoDrop® ND-1000 Spectrophotometer (Thermo Fisher Scientific, Wilmington DE, USA). Purified PCR product and the respective primer (NL1, NL4, ITS1, ITS4, 1F or 5R) were transferred to separate Eppendorf tubes in recommended amounts according to instructions from the company performing the sequencing, depending on the concentration of the DNA, and sent to Eurofins Genomics (Ebersberg, Germany) for LIGHTrun Sanger sequencing.

The software BioEdit Sequence Alignment Editor (BioEdit, version 7.0.5.3) was used to edit and align sequences. Bad sequences were removed. The sequence was then analyzed and compared to other sequences in "Nucleotide Basic Local Alignment Search Tool" (nBLAST), a nucleotide database, to identify the genus and species of the isolated sample. nBLAST was accessed from the website of the National Center for Biotechnology (NCBI).

3.5 Purity testing by NanoDrop

The purity of the samples were controlled using NanoDrop spectrophotometer (NanoDrop™ 2000, Thermo Fisher Scientific Inc.) and accompanying software. 2 µL of a blank sample of sterilized water was used to calibrate the spectrophotometer.

The values for the ratio of sample absorbance at 260 and 280 nm ($A_{260/280}$), the ratio of sample absorbance at 260 and 230 nm ($A_{260/230}$), and the sample concentration in ng/µL were measured for the samples.

3.6 Microbiota analysis

The kveik samples had to be prepared in advance of the microbiota assay. 1.001 g of Sigmund Gjernes, 1.297 g of Midtbust, 1.005 g of Ebbegarden, 1.188 g of Tormodgarden, 1.110 g of Ørjasæter, 1.082 g of Stalljen, 1.207 g of Gausemel, 1.198 g of Otterdal, 1.193 g of Gamlegrua and 1.009 g of Espe were weighed in sterile containers separately and diluted 1:10 with sterile wort. The cultures were incubated overnight at 30 °C, before a second inoculation (2%) and incubation overnight was done. These cultures were used in the microbiota analysis.

To prepare for the study, 10 mL of each of the cultures were centrifuged at 4500 rpm for 15 minutes. The supernatants were decanted away, the pellets were resuspended in 1 mL of Ringers solution and moved to Eppendorf tubes. The washing procedure was repeated twice. After being centrifuged at 3600 rpm for 10 minutes, the remaining supernatant was removed completely with a pipet tip. The pellets were frozen at -20 °C.

The DNA was extracted by the DNeasy® PowerFood® Microbial kit (Qiagen, Hilden, Germany). The pellet was resuspended in 450 µL of solution MBL and was then transferred to a PowerBead Tube. The tube was secured horizontally to a Vortex Adapter and vortexed at maximum speed for 10 minutes. The tubes were centrifuged at 13,000 x g for 1 minute at room temperature, before transferring the supernatant to a clean 2 mL collection tube. 100 µL of Solution IRS was added and vortexed briefly to mix. The samples were incubated at 4 °C for 5 minutes. The tubes were centrifuged again at 13,000 x *g* for 1 minute, before transferring the supernatant to a clean 2 mL collection tube. 900 µL of Solution MR was added to the tubes and they were vortexed to mix the solutions. 650 µL of supernatant was loaded onto a MB Spin Column and the samples were centrifuged at 13,000 x *g* for 1 minute. The flow-through was discarded and the step was repeated until all the supernatant had been loaded onto the column. The column was placed into a clean 2 mL collection tube. 650 µL of solution PW was added to the column, before centrifuging at 13,000 x *g* for 1 minute. The flow-through was discarded and 650 µL of ethanol was added to the column. It was then centrifuged for 1 minute, the flow-though was discarded, and the column was centrifuged for 2 minutes. The MB Spin Column was placed in a clean 2 mL collection tube and 100 µL of Solution EB was added to the center of the white filter membrane. The MB Spin Column was centrifuged for 1 minute, the flow-through containing the DNA was retained and the column was discarded.

Nanodrop was used to measure the amount of DNA in the samples after extraction. The value for A_{260}/A_{280} should ideally be approximately 1.8 and the value for A_{260}/A_{230} should ideally be approximately 2.3. The DNA samples were frozen at -20 °C.

This process was performed on all ten types of Kveik.

3.7 Antibacterial effect of Einerlog

Ripe berries, unripe berries, twigs and needles of juniper were used to make four types of extracts. 10 grams of sample and 100 mL of distilled water were added to containers, placed in a boiling water bath for 1 hour before the extracts were transferred to sterile bottles. After testing the antibacterial effect of these extracts, in the way described below, each extract was concentrated down to a volume of approximately 5 mL and the antibacterial effect of the concentrated extract was tested. The original test was conducted using 0.1 g/mL concentration of juniper and the second test was conducted using a 2 g/mL concentration. The method used in this experiment was a modified version of the method from Diep et al. (2019).

100 µL of MRS broth was added to all the wells in a 96 well microtiter plate (Sarstedt AG & Co, Nürnbrecht). 100 µL of einerlog was added to well number one using an automatic pipette. The content of the well was mixed and 100 µL of the content were transferred to well number 2. This procedure was repeated up to well number 11. After the content was mixed in well number 11, 100 µL was transferred from the well and discarded. Well number 12 of each row served as a negative control with no einerlog (Diep et al., 2019). Three rows were made with each type of juniper extract. The antibacterial effect of each type of extract was tested against *Lactobacillus* strains.

Overnight bacterial cultures were used to inoculate MRS broth, resulting in 2% inoculations. Three types of bacterial cultures were used, containing *Lb. brevis* BSO0464, *Lb. buchneri* CDO34 and *Lb. plantarum* (Wildbrew^{TM} Sour Pitch, Lallemand) (Dysvik et al., 2020b). 100 µL of bacterial culture was added to well 1-11, one row of each of the three bacterial cultures for every type of einerlog. The optical density of the wells was measured at 600 nm.

The microtiter plates were incubated at 30 °C for 24 hours. The OD at 600 nm was measured after the incubation was completed and the difference from the first measurement to the second was calculated to determine the bacterial growth in each well.

3.8 Growth and metabolism of kveik at different temperatures

3.8.1 Preparation of inocula

Fresh cultures were made of the kveik types Gausemel, Ørjasæter and Gamlegrua. This was done by inoculating 100 µL of thawed frozen culture, containing 15% glycerol, in 10 mL of wort. The culture was incubated with shaking at room temperature for approximately 48 hours. Before use, the amount of yeast cells in the culture was counted using a Bürker counting chamber (Marienfeld, Germany). The culture was diluted 1:50 using sterile water. A pipette was used to fill the counting chamber, and a 40x objective was used to determine the amount of cells in B squares. For each sample, the amount of yeast cells in 12 B-squares were counted and the average number of yeast cells in one B-square was calculated. The following equation was used to calculate yeast cells per mL from the average number of cells:

$$\text{cells per mL} = (\bar{x} \times 160) \times 10^3 \times \text{dilution factor}$$

After the amount of cells per mL was calculated, the following equation was used to calculate how many mL of culture was needed to reach a specific cell concentration:

$$C_1 \times V_1 = C_2 \times V_2$$

The goal was to make 500 mL of culture containing 1×10^6 cells per mL.

3.8.2 Inoculation

After inoculating the wort with the correct amount of culture, the wort was carefully mixed and distributed to six 50 mL Pyrex bottles and three 100 mL Pyrex bottles. The bottles were then incubated at the correct temperatures, respectively 22 °C, 30 °C and 37 °C. Growth experiments were done in triplicate.

3.8.3 Sampling

Measurements were taken from the samples after 0, 2 and 7 days. The parameters that were analyzed on every outtake were pH, cell-count, sugar content (°Plato) and HSGC analysis. The sugar content was measured using a digital refractometer (ATAGO, USA), and the pH was measured using a Radiometer PHM 92 Lab pH meter (Radiometer Analytical A/S, Denmark). In addition to this, a beer alcolyzer (Anton Paar Alcolyzer DMA 4500 M) was used to analyze the sugar content and the alcohol content after 7 days of incubation.

The cell-count was analyzed by mixing the samples carefully, before withdrawing 1 mL of sample from the bottle and adding this to a reagent tube containing 9 mL of Ringers solution. The Ringers tube was vortexed, and 1 mL was transferred to another reagent tube, thus making a dilution series. On the first day, the following dilutions were used: 10^{-3}, 10^{-4} and 10^{-5}. On the second outtake, the dilutions 10^{-5}, 10^{-6} and 10^{-7} were used, and the dilutions 10^{-4}, 10^{-5} and 10^{-6} were used for the last outtake. The samples were pour-plated in YM nutrient agar and incubated at 30 °C for 2 days, after which the colonies were counted and the colony-forming units (CFU) per mL were calculated. Parallel plates of all dilutions were used.

3.9 Modern pilot-scale brewing with kveik

The pilot-scale brewing performed in this study was done using modern methods and equipment, as opposed to using traditional Norwegian methods and equipment. The reason for this was to use the kveik in a brewing process that would be accessible and familiar to people that have prior experience with brewing, and to make the results more comparable to modern ale.

3.9.1 Pre-trial

A pre-trial run of brewing was performed to test the fermentation activity when using an amount of kveik culture that was thought to be ideal, to test the fermenting buckets (10 L, Bryggselv AS, Oppegård, Norway) that was to be used for the trial, and to decide which fermentation temperatures to use during the trial. The pre-trial fermentation temperatures were 22 °C and 30 °C, but as the fermentations at 22 °C were very time-demanding, it was decided to use 30 °C and 37 °C as fermentation temperatures for the kveik. The fermentation temperature for the control yeast, *Saccharomyces cerevisiae* SafAle US-05 (Fermentis, France), was kept at 22 °C and 30 °C . The ideal fermentation temperature for this ale yeast is 18-28°C (Fermentis, 2018), and it was thought to be wise to maintain fermentation temperatures within this range to properly be able to use this yeast as a control. The wort (9 L) was inoculated with 10 mL of kveik culture in the pre-trial. The amount of starter culture was chosen based on experience from the growth and metabolism study.

3.9.2 Trial

Table 3.9 shows an overview of the brews done in this experiment. In the cases where two temperatures are stated for the brew, then the lowest temperature applies to the control (US-05) and the highest temperature applies to the kveiks.

Table 3.9. *Overview of the number of brews, batch sizes, fermentation temperatures, additives and which types of yeast/kveik were used in each brew. Where two fermentation temperatures are stated for a brew, the lowest applies to the control yeast (US-05) and the highest temperatures applies to the kveiks (Gamlegrua, Ørjasæter and Gausemel). The variation in batch size is due to adjustment of the batch volume according to the measured gravity of the wort, which was measured using a digital refractometer.*

Brew (No.)	Batch size (L)	Fermentation temperature (°C)	Additives	Yeast/kveik
1	40	30	-	Gamlegrua, Ørjasæter, Gausemel and US-05
2	45	37 and 22	-	Gamlegrua, Ørjasæter, Gausemel and US-05
3	50	30	Einerlog	Gamlegrua and US-05
4	45	30	-	Gamlegrua, Ørjasæter, Gausemel and US-05
5	40	37 and 22	-	Gamlegrua, Ørjasæter, Gausemel and US-05
6	40	30	-	Gamlegrua, Ørjasæter, Gausemel and US-05
7	40	37 and 22	-	Gamlegrua, Ørjasæter, Gausemel and US-05
8	20	22	Einerlog	Gamlegrua

The goal for each brew was to reach an approximate original gravity in the wort of 1.080. This was done to replicate the gravity used in traditional brewing, and to get a finished product with a similar composition as traditional, Norwegian ale. This goal resulted in different batch volumes in the brews.

As the goal with the study was to compare the aroma derived from the yeast metabolism at different temperatures, the brewing was done using bitter hops along with a neutral pilsner malt, as opposed to using more aromatic malts and hops. 3.4 kg of malt was used per 10 L of water and 0.67 g of hops were used per liter of finished wort. The malt used in the brewing was Extra Pale Premium Pilsner malt from Weyermann (Bamberg, Germany). The mashing was done at 58 °C for 5 minutes, followed by 65 °C for 60 minutes and 78 °C for 2 minutes. The hops used in the brewing was Magnum, a German type of hops with an α-acid content of 13.4%; supplied from Bryggeland.no (Oslo, Norway). The IBU of the finished beer was calculated to be 18. The hop was added at a 60 minute boil.

Brews number 3 and 8 were made using einerlog as a mashing liquid instead of water in the brewing process. 1 kilo of juniper twigs and branches were used per 10 liters of finished wort, where the juniper was boiled for 1 hour prior to the mashing. The amount of juniper used in the brewing, as well as the fractions of the juniper that were used, was decided from the result of the preliminary experiment that tested the antibacterial effect of einerlog. The resulting concentration of the einerlog in the pilot scale brewing was 0.1 g/mL, which is within the lowest part of the range where the antimicrobial effect was evident for twigs and needles in the preliminary studies. The einerlog was transferred from the boiling vessel to the mashing vessel, and the mashing and the rest of the brewing process was done in an identical way as to the other brews.

In the brewing experiment, a 60L PRO pilot scale brewery from CoEnCo (Oostkamp, Belgium, 2014) was used for brews 1-7. For brew number 8, Beer Brew 30 L (Ølbrygging AS, Grimstad, Norway) was used. Beer fermenting buckets with a volume of 10L were utilized, and each bucket were filled with approximately 9L of wort, leaving a sufficient headspace for the fermentation. The buckets were placed in temperature-controlled water-baths to ensure a stable temperature throughout the fermentation process. The fermentation was considered to be complete when the krausen had completely dissipated and the yeast had flocculated at the bottom of the fermentation vessel. The fermentation time was calculated from the time when the starter culture was inoculated in the wort, until the time when the krausen had dissipated.

Traditional brewers use a low pitch rate for kveik fermentation. Some kveiks have an increased aroma intensity when they are underpitched, but this is not necessarily the case for all kveiks (Preiss & Netto, 2019a). This study underpitched the kveiks in the pilot-scale brewing in an attempt to get the aroma intensity to be as high as possible.

The kveik cultures that were to be used in the brewing experiment were made 24-48 hours in advance of the brewing. This was done by inoculating 100 µl of thawed overnight culture containing 15% Glycerol from a -20 °C (short-term storage) or -80 °C (long-term storage) freezer storage in 10 mL of autoclaved wort. The culture was incubated with shaking at room temperature. After the wort was prepared on the brewing day and the temperature of the wort was sufficiently lowered to the fermentation temperature, the kveik starter cultures were added to each of the pre-labelled fermenting buckets. The control yeast, SafAle US-05, was directly pitched using dry yeast

in the fermentation bucket on the surface of the wort (5 g/9 L) in accordance with the pitching recommendations from the supplier (5-8 g/10 L) (Fermentis, 2018).

After the fermentation was complete, the beer was bottled. Table sugar (sucrose) was used as priming sugar, and 5 g/L was added to the beer before bottling. The beer was then stored at room temperature for 14 days to allow for secondary fermentation to take place, and then the beer was moved to a cold storage with a temperature of 4 °C. Samples were analyzed using an Anton Paar alcolyzer and HSGC. Analysis was done of all the beers at the following stages: before fermentation, after fermentation and after maturation (2 weeks of secondary fermentation and 2 weeks of cold storage).

The brewing was done in triplicate, except brew number 8 with einerlog that was fermented at 22 °C, which was only done in duplicate, and the US-05 control without einerlog, which was done in quadruplicate.

3.10 Chemical analysis

3.10.1 Phenolic content

Total phenols can be analyzed by the Folin Ciocalteu method (ISO14502-1). 0.5 mL of diluted beer sample (1:10) and 2.5 mL of 10% Folin Ciocalteu reagent dissolved in water were mixed together, along with 2.0 mL 7.5% Na_2CO_3 buffer. The samples were incubated in triplicate at an ambient temperature for 60 min, and the absorbance at 765 nm was read. A standard curve (gallic acid (1 g/L)) was constructed by preparing gallic acid samples in triplicate. The resulting standard curve is shown in appendix E-11. Quantifications are done by the calibration curve of gallic acid, expressed as mg/L gallic acid equivalent (GAE). The absorbance of several beers were measured. The samples were diluted again (1:2) to fit in the standard curve.

3.10.2 Beer alcolyzer

Samples from the growth- and metabolism experiment and samples from the pilot-scale brewing were analyzed using a beer alcolyzer, the instrument Anton Paar Alcolyzer DMA 4500 M. It is well suited to analyze beers with a light color. The standard parameters for analysis are; sugar content, gravity, color, CO_2, haze, alcohol (%ABV), unfermented substances (Ea (%w/w)) and attenuation (ADF (%w/w)). The temperature of the samples were adjusted to room temperature prior to the start of analysis.

3.10.3 Headspace Gas Chromatography

Headspace Gas Chromatography (HSGC) is used to measure the amount of volatile compounds in beer. The samples were analyzed using a method previously described by (Dysvik et al., 2020a).

The samples of beer are filtered through a folded filter to remove CO_2. They are placed in a 4 °C cold storage room during the time of filtration.

10.00 g of the filtrate were weighed into headspace-bottles (Machery Nagel, Dueren, Germany). The headspace-bottles were sealed with Teflon coated septa with an aluminum ring (PFTA/Si septa, Agilent Technologies, Wilmington, DE, USA). The samples were frozen at -20 °C, and they were thawed before they were processed further. They were placed in an Agilent Technologies 7679A automatic headspace sampler with a 6890 GC system (Agilent Technologies) and a flame ionization detector. The software used was Open LAB EZChrom (Agilent Technologies).

As a carrier gas, helium 6.0 (AGA, Norge) with a flow of 5.0 mL/min was used. The headspace bath temperature was 50 °C and the manifold temperature was 60 °C. The equilibrium time was 45 minutes, and the samples were mixed during heating with 70 shakes/min. The headspace bottles were pressurized to 10 PSI before injection, and the injection time was 0.5 minutes.

A CP-SIL 5CB GC column (Varian, Middelburg, Netherlands) was used to separate the components. The column had a length of 25 meters, with an inner diameter of 0.53 mm and a film thickness of 5.0 µm. The following temperature program was used during the analysis: 35 °C for 5 min; an increase of 10 °C min^{-1} to 40 °C for 2 min; an increase of 15 °C min^{-1} to 70 °C for 2 min; an increase of 30 °C min^{-1} to 130 °C for 4 min; an increase of 30 °C min^{-1} to 160 °C for 4 min; an increase of 10 °C min^{-1} to 180 °C for 2 min; an increase of 10 °C min^{-1} to 200 °C for 2 min.

The volatile compounds were separated based on the volatility of the components and the affinity of the components to the stationary phase of the column. Identification and quantification of the various compounds were carried out by calibration with standard solutions of known concentrations of the following components from Merck (Germany) if other not stated: acetaldehyde, diacetyl, ethyl acetate, 2-butanon, 2-hexanol, 2-methyl-butanal, 2-methyl-1-butanol, 2-methyl-1-propanal, 3-methyl-butanal, 3-methyl-1-butanol, isobutyl acetate, hexanal, isoamyl acetate, ethyl hexanoate, 3-carene, R(+)-limonene, ethyl heptanoate, ethyl octanoate, β-citronellol,

ethyl nonanoate, ethyl decanoate, phenylethyl alcohol (Sigma-Aldrich), acetoin, acetone, ethanol, 1-butanol, 1-propanol, 2-butanol, dimethyl sulphide and 2,3-pantadion.

3.11 Statistical analysis

Microsoft Office 365 ProPlus Excel version 1908 was used to calculate the averages and standard deviation of the results from the growth, metabolism and brewing experiments. In addition to this, ANOVA in RStudio version 1.2.5033 (©2009-2019, RStudio Inc., Boston, MA, USA) was used to analyze the statistical significances of time, culture type and temperature in several of the results. ANOVA type III was used, as the number of observations in the groups were unbalanced. If a statistical significance was found, a Tukey post hoc test was used to discover which of the comparisons were significantly different. The resulting p-values indicated whether or not the difference between any two variables of the analyzed dataset were significant, with the H_0 hypothesis being that there is no significant difference, and a H_1 hypothesis being that there is a significant difference. A significance level of $p < 0.05$ was used. An example of a resulting output from a type III ANOVA is shown in appendix G-1, and an example of a resulting output from Tukey post hoc is shown in appendix G-2.

3.12 Sensory analysis

A trained panel (n = 5) of Norbrygg judges performed the sensory analysis. They were each given 11 samples of beer; one for each yeast/kveik type used at the different fermentation temperatures, with and without einerlog. The judges were not calibrated beforehand, and they were allowed to choose the time, order and duration of tasting for themselves. Individual evaluation sheets were filled out for each beer. The evaluation sheet (see appendix F) included appearance (clear, hazy, turbid, color, foam), aroma (intensity, malt, hops, yeast, oxidation, diacetyl, dimethyl sulphide (DMS)), flavor (sweet, bitter, acidity, malt, hops, yeast, body, concentration, additives, alcohol-level) and a conclusion on the quality of the beer. A summary of selected parameters included in the sensory evaluation sheet are presented in the result section.

4 Results

4.1 Identification of kveik

4.1.1 Microbiological analysis of kveik

Four types of kveik were studied to identify and isolate pure strains. The resulting log CFU/mL values for the kveiks Ørjasæter, Otterdal, Gausemel and Gamlegrua after plating on various agar plates from dilution series of overnight cultures are shown in Table 4.1. The raw data can be found in appendix C.

Table 4.1. *Cell count values (log CFU/mL) for the kveik types Ørjasæter, Otterdal, Gausemel and Gamlegrua after the plating of overnight cultures on different types of agar plates.*

Agar	Growth Ørjasæter (log CFU/mL)	Growth Otterdal (log CFU/mL)	Growth Gausemel (log CFU/mL)	Growth Gamlegrua (log CFU/mL)
PCA	8.6	7.9	7.8	7.8
MRS	8.5	7.9	7.8	7.7
YM	8.6	7.9	7.8	7.8
Rose-Bengal	8.6	7.9	7.9	7.8
MRS + cycloheximide [a]	< 2	< 1	1.8	5.1
PCA + cycloheximide [a]	2 [b]	< 2	2.0	5.0

[a] *25 mg/L.*
[b] *Some growth, but not countable.*

Ørjasæter had 8.6 log CFU/mL on PCA, YM and Rose-Bengal agar, and 8.5 log CFU/mL on MRS agar. There was no growth on the lowest dilution on MRS agar containing cycloheximide (<1 CFU/mL). There was growth on both of the PCA with cycloheximide plates with 10^{-2} dilution, but it was not possible to get a read on the number of colonies. Looking at this growth in a microscope showed a mixture of yeast and rod-shaped bacteria, both motile and non-motile. The yeast in GYP was round, and several cells were budding when the culture was examined in the microscope. Some of the colonies on Rose-Bengal agar had taken up the color of the agar. The color of these colonies started in the center of the colony and spread outwards.

The resulting cell count for Otterdal on PCA, MRS, YM and Rose-Bengal was 7.9 log CFU/mL. There were no colonies formed at the lowest dilution on MRS + cycloheximide and PCA + cycloheximide, the resulting cell count was therefore <1 log CFU/mL and <2 log CFU/mL

respectively. The isolates from YM had very homogenous colonies. The morphology of the colonies on MRS agar as a whole were very homogenous but differed slightly in size.

The resulting cell count for Gausemel were 7.8 log CFU/mL on PCA, MRS and YM, and 7.9 log CFU/mL on Rose-Bengal agar. The MRS + cycloheximide agar had 1.8 log CFU/mL and the PCA + cycloheximide agar had 2.0 log CFU/mL. The yeast from PCA + cycloheximide had morphologically bigger colonies than the bacteria colonies on this agar. Rod- and cocci-shaped bacteria were observed by microscopy in the yeast-samples from MRS + cycloheximide and PCA + cycloheximide during isolation. Several of the small colonies from these agars consisted of rod-shaped bacteria.

The resulting amounts of colonies for Gamlegrua were 7.8 log CFU/mL for PCA, Rose-Bengal and YM agar. MRS agar had 7.7 log CFU/mL. The result for MRS + cycloheximide was 5.1 log CFU/mL and for PCA + cycloheximide the result was 5.0 log CFU/mL. The isolates from YM were morphologically very similar. In the second round of the isolation, the colonies from YM agar and Rose-Bengal agar were white and had a homogenous appearance. The MRS + cycloheximide isolates all contained small, white and morphologically homogenous colonies.

4.1.2 Identification of isolated strains

The resulting sequences from LIGHTrun Sanger sequencing were analyzed using BioEdit Sequence Alignment Editor and BLAST. Table 4.2 shows an overview of the number of isolates, as well as results from microscopy and Sanger sequencing.

Table 4.2. *Overview of the number of isolates from each type of nutrient agar for each of the four analyzed kveik samples. The number of yeast and/or bacteria isolates for each type of nutrient agar, decided by microscopy, is stated as a fraction of the total number of isolates. The identification done by 26S rRNA, ITS1-5.8S rRNA-ITS2 or 16S rRNA sequencing is stated as genus and species, along with the number of isolates that were sequenced.*

Kveik	Media	Isolates	Microscopy	Identification			
				26S rRNA & ITS1-5.8S rRNA-ITS2		16S rRNA	
				Yeast	Isolates	Bacteria	Isolates
Ørjasæter	**R&B**	10	Yeast	*S. cerevisiae*	4 of 10	-	-
	YM	10	Yeast	*S. cerevisiae*	5 of 10	-	-
	MRS	5	Yeast	*S. cerevisiae*	5 of 5	-	-
	PCA+ [a]	3	Yeast	*S. cerevisiae*	3 of 3	-	-
	MRS+ [a]	0	-	-	-	-	-
Gausemel	**R&B**	10	Yeast	*S. cerevisiae*	2 of 10	-	-
	YM	10	Yeast	*S. cerevisiae*	2 of 10	-	-
	MRS	10	Yeast	*S. cerevisiae*	3 of 10	-	-
	PCA+ [a]	4	Yeast (2 of 4); Bacteria (2 of 4)	*S. cerevisiae*	1 of 2	-	0 of 2
	MRS+ [a]	14	Yeast (4 of 14); Bacteria (10 of 14)	*S. cerevisiae*	2 of 4	*Acetobacter malorum*; *Lactobacillus plantarum*	1 of 10; 1 of 10
Gamlegrua	**R&B**	10	Yeast	*S. cerevisiae*	2 of 10	-	-
	YM	10	Yeast	*S. cerevisiae*	1 of 10	-	-
	MRS	10	Yeast (9 of 10); Bacteria (1 of 10)	*S. cerevisiae*	3 of 9	-	0 of 1
	PCA+ [a]	10	Yeast (5 of 10); Bacteria (5 of 10)	*S. cerevisiae*	2 of 5	*Lb. plantarum*	3 of 5
	MRS+ [a]	10	Bacteria	-	-	-	0 of 10
Otterdal	**R&B**	10	Yeast	*S. cerevisiae*	4 of 10	-	-
	YM	10	Yeast	*S. cerevisiae*	3 of 10	-	-
	MRS	10	Yeast	*S. cerevisiae*	3 of 10	-	-
	PCA+ [a]	0	-	-	-	-	-
	MRS+ [a]	0	-	-	-	-	-

[a] *+ = 25 mg/L cycloheximide.*

The yeast *S. cerevisiae* was found in all of the kveiks, and no other yeast was isolated. Four out of the total of five bacteria isolates were identified as *Lb. plantarum* and the fifth isolate was

identified as *Acetobacter malorum*. Strains of both bacteria and yeast were isolated from the kveiks Gamlegrua and Gausemel, but only yeast strains were isolated from the kveiks Ørjasæter and Otterdal.

Rose-Bengal sample number seven of Ørjasæter kveik showed divergent growth and flocculation from the rest of the Rose-Bengal samples. MRS sample number 10 exhibited very good growth, compared to the rest of the MRS samples.

The Gamlegrua Rose-Bengal agar colonies 1-5 were pink and colonies 6-10 were white. The colonies from YM agar and Rose-Bengal agar were white and had a homogenous appearance. Isolate number five from PCA + cycloheximide showed a different colony morphology compared to other isolates from this agar. Isolate number 1 from MRS agar had the same morphology as the MRS + cycloheximide colonies; the rest of the MRS isolates (2 through 10) consisted of bigger, white colonies.

Otterdal had a lot of pink colonies on the Rose-Bengal agar plates, and the colonies of isolates 1-5 were completely pink. The colonies of isolates 6-10 were a mixture of white and pink.

Gausemel isolate 1-5 from Rose-Bengal agar were pink and isolate 6-10 were white. The colonies on YM agar had different morphologies. MRS + cycloheximide isolate number four had a different morphology than the other MRS + cycloheximide isolates. This isolate had colonies containing both yeast and cocci-shaped bacteria, paired together two-and-two. This isolate also had a completely clear broth with no pellet-formation.

Figure 4.1 shows the result for the total amount of sequenced isolates, not taking the type of kveik it was isolated from into consideration. The percentages out of the total amount of sequenced isolates are shown in the figure. A total of 5 bacterial and 45 yeast isolates were sequenced.

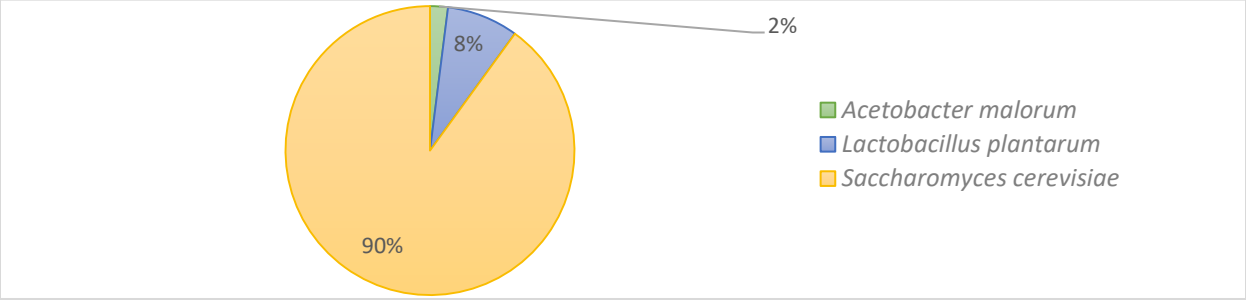

***Figure 4.1**. Distribution of bacteria (n = 5) and yeasts (n = 45) of the four kveik samples tested using Sanger Sequencing.*

The figure shows a clear majority of *S. cerevisiae* yeast in the sequencing results.

4.2 The antibacterial effect of einerlog

The initial concentration of the einerlog used in this experiment was 2 g/mL, and it was diluted 2x for every consecutive well it was placed in. Table 4.3 and Table 4.4 shows the resulting measurements at OD_{600}, where the values are the difference from day 0 to day 1. A low value indicates the lack of bacterial growth and a presence of an antibacterial effect.

Table 4.3. *The result from microtiter plate assay, involving twigs and needles from juniper and three LAB: Lb. plantarum, Lb. brevis and Lb. buchneri. From the first well to the eleventh well, the juniper extract is diluted 2x. The concentration of extract shown in the table indicates the concentration in the respective well relative to the concentration of the original extract (2g/ml). Well number 12 is used as a control, with no juniper extract added to the well. The data shown is the difference in OD_{600} from day 0 to day 1 (day 1 minus day 0), after incubation at 30 °C for 24 hours.*

Extract type		Twig			Needle		
		Lb. plantarum	***Lb. brevis***	***Lb. buchneri***	***Lb. plantarum***	***Lb. brevis***	***Lb. buchneri***
Concentration of extract (g/mL)	**1.0**	0.244	0.051	0.063	0.224	0.139	0.2
	0.5	0.164	0.141	0.171	0.833	0.393	0.656
	0.25	0.454	0.342	0.467	0.154	-0.88	-0.447
	0.125	0.57	0.356	0.488	1.372	0.309	0.77
	0.063	0.89	0.372	0.825	1.506	0.333	0.842
	0.031	1.39	0.323	0.949	1.507	0.361	0.848
	0.016	1.525	0.321	0.882	1.53	0.341	0.821
	0.008	1.525	0.307	0.895	1.542	0.354	0.817
	0.004	1.531	0.337	0.952	1.531	0.363	0.864
	0.002	1.519	0.367	0.931	1.557	0.339	0.836
	0.001	1.522	0.345	0.896	1.529	0.379	0.851
	0	1.51	0.381	0.697	1.492	0.369	0.788

Juniper twigs seems to have a considerable inhibitory effect on *Lactobacillus plantarum* at the concentrations 0.031-1.0 g/mL, on *Lactobacillus brevis* at the concentrations 0.5-1.0 g/mL and on *Lactobacillus buchneri* at the concentrations 0.125-1.0 g/mL. Juniper needles doesn't seem to be as efficient as twigs, but there are still some inhibitory effects on *Lb. plantarum* at the concentrations 0.125-1.0 g/mL and on *Lb. brevis* and *Lb. buchneri* on the strongest concentration used in this experiment (1.0 g/mL).

Table 4.4. *The result from microtiter plate assay, involving unripe and ripe berries from juniper and three LAB: Lb. plantarum, Lb. brevis and Lb. buchneri. From the first well to the eleventh well, the juniper extract is diluted 2x. The concentration of extract shown in the table indicates the concentration in the respective well relative to the concentration of the original extract. Well number 12 is used as a control, with no juniper extract added to the well. The data shown is the difference in* OD_{600} *from day 0 to day 1 (day 1 minus day 0), after incubation at 30 °C for 24 hours.*

Extract type		Unripe berries			Ripe berries		
		Lb. plantarum	*Lb. brevis*	*Lb. buchneri*	*Lb. plantarum*	*Lb. brevis*	*Lb. buchneri*
Concentration of extract (g/mL)	**1.0**	1.499	0.389	0.375	1.547	0.404	0.977
	0.5	1.602	0.297	0.692	1.504	0.425	0.838
	0.25	1.517	0.29	0.731	1.532	0.337	0.904
	0.125	1.541	0.343	0.712	1.522	0.333	0.921
	0.063	1.547	0.362	0.769	1.543	0.347	0.846
	0.031	1.52	0.366	0.74	1.548	0.333	0.769
	0.016	1.517	0.353	0.733	1.536	0.305	0.771
	0.008	1.516	0.354	0.724	1.545	0.331	0.766
	0.004	1.5	0.342	0.702	1.563	0.348	0.842
	0.002	1.53	0.339	0.706	1.565	0.384	0.848
	0.001	1.537	0.342	0.727	1.548	0.357	0.858
	0	1.534	0.376	0.711	1.518	0.373	0.832

Unripe berries seemed to have a slight inhibitory effect on *Lb. buchneri* at 1 g/mL, but no inhibition was seen for the other two LAB. Ripe berries does not seem to have any inhibitory effect on any of the three LAB used in this experiment.

4.3 Growth and metabolism of kveik at different temperatures

Unless other information is stated, the results from the growth experiments are presented as an average of three parallels. The experiment was conducted using the three temperatures 22 °C, 30 °C and 37 °C, and the three kveiks Gamlegrua, Gausemel and Ørjasæter, and the control yeast US-05. Read-outs were taken at 0 hours, 2 days and 7 days. The raw data can be found in appendix A.

4.3.1 Cell counts

The cell count of all the samples were measured on day 0, 2 and 7. All results are shown as averages of three measurements, except US-05 on day 7, which is an average of two measurements. Figure 4.2, Figure 4.3, Figure 4.4 and Figure 4.5 show line charts of the four cultures incubated at 22 °C, 30 °C and 37 °C. The raw data can be found in appendix A-2.

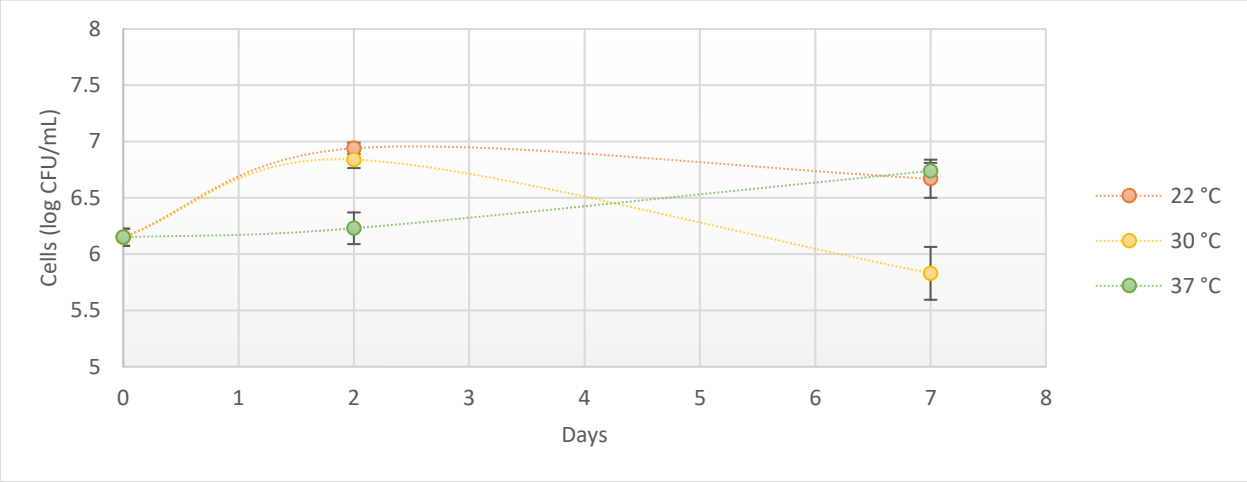

Figure 4.2. *US-05 cell counts from the growth experiment. The result is given as log CFU/mL. The samples were incubated at 22 °C, 30 °C and 37 °C. Read-outs were taken at 0, 2 and 7 days. Values are averages of n = 3, except for day 7 at 37 °C (n = 2). The figure shows the standard deviation for each value.*

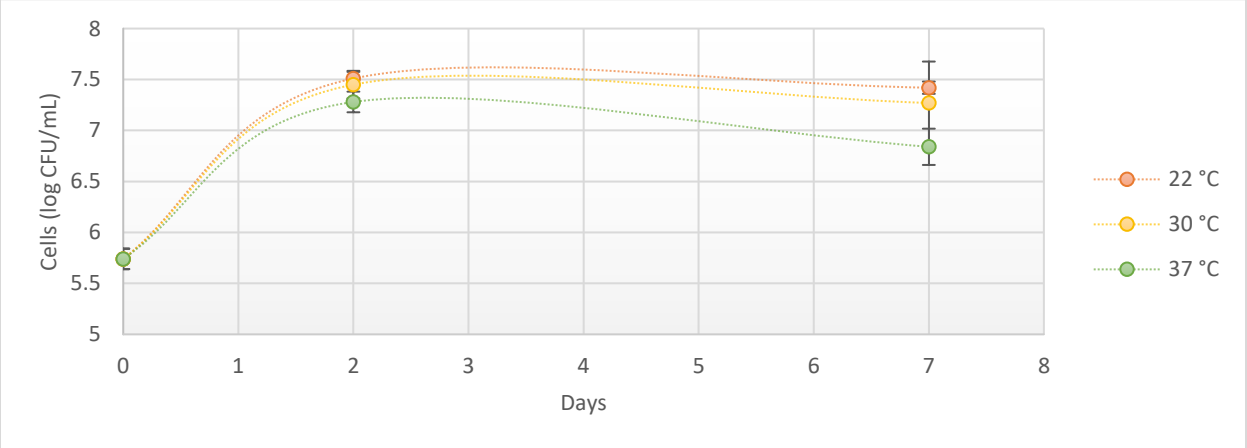

Figure 4.3. *Ørjasæter cell counts from the growth experiment. The result is given as log CFU/mL. The samples were incubated at 22 °C, 30 °C and 37 °C. Read-outs were taken at 0, 2 and 7 days. Values are averages of n = 3. The figure shows the standard deviation for each value.*

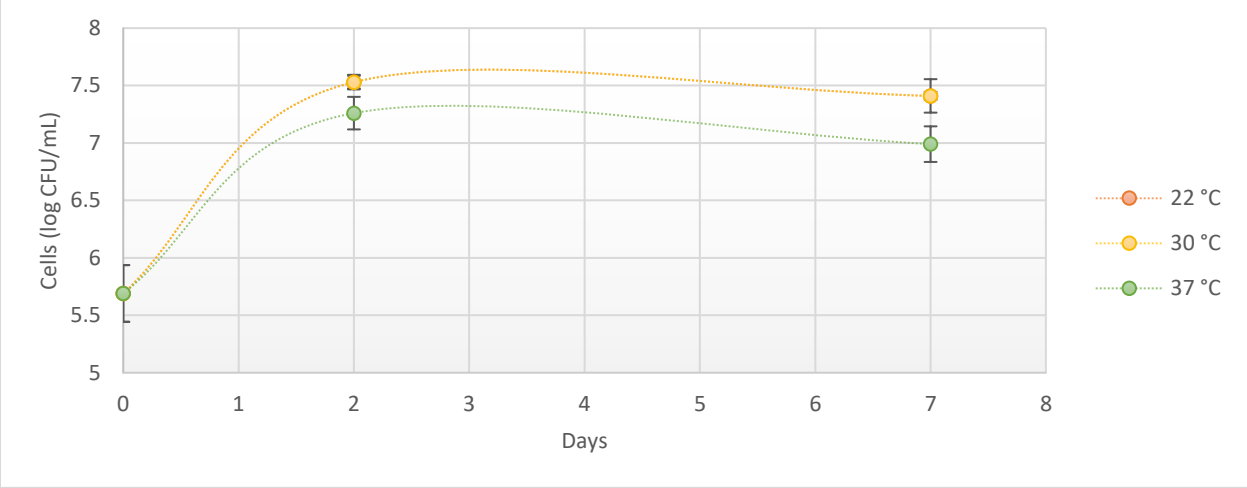

Figure 4.4. *Gamlegrua cell counts from the growth experiment. The result is given as log CFU/mL. The kveik was incubated at three different temperatures: 22 °C, 30 °C and 37 °C. Read-outs were taken at 0, 2 and 7 days. Values are averages of n = 3. The figure shows the standard deviation for each value.*

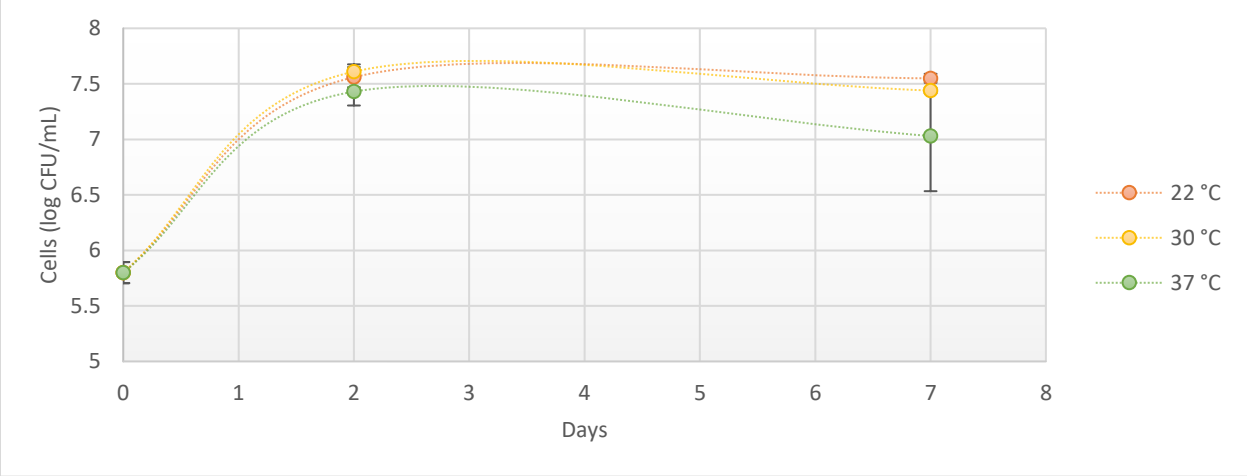

Figure 4.5. *Gausemel cell counts from the growth experiment. The result is given as log CFU/mL. The kveik was incubated at three different temperatures: 22 °C, 30 °C and 37 °C. Read-outs were taken at 0, 2 and 7 days. Values are averages of n = 3. The figure shows the standard deviation for each value.*

The cell count for all three kveiks incubated at 37 °C was lower than the cell counts for the kveiks incubated at 22 °C and 30 °C on both day 2 and 7. The difference in cell counts between 22 °C and 30 °C is very small for all three kveiks. Gausemel and Ørjasæter both had the highest cell counts in the samples incubated at 22 °C on day 7 with 7.6 and 7.4 log CFU/mL respectively. The three kveiks had cell counts of 6.8 to 7.6 log CFU/mL at the three temperatures on day 7, whilst US-05 had cell counts of 5.8 to 6.7 log CFU/mL at the three temperatures on day 7.

The statistical significance of the cell count (log CFU/mL) values for all four cultures on the last outtake on day 7 were analyzed. The resulting p-values are listed in Table 4.5.

Table 4.5. *Statistical significance for cell count (log CFU/mL) value on day 7 for all yeast/kveik cultures in the growth and metabolism study at different temperatures. A p-value < 0.05 indicates a significant statistical difference in the compared values. A 95% confidence interval is used. The p-values of comparisons found to be statistically significant are highlighted in bold letters.*

Comparison:	Individual p-value	Combined p-value
Temperature vs. temperature	-	0.2274
Gausemel vs. Gamlegrua	0.9705816	**0.00001045**
US-05 vs. Gamlegrua	**0.0000752**	
Ørjasæter vs. Gausemel	0.7595278	
US-05 vs. Ørjasæter	**0.0003322**	
Ørjasæter vs. Gamlegrua	0.9481295	
US-05 vs. Gausemel	**0.0000222**	

The differences of cell counts between the temperature groups were not significant, but the difference between the cultures were significant. Tukey's method showed that the cell counts of US-05 vs. Gamlegrua, US-05 vs. Gausemel and US-05 vs. Ørjasæter were significantly different from each other at day 7.

4.3.2 pH measurements

The pH of all samples were measured on day 0, 2 and 7. All results are shown as an average of three measurements, except US-05 on day 7, which is an average of two measurements. Figure 4.6 to Figure 4.9 show line charts with standard deviation of the four cultures. The raw data can be found in appendix A-1.

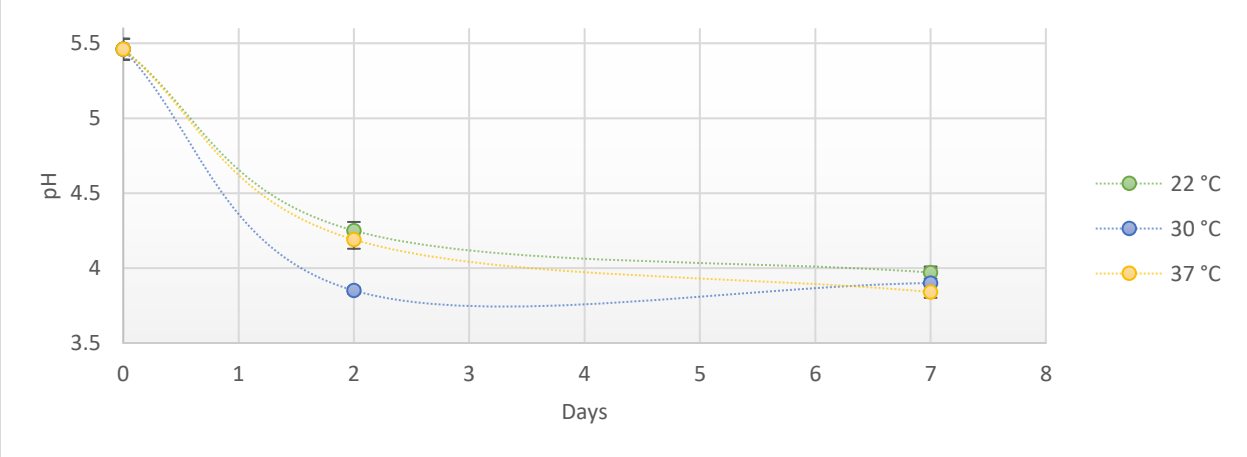

Figure 4.6. *pH results for US-05 from the growth and metabolism experiment with standard deviation. Values are averages of n = 3, except day 7 at 37 °C (n = 2). Three incubation temperatures were used: 22 °C, 30 °C and 37 °C. The pH was measured using a pH meter.*

The lowest pH value for US-05 on day 2 was observed at 30 °C with pH 3.9. On day 7, the lowest pH value was observed at 37 °C, however the pH values of all temperatures were very similar (pH 3.8-4.0). The highest pH values on both day 2 and 7 were observed at 22 °C with pH values 4.3 and 4.0, respectively.

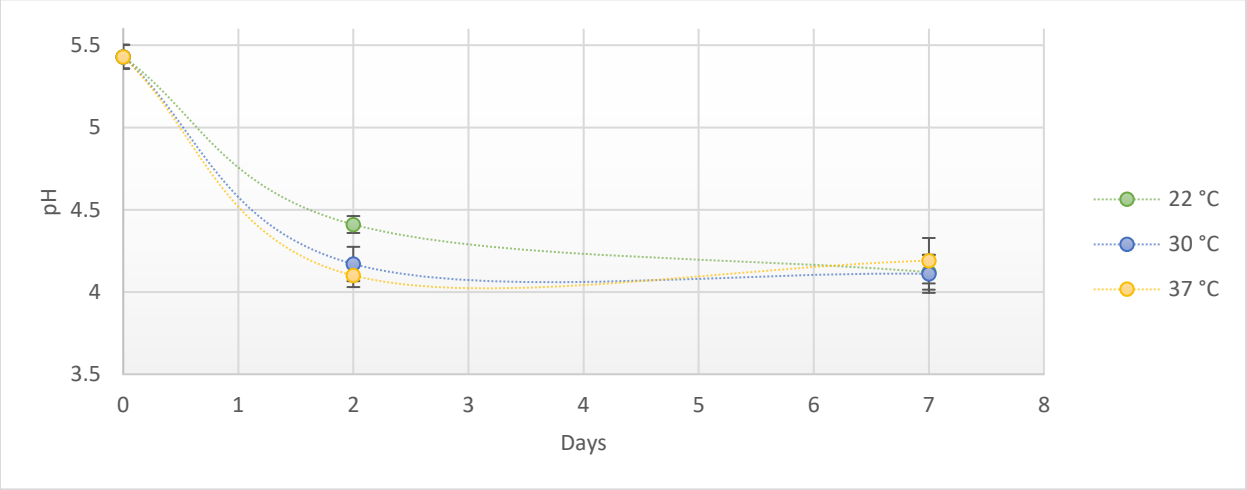

Figure 4.7. *pH results for Ørjasæter from the growth and metabolism experiment with standard deviation. Values are averages of n = 3. Three incubation temperatures were used: 22 °C, 30 °C and 37 °C. The pH was measured using a pH meter.*

The Ørjasæter samples on day 7 had very similar values. On day 2, the samples incubated at 37 °C had the lowest pH value with pH 4.2, and the samples incubated at 22 °C had the highest pH value with pH 4.4. On day 7, the samples incubated at 37 °C had the highest pH value with pH 4.2, and the samples incubated at 22 °C and 30 °C had the lowest pH values with pH 4.1.

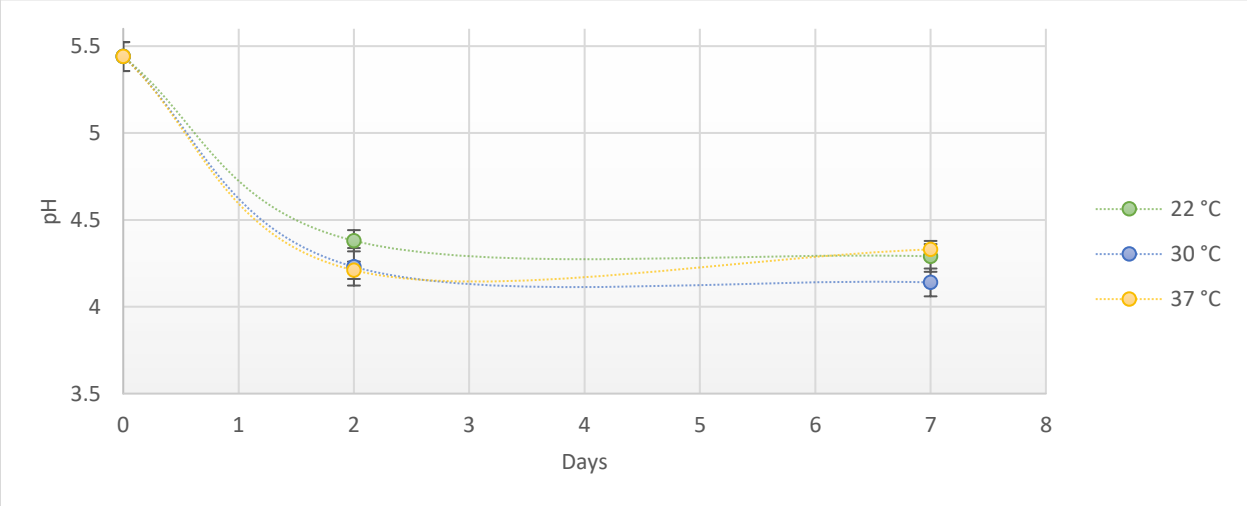

Figure 4.8. *pH results for Gamlegrua from the growth and metabolism experiment with standard deviation. Values are averages of n = 3. Three incubation temperatures were used: 22 °C, 30 °C and 37 °C. The pH was measured using a pH meter.*

The Gamlegrua cultures samples incubated at 37 °C had the lowest pH levels on day 2 with pH 4.2 and the highest pH values on day 7 with pH 4.3. The samples incubated at 22 °C had the highest pH level out of the Gamlegrua samples on day 2 with pH 4.4. On day 7, the lowest pH level was the samples incubated at 30 °C with pH 4.1.

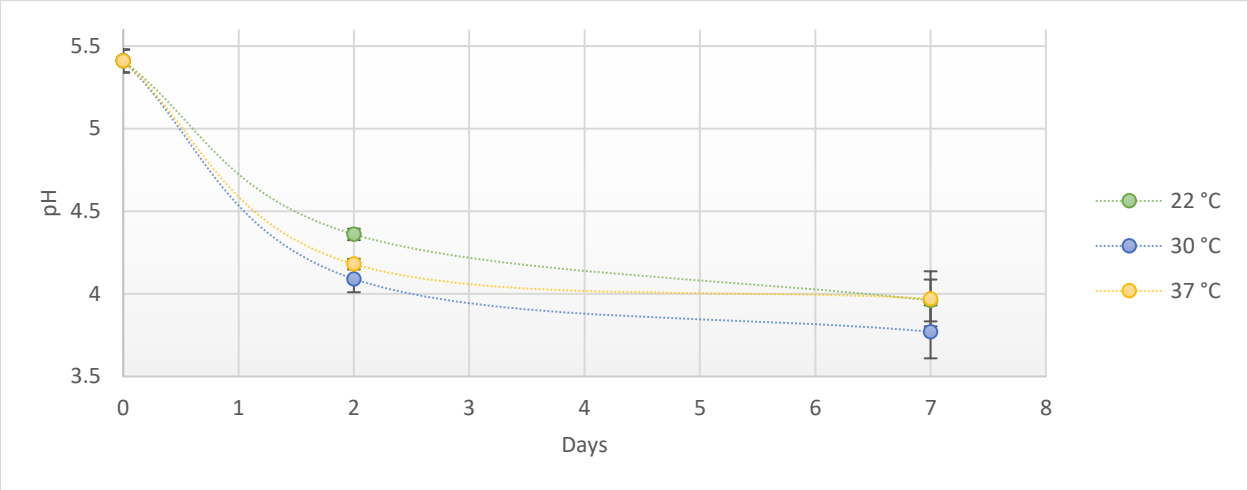

Figure 4.9. *pH results for Gausemel from the growth and metabolism experiment with standard deviation. Values are averages of n = 3. Three incubation temperatures were used: 22 °C, 30 °C and 37 °C. The pH was measured using a pH meter.*

The Gausemel samples incubated at 30 °C had the lowest pH value on both day 2 and 7 with pH 4.1 and 3.8, respectively. The sample incubated at 22 °C had the highest pH value on day 2 with pH 4.4, and the samples incubated at 22 °C and 37 °C had the highest pH values on day 7 with pH 4.0.

The statistical significance of the difference in pH values between the cultures and between the temperatures were investigated. The respective p-values are listed in Table 4.6.

Table 4.6. *Statistical significance of the pH values observed on day 7 for all yeast/kveik cultures in the growth and metabolism study, where samples had been incubated at three different temperatures: 22 °C, 30 °C and 37 °C. The kveiks Gausemel, Gamlegrua and Ørjasæter, and the control yeast SafAle US-05, were analyzed. A p-value < 0.05 indicates a significant statistical difference in the compared values. A 95% confidence interval is used. The p-values of comparisons found to be statistically significant are highlighted in bold letters.*

Comparison:	Individual p-value	Combined p-value
Temperature vs. temperature	-	0.246
Gausemel vs. Gamlegrua	**0.0000035**	**0.00000043**
US-05 vs. Gamlegrua	**0.0000095**	
Ørjasæter vs Gausemel	**0.0010255**	
US-05 vs. Ørjasæter	**0.0022943**	
Ørjasæter vs. Gamlegrua	0.2055251	
US-05 vs. Gausemel	0.9982708	

The cultures Gausemel vs. Gamlegrua, US-05 vs. Gamlegrua, Ørjasæter vs. Gausemel and US-05 vs. Ørjasæter had statistically significant differences in the pH values at day 7. No significant statistical difference could be found when the incubation temperatures were compared to one another.

4.3.3 Alcohol and sugar content

The alcohol and the sugar level in the samples were measured using Anton Paar alcolyzer on day 7 of fermentation, the last outtake. The results are shown as percentage of alcohol by volume (% ABV) and gravity in Figure 4.10, and the raw-data can be found in appendix A-3.

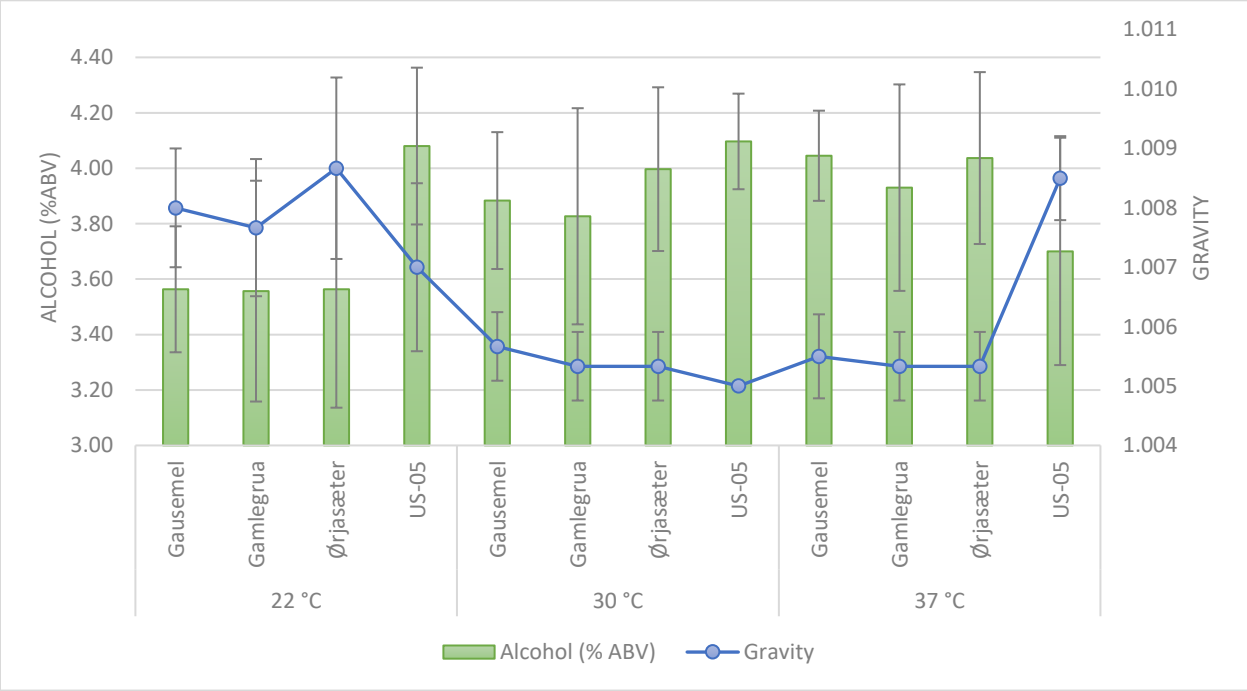

Figure 4.10. *The resulting alcohol content and gravity of three types of kveik and the US-05 control at three different temperatures in the growth and metabolism experiment. The samples were measured using an Anton Paar beer alcolyzer after being incubated at the respective temperature for 7 days. All measurements are averages of n = 3, except Gausemel at 37 °C and US-05 at 22 °C and 37 °C (n = 2).*

The kveiks were lower in alcohol level than US-05 for the samples incubated at 22 °C, and the opposite was observed for the samples incubated at 37 °C. The control yeast had a higher alcohol content at 30 °C, but the differences at this temperature were smaller than on the other two temperatures. Gamlegrua was observed to have the lowest alcohol level of the kveiks at 30 °C and 37 °C with 3.8% and 3.9% respectively.

The statistical significances of alcohol content and gravity were analyzed on day 7. The p-values are listed in Table 4.7.

Table 4.7. *Statistical significances for alcohol content (% ABV) and gravity on day 7 for all yeast/kveik cultures in the growth and metabolism study at different temperatures. A p-value < 0.05 indicates a significant statistical difference in the compared values. A 95% confidence interval is used. Analyzed using ANOVA in RStudio. The p-values of comparisons found to be statistically significant are highlighted in bold letters.*

Comparison	Alcohol (% ABV)	Gravity	
	Combined p-value	Combined p-value	Individual p-value
Yeast/kveik vs. kveik	0.6517	0.9377	-
30 °C vs. 22 °C	0.05657	**0.00001163**	**0.0000104**
37 °C vs. 22 °C			**0.0010464**
37 °C vs. 30 °C			0.3387920

No significant differences in alcohol levels were found when comparing cultures or temperatures. Statistically significant differences were found between the temperatures 30 °C vs. 22 °C and 30 °C vs. 22 °C samples when comparing the gravity on day 7. No significant differences in gravity were found when the cultures were compared.

The sugar content of the samples were also measured using a digital refractometer (ATAGO, USA) on all outtakes. A conversion table between specific gravity, °Plato and °Brix can be found in appendix E-10. The results are depicted in Figure 4.11, and the raw-data for these results can be found in appendix A-1.

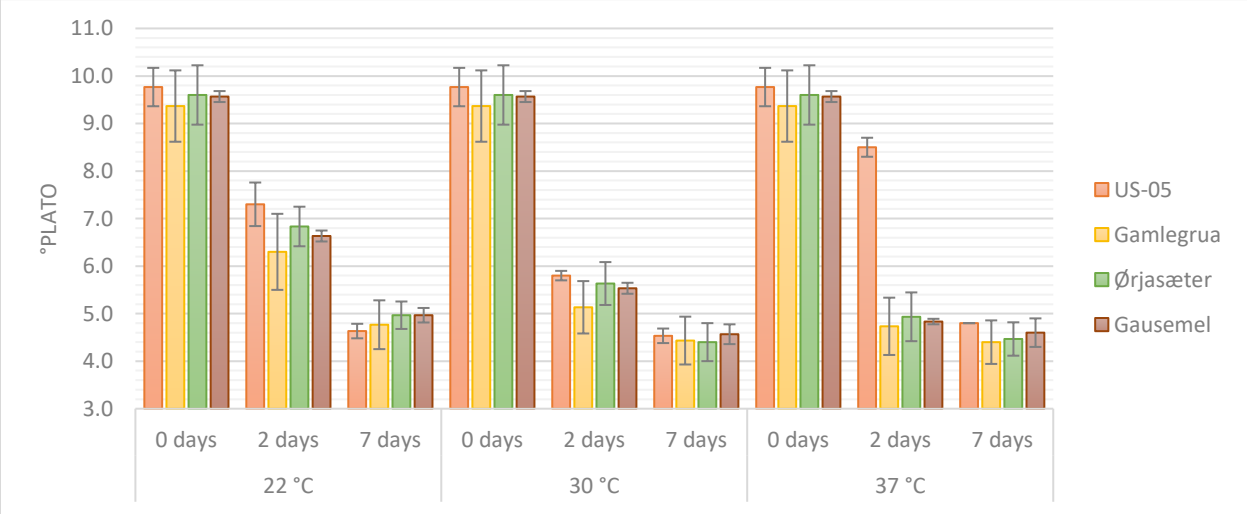

Figure 4.11. *Results from analysis of sugar content in the growth and metabolism study. The yeast SafAle US-05 and the kveiks Gamlegrua, Ørjasæter and Gausemel were analyzed at three temperatures: 22 °C, 30 °C and 37 °C. Measurements were taken on day 0, day 2 and day 7. The bar chart illustrates all of the samples on all outtakes, with standard deviation. The values are averages of n = 3, except the value for US-05 at 37 °C on day 7 (n = 2).*

The bar chart shows higher sugar content for all of the cultures at 22 °C on day 7 compared to 30 °C and 37 °C. The °Plato observations on US-05 at 37 °C on day 2 was measured to be a lot higher than all of the other observations on day 2.

The statistical significance of the °Plato values from day 7 was analyzed. The respective p-values are listed in Table 4.8.

Table 4.8. *Statistical significance for sugar content (°Plato) on day 7 for all yeast/kveik cultures in the growth and metabolism study at different temperatures, calculated using type III ANOVA and Tukey's method in RStudio. A p-value < 0.05 indicates a significant statistical difference in the compared values. A 95% confidence interval is used. The p-values of comparisons found to be statistically significant are highlighted in bold letters.*

Comparison:	Individual p-value	Combined p-value
Yeast/kveik vs. kveik	-	0.7574
22 °C vs. 30 °C	**0.0256339**	**0.02262**
30 °C vs. 37 °C	0.8824427	
22 °C vs. 37 °C	0.0843757	

No statistical significance could be found when the cultures were compared with each other. The differences between the observations at 22 °C and 30 °C was found to be significant.

4.3.4 Aromatic components

The content of selected aromatic components were measured in a growth experiment that included the kveiks Gamlegrua, Gausemel and Ørjasæter, as well as SafAle US-05 yeast that served as a control. Three different incubation temperatures (22 °C, 30 °C and 37 °C) were used. Outtakes were taken on day 0, 2 and 7 to map the composition of the samples and the development of aromatic components. The aromatic components are categorized in subchapters based on their functional group.

The sulphur component dimethyl sulphide (DMS) was found in quantities of 0.00 to 0.01 ppm and the differences between the cultures and temperatures were very small. This aromatic component is therefore not referred to further in this section.

4.3.4.1 Esters

The esters isobutyl acetate and ethyl heptanoate were found in quantities of 0.00 to 0.02 ppm, with very small differences between the different cultures and temperatures, and the results from these volatile components were therefore not included in the result section of this thesis. Figure 4.12, Figure 4.13, Figure 4.14 and Figure 4.15 illustrates the content and development of four esters, respectively ethyl acetate, isoamyl acetate, ethyl hexanoate and ethyl octanoate.

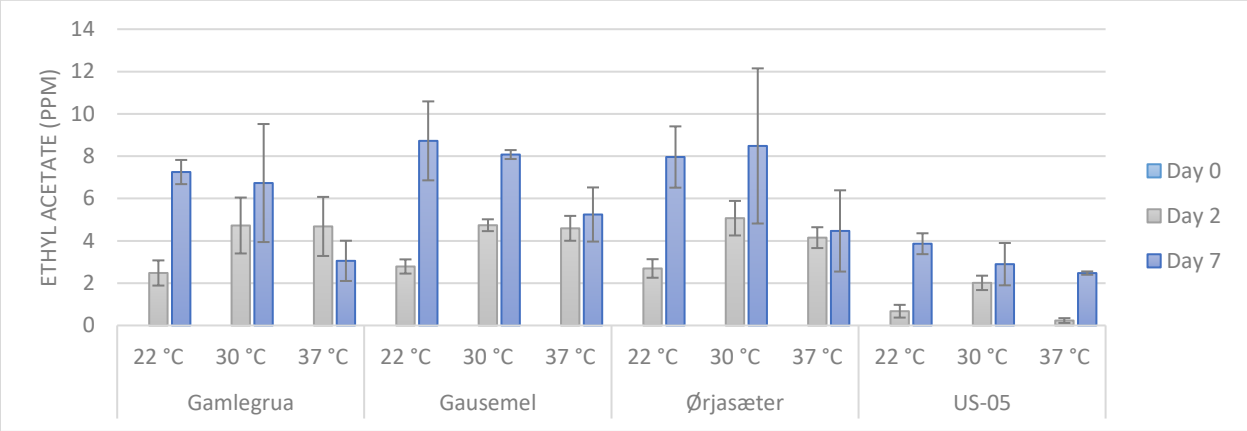

Figure 4.12. *Ethyl acetate results from HSGC analysis in the growth and metabolism experiment, with standard deviation. The kveiks Gamlegrua, Gausemel and Ørjasæter were analyzed, along with the commercial yeast SafAle US-05. Measurements were taken on day 0, day 2 and day 7. The values are averages of n = 3, except for the 7 day measurement of US-05 at 37 °C (n = 2). Three incubation temperatures were used; 22 °C, 30 °C and 37 °C.*

The content of ethyl acetate was higher on day 7 at 22 °C than at 30 °C and 37 °C for Gamlegrua, Gausemel and US-05. The highest content on day 7 for Ørjasæter was at 30 °C. However, the contents were quite similar on day 7 at 22 and 30 °C. The content of the ester at 37 °C was the lowest for all of the cultures on day 7.

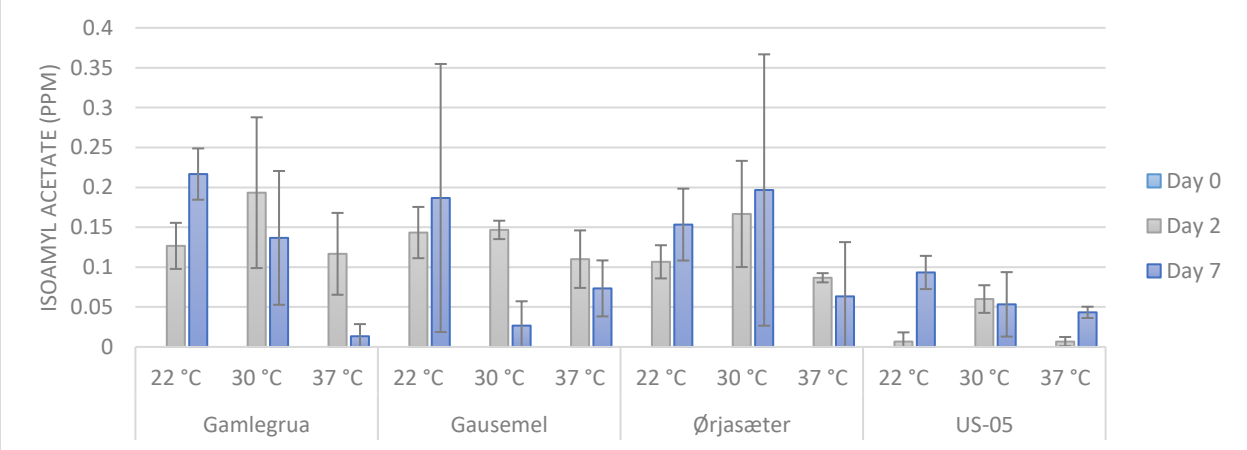

Figure 4.13. *Isoamyl acetate results from HSGC analysis in the growth and metabolism experiment, with standard deviation. The kveiks Gamlegrua, Gausemel and Ørjasæter were analyzed, along with the commercial yeast SafAle US-05. Measurements were taken on day 0, day 2 and day 7. The values are averages of n = 3, except for day 7 of US-05 at 37 °C (n = 2). Three incubation temperatures were used; 22 °C, 30 °C and 37 °C.*

Gamlegrua, Gausemel and US-05 had their highest contents of isoamyl acetate on day 7 at 22 °C. Ørjasæter had its highest content at 30 °C on day 7. The highest content on day 7, in total, was found in Gamlegrua at 22 °C. There are big standard deviations in the measured values for Gausemel on day 7 at 22 °C and for Ørjasæter on day 7 at 30 °C.

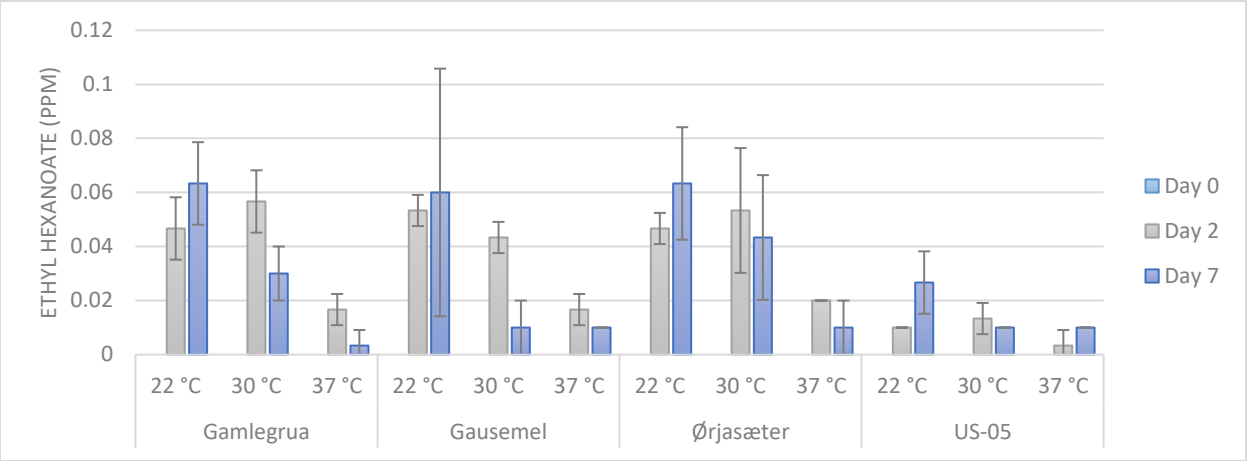

Figure 4.14. *Ethyl hexanoate results from HSGC analysis in the growth and metabolism experiment, with standard deviation. The kveiks Gamlegrua, Gausemel and Ørjasæter were analyzed, along with the commercial yeast SafAle US-05. Measurements were taken on day 0, day 2 and day 7. The values are averages of n = 3, except for day 7 of US-05 at 37 °C (n = 2). Three incubation temperatures were used; 22 °C, 30 °C and 37 °C.*

US-05 had the lowest content of ethyl hexanoate overall. The highest values for all of the cultures were observed at 22 °C on day 7, with 0.03 ppm for US-05 and 0.06 ppm for the three kveiks.

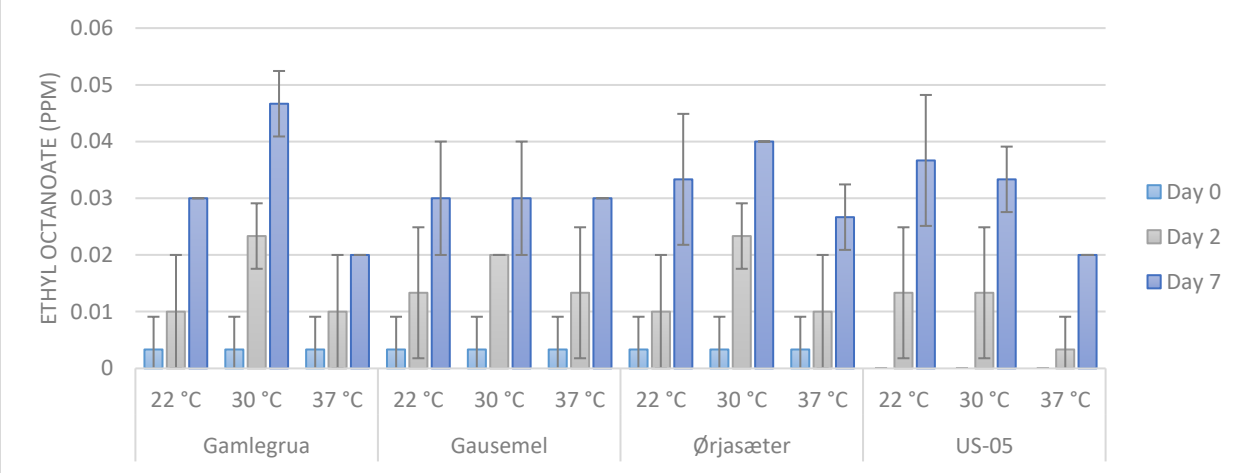

Figure 4.15. *Ethyl octanoate results from HSGC analysis in the growth and metabolism experiment, with standard deviation. The kveiks Gamlegrua, Gausemel and Ørjasæter were analyzed, along with the commercial yeast SafAle US-05. Measurements were taken on day 0, day 2 and day 7. The values are averages of n = 3, except for day 7 of US-05 at 37 °C (n = 2). Three incubation temperatures were used; 22 °C, 30 °C and 37 °C.*

The content of ethyl octanoate increased from day 0 to day 2 and from day 2 to day 7 for all of the cultures at all incubation temperatures. The highest content was observed on day 7 for Gamlegrua at 30 °C with 0.047 ppm.

4.3.4.2 *Higher alcohols*

Figure 4.16 to Figure 4.20 illustrates the content and development of five higher alcohols in samples of three kveiks and one commercial yeast incubated at three different temperatures. The higher alcohols included in this study are 1-propanol, 2-methyl-1-propanol, 3-methyl-1-butanol, 2-methyl-1-butanol and 2-hexanol.

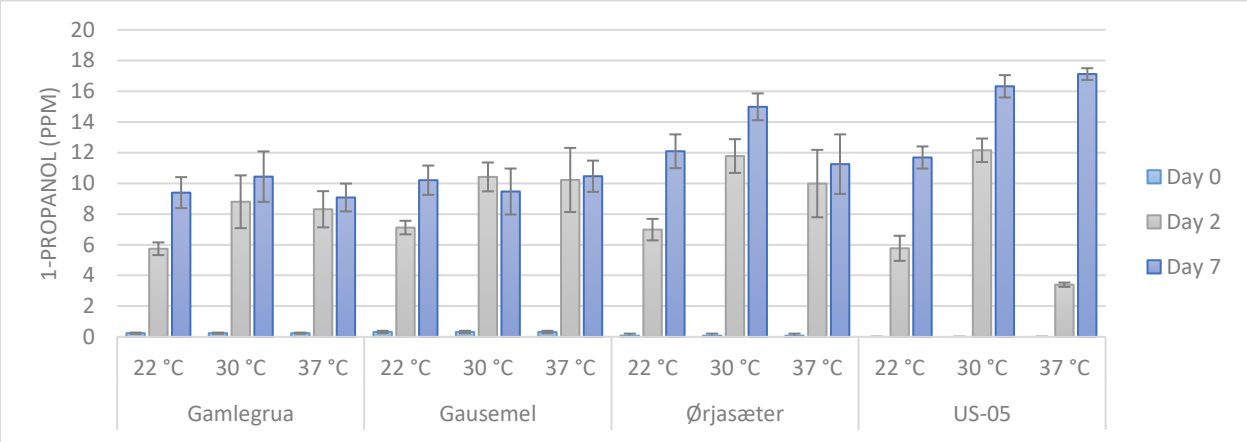

Figure 4.16. *1-propanol results from HSGC analysis in the growth and metabolism experiment, with standard deviation. The kveiks Gamlegrua, Gausemel and Ørjasæter were analyzed, along with the commercial yeast SafAle US-05. Measurements were taken on day 0, day 2 and day 7. The values are averages of n = 3, except for the 7 day measurement of US-05 at 37 °C (n = 2). Three incubation temperatures were used; 22 °C, 30 °C and 37 °C.*

The content of 1-propanol increased from day 0 to day 2 in all of the samples. All of the samples, except for Gausemel at 30 °C, had an increase of 1-propanol from day 2 to day 7. On day 7, the largest content was found in US-05 at 37 °C with 17.1 ppm, and the day 7 content in the kveiks at 37 °C was in the range of 9.1-11.3 ppm.

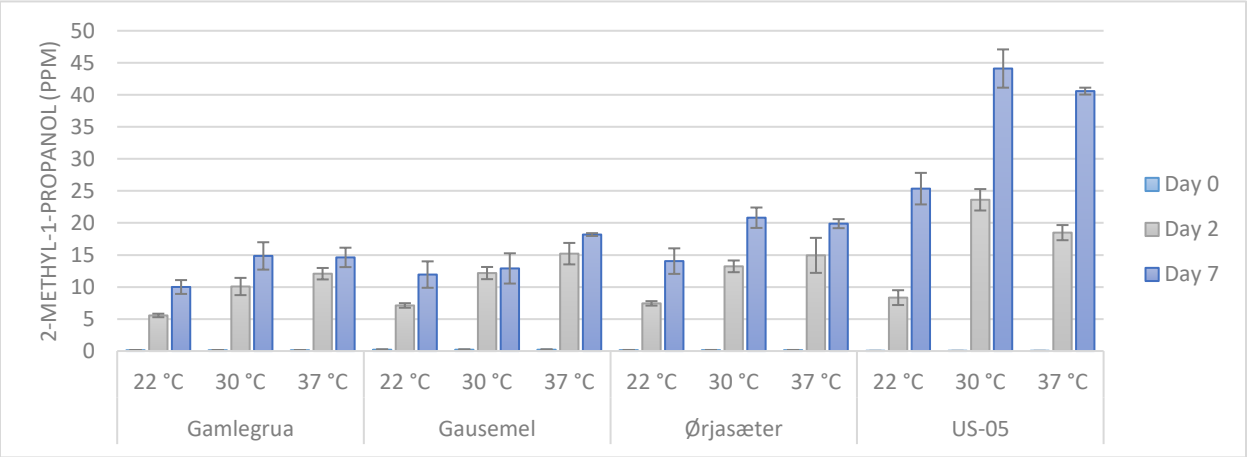

Figure 4.17. *2-methyl-1-propanol results from HSGC analysis in the growth and metabolism experiment, with standard deviation. The kveiks Gamlegrua, Gausemel and Ørjasæter were analyzed, along with the commercial yeast SafAle US-05. Measurements were taken on day 0, day 2 and day 7. The values are averages of n = 3, except for the 7 day measurement of US-05 at 37 °C (n = 2). Three incubation temperatures were used; 22 °C, 30 °C and 37 °C.*

The content of 2-methyl-1-propanol increased from day 0 to day 2, and from day 2 to day 7 for all of the samples. The control US-05 had a larger content of this alcohol at all incubation temperatures compared to the kveiks, with contents in the range of 25.4-44.1 ppm, and the largest content was found at 30 °C (44.1 ppm). The content in the kveiks on day 7 at the three temperatures ranged from 10.0 ppm to 20.8 ppm.

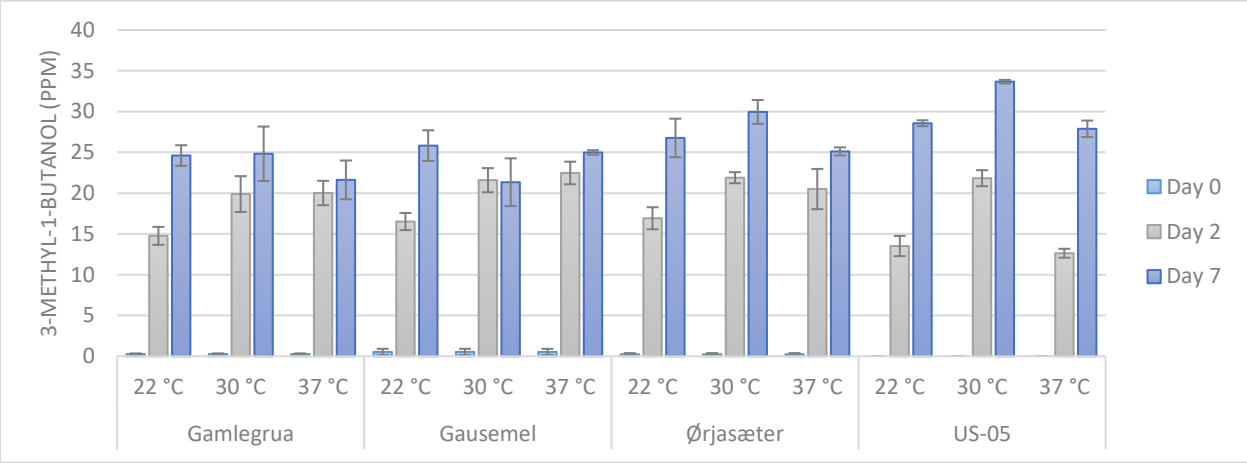

Figure 4.18. *3-methyl-1-butanol results from HSGC analysis in the growth and metabolism experiment, with standard deviation. The kveiks Gamlegrua, Gausemel and Ørjasæter were analyzed, along with the commercial yeast SafAle US-05. Measurements were taken on day 0, day 2 and day 7. The values are averages of n = 3, except for the 7 day measurement of US-05 at 37 °C (n = 2). Three incubation temperatures were used; 22 °C, 30 °C and 37 °C.*

The content of 3-methyl-1-butanol increased from day 0 to day 2 and from day 2 to day 7 for all of the samples except for Gausemel at 30 °C. The largest content was found in US-05 on day 7 at 30 °C with 33.7 ppm. The lowest content on day 7, by a small margin, was found in Gausemel at 30 °C with 21.3 ppm.

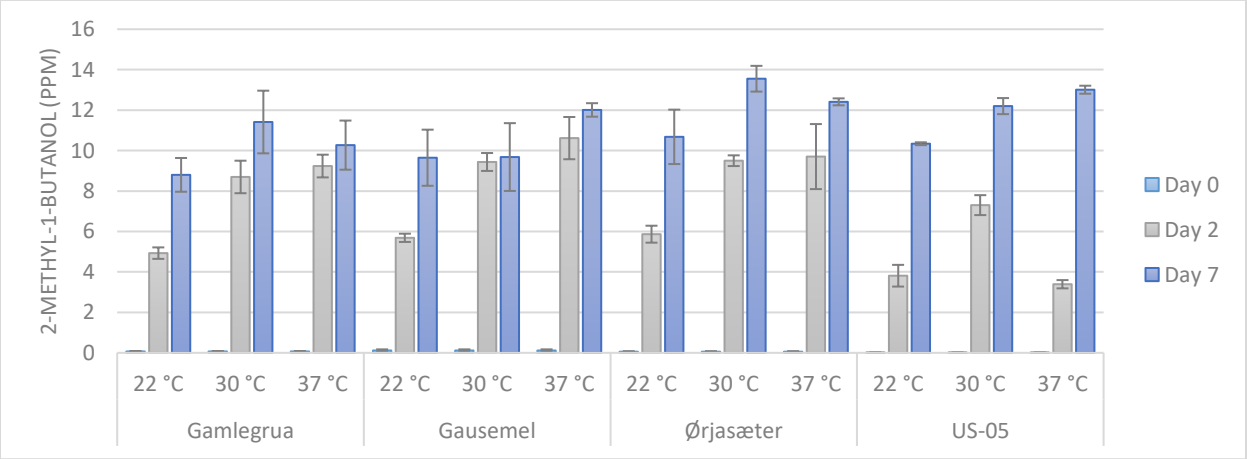

Figure 4.19. *2-methyl-1-butanol results from HSGC analysis in the growth and metabolism experiment, with standard deviation. The kveiks Gamlegrua, Gausemel and Ørjasæter were analyzed, along with the commercial yeast SafAle US-05. Measurements were taken on day 0, day 2 and day 7. The values are averages of n = 3, except for the 7 day measurement of US-05 at 37 °C (n = 2). Three incubation temperatures were used; 22 °C, 30 °C and 37 °C.*

The content of 2-methyl-1-butanol increased from day 0 to day 2 and from day 2 to day 7 in all of the samples and at all of the temperatures. The largest content on day 7, by a small margin, was found in Ørjasæter at 30 °C with 13.6 ppm. The lowest content on day 7 for each culture was found at 22 °C, with contents ranging from 8.8 to 10.7 ppm.

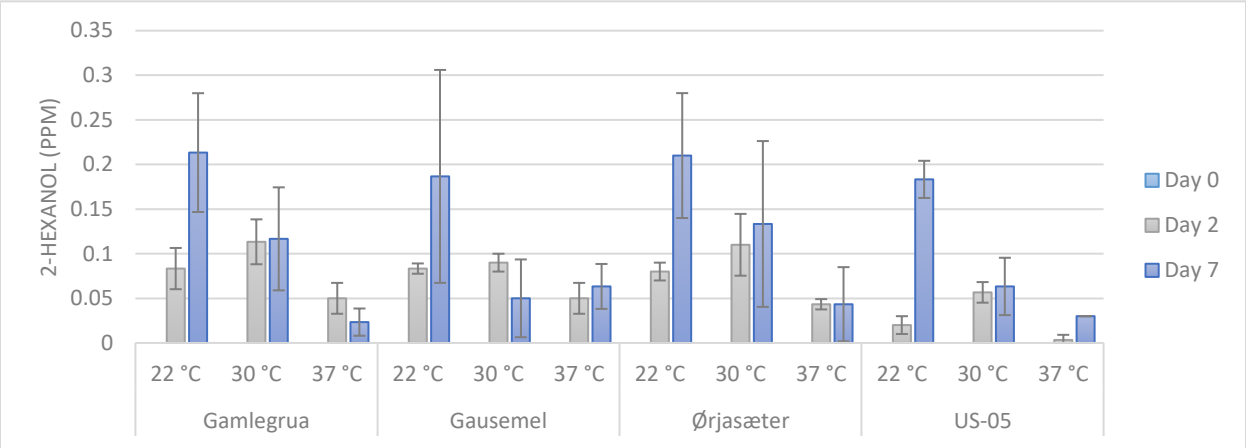

Figure 4.20. *2-hexanol results from HSGC analysis in the growth and metabolism experiment, with standard deviation. The kveiks Gamlegrua, Gausemel and Ørjasæter were analyzed, along with the commercial yeast SafAle US-05. Measurements were taken on day 0, day 2 and day 7. The values are averages of n = 3, except for the 7 day measurement of US-05 at 37 °C (n = 2). Three incubation temperatures were used; 22 °C, 30 °C and 37 °C.*

The content of 2-hexanol was higher at 22 °C on day 7 and decreased with an increase in the incubation temperature. The exception to this is Gausemel, who had a lower content on day 7 at 30 °C than at 37 °C. The highest contents were found in Gamlegrua and Ørjasæter at 22 °C on day 7 with 0.21 ppm.

4.3.4.3 Aldehydes

Figure 4.21 to Figure 4.24 illustrates the content and development of four aldehydes in samples of three kveiks and one commercial yeast incubated at three different temperatures. The aldehydes included in this study are acetaldehyde, 2-methyl-propanal, 3-methyl-butanal and 2-methyl-butanal. Hexanal was found in quantities of 0.00 to 0.01 ppm and the differences between the cultures and temperatures were very small. This aromatic component is therefore not referred to further in this section.

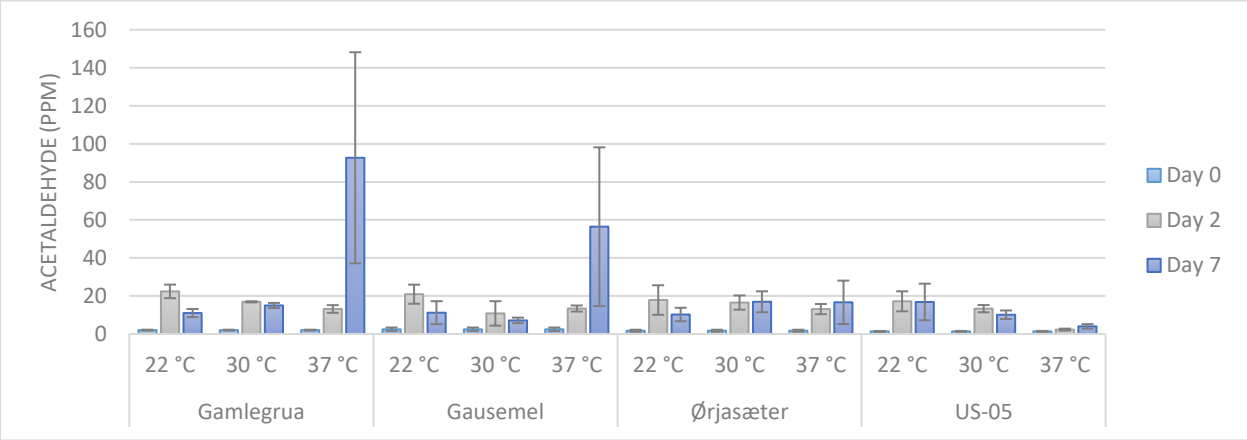

Figure 4.21. *Acetaldehyde results from HSGC analysis in the growth and metabolism experiment, with standard deviation. The kveiks Gamlegrua, Gausemel and Ørjasæter were analyzed, along with the commercial yeast SafAle US-05. Measurements were taken on day 0, day 2 and day 7. The values are averages of n = 3, except for the 7 day measurement of US-05 at 37 °C (n = 2). Three incubation temperatures were used; 22 °C, 30 °C and 37 °C.*

The largest content of acetaldehyde was found in Gamlegrua and Gausemel incubated at 37 °C at day 7 with 92.7 ppm and 56.4 ppm, respectively. The other temperatures and cultures had contents of the aldehyde at day 7 in the range 4.0-17.0 ppm.

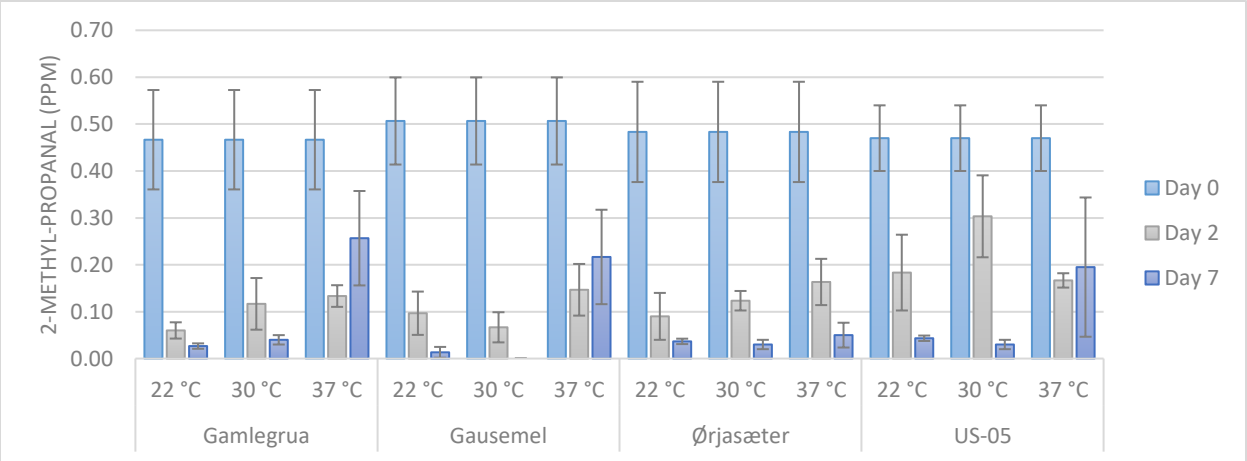

Figure 4.22*. 2-methyl-propanal results from HSGC analysis in the growth and metabolism experiment, with standard deviation. The kveiks Gamlegrua, Gausemel and Ørjasæter were analyzed, along with the commercial yeast SafAle US-05. Measurements were taken on day 0, day 2 and day 7. The values are averages of n = 3, except for the 7 day measurement of US-05 at 37 °C (n = 2). Three incubation temperatures were used; 22 °C, 30 °C and 37 °C.*

The content of 2-methyl-propanal decreased in all of the samples from 0.47-0.51 ppm at day 0 to 0.06-0.3 ppm at day 2, but it increased from day 2 to day 7 in the samples incubated at 37 °C of Gamlegrua (0.26 ppm), Gausemel (0.22 ppm) and US-05 (0.20 ppm).

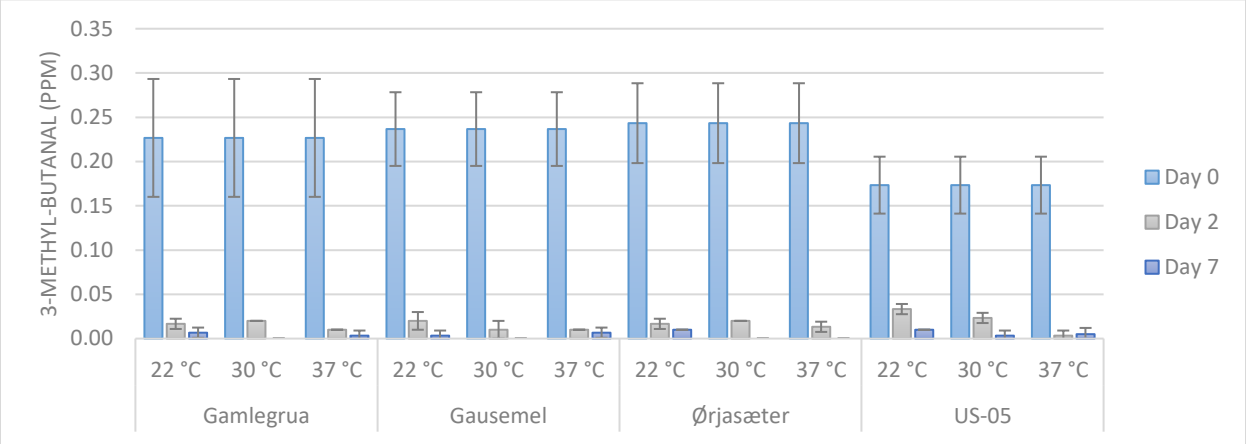

Figure 4.23. *3-methyl-butanal results from HSGC analysis in the growth and metabolism experiment, with standard deviation. The kveiks Gamlegrua, Gausemel and Ørjasæter were analyzed, along with the commercial yeast SafAle US-05. Measurements were taken on day 0, day 2 and day 7. The measurements were n = 3, except for the 7 day measurement of US-05 at 37 °C (n = 2). Three incubation temperatures were used; 22 °C, 30 °C and 37 °C.*

The content of 3-methyl-butanal decreased sharply in all of the samples from 0.24-0.17 ppm to 0.003-0.03 ppm at all temperatures from day 0 to day 2, and it decreased further from day 2 to day 7 to levels around 0.00-0.01 ppm.

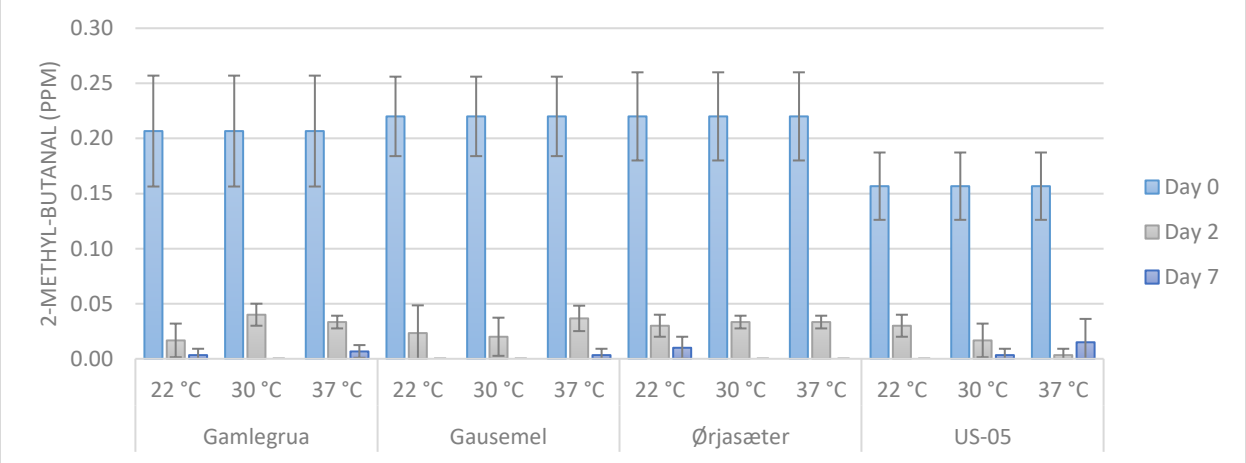

Figure 4.24. *2-methyl-butanal results from HSGC analysis in the growth and metabolism experiment, with standard deviation. The kveiks Gamlegrua, Gausemel and Ørjasæter were analyzed, along with the commercial yeast SafAle US-05. Measurements were taken on day 0, day 2 and day 7. The measurements were n = 3, except for the 7 day measurement of US-05 at 37 °C (n = 2). Three incubation temperatures were used; 22 °C, 30 °C and 37 °C.*

The content of 2-methyl-butanal in all of the samples and at all temperatures decreased drastically from day 0 to day 2. In many cases the content of the aldehyde was not detected in the sample on day 7. The only case where there was an increase from day 2 to day 7 was for US-05 at 37 °C.

4.3.4.4 Ketones

Figure 4.25 and Figure 4.26 illustrates the content and development of two ketones in samples of three kveiks and one commercial yeast incubated at three different temperatures. The ketones included in this study are acetone and diacetyl.

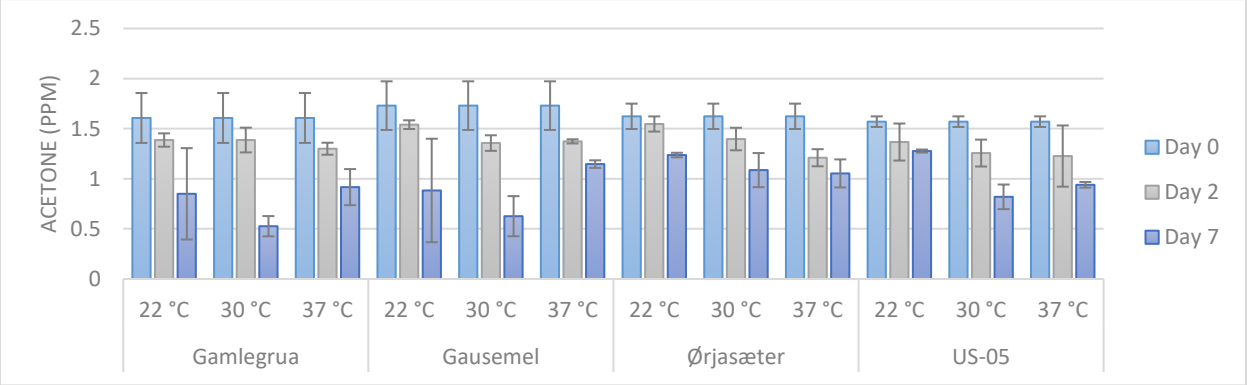

Figure 4.25. *Acetone results from HSGC analysis in the growth and metabolism experiment, with standard deviation. The kveiks Gamlegrua, Gausemel and Ørjasæter were analyzed, along with the commercial yeast SafAle US-05. Measurements were taken on day 0, day 2 and day 7. The values are averages of n = 3, except for the 7 day measurement of US-05 at 37 °C (n = 2). Three incubation temperatures were used; 22 °C, 30 °C and 37 °C.*

The content of acetone in all samples and at all temperatures was descending from day 0 to day 7. The largest decrease in content for Gamlegrua, Gausemel and US-05 was seen in the samples at 30 °C, and the largest decrease in content for Ørjasæter, by a small margin, was seen in the samples at 37 °C.

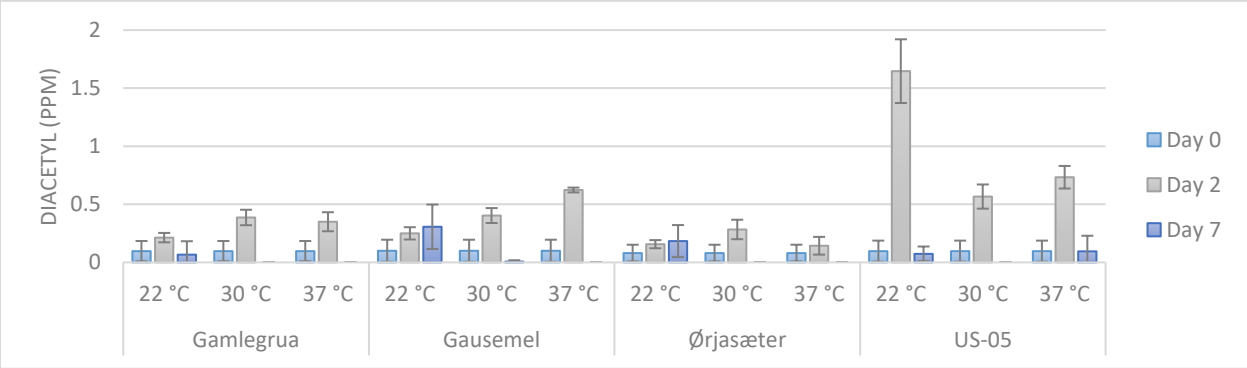

Figure 4.26. *Diacetyl results from HSGC analysis in the growth and metabolism experiment, with standard deviation. The kveiks Gamlegrua, Gausemel and Ørjasæter were analyzed, along with the commercial yeast SafAle US-05. Measurements were taken on day 0, day 2 and day 7. The values are averages of n = 3, except for the 7 day measurement of US-05 at 37 °C (n = 2). Three incubation temperatures were used; 22 °C, 30 °C and 37 °C.*

For the majority of the samples the diacetyl content spiked on day 2, ranging from 0.14 to 1.65 ppm, and decreased to little or no content on day 7. Gausemel and Ørjasæter at 22 °C had an increase in diacetyl content from day 2 to day 7. The highest level was measured on day 2 at 22 °C for US-05 with 1.65 ppm. The highest level of diacetyl measured in kveik was on day 2 at 37 °C for Gausemel with 0.62 ppm.

4.4 Microbiota analysis

4.4.1 Prokaryote

The result from 16S rRNA bacterial microbiota assay is illustrated in Figure 4.27. The raw data for the analysis can be found in appendix D-2.

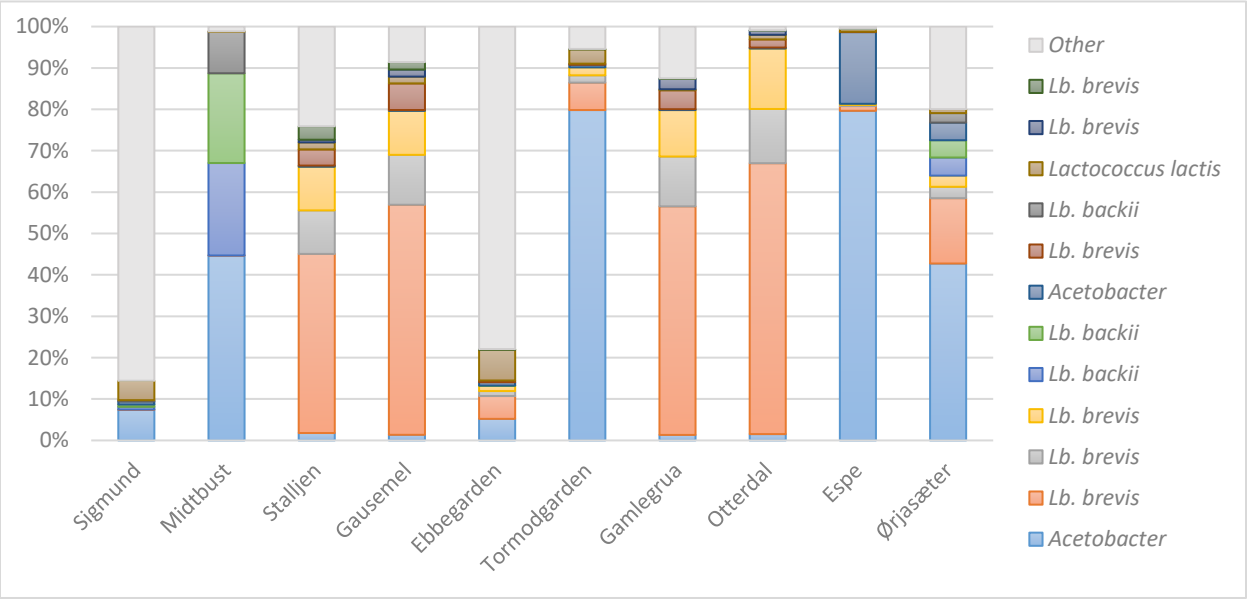

***Figure 4.27**. Bar chart showing the relative abundance of bacterial species identified from 16S rRNA microbiota assay. The abundance of the most found bacteria in the samples are stated by genus- and species name, the rest falls under the category "other". The difference between the species with the same names shown in different colors is that they are sequence variants of the species.*

One sequence variant of *Acetobacter* spp. is found in a high abundance in four kveiks, where it accounts for 80% of the bacterial microbiota of Tormodgarden and Espe, and approximately 45% of Midtbust and Ørjasæter. In addition to this, approximately 18% of the remaining bacterial microbiota in Espe is another sequence variant of *Acetobacter*.

For Sigmund and Ebbegarden, the bacteria found in the largest quanta goes under the category “other”.

Stalljen, Gausemel, Gamlegrua and Otterdal all contain the same four sequence variants of *Lb. brevis*. The total content of these vary in levels of approximately 70-95% out of the total amount of bacteria found in these samples. Tormodgarden and Ørjasæter also contains *Lb. brevis*, which accounts for 10% and 20%, respectively, of the bacterial microbiota in these kveiks.

The bacterial microbiota of Midtbust consists of approximately 55% *Lb. backii*. Three sequence variants of this bacteria was found in large quantities in the kveik sample. Approximately 10% of Ørjasæter consists of *Lb. backii*.

4.4.2 Eukaryote

The ITS microbiota result is presented in Figure 4.28. Raw data can be found in appendix D-3. The organism found in the ITS microbiota analysis is *Saccharomyces cerevisiae*. The *S. cerevisiae* illustrated in different colors have different sequences, and they are therefore different sequence variants.

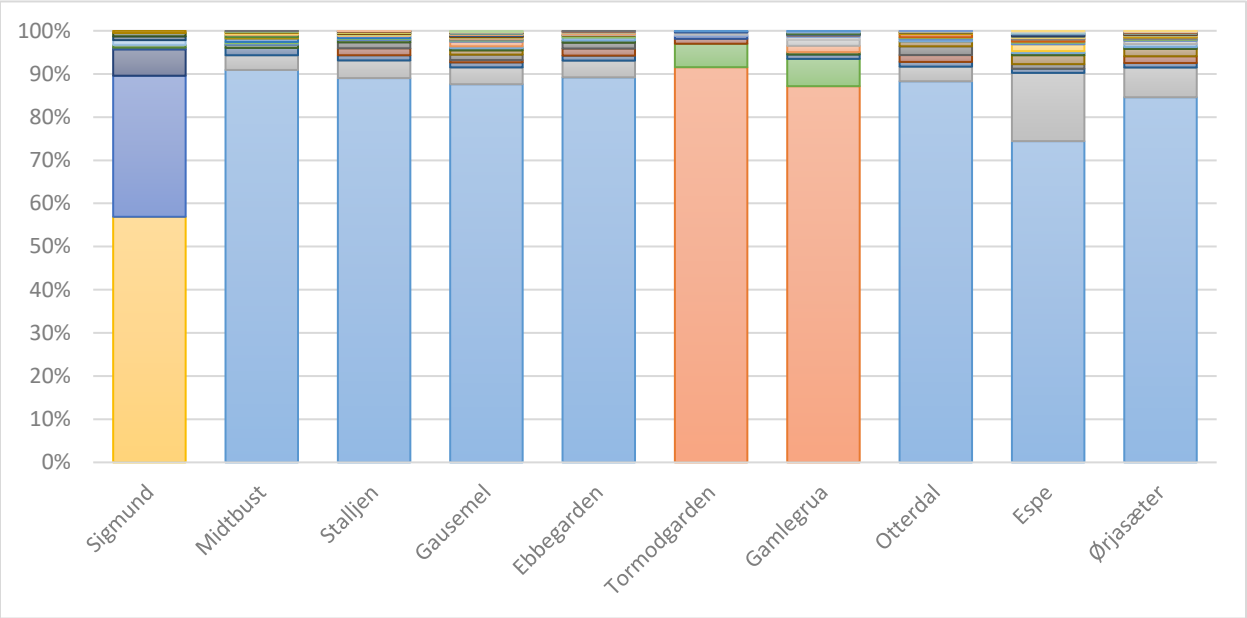

Figure 4.28. Bar chart showing the relative abundance of *Saccharomyces cerevisiae* identified from ITS microbiota assay. The different colors in the bar chart represents different sequence variants of *S. cerevisiae*.

The results from the ITS microbiota analysis suggests that Midtbust, Stalljen, Gausemel, Ebbegarden, Otterdal, Espe and Ørjasæter in large part consists of the same sequence variants of *S. cerevisiae* (light blue and grey). Sigmund stands out from the rest of the analyzed kveik, as it consists of two sequence variants of *S. cerevisiae* that is not found in the other nine kveiks. Tormodgarden and Gamlegrua mainly consists of two sequence variants of *S. cerevisiae* (red and green). These two sequence variants were not found in the other eight kveiks.

The total number of sequence variants of *S. cerevisiae* found in the kveik samples, from the lowest to the highest, are: Tormodgarden (8), Sigmund (9), Otterdal (14), Midtbust (15), Gamlegrua and Ebbegarden (16), Stalljen (21), Espe (23), Ørjasæter (25) and Gausemel (28).

The results from the ITS and 16S RNA extractions were measured using Nanodrop. These measurements can be found in appendix D-1.

4.5 Modern pilot-scale brewing with kveik

In the pilot-scale brewing, 11 different types of beer were brewed. Figure 4.29 illustrates the average fermentation time for each type of brew along with the standard deviation. The calculated p-values are listed in Table 4.10.

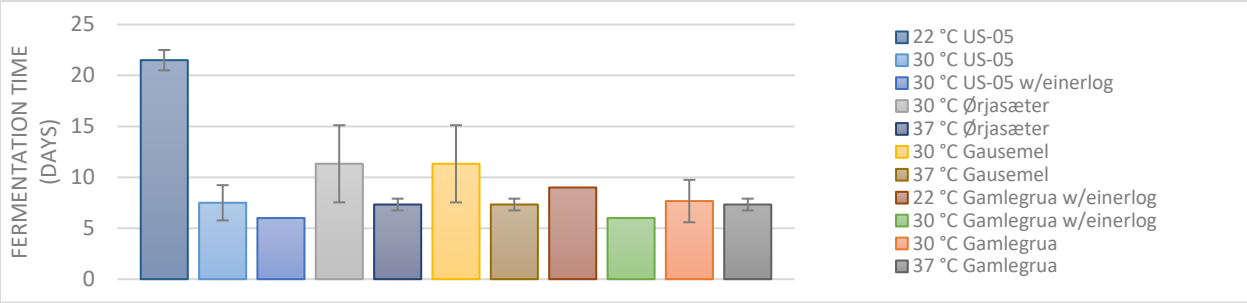

Figure 4.29. *Bar chart showing the average number of fermentation days in the pilot-scale brewing for each type of brew, with standard deviation.*

Two of the ales fermented at 30 °C with einerlog, i.e. Gamlegrua and US-05, had the shortest fermentation time with 6 days, and US-05 fermented at 22 °C had the longest fermentation time with 22 days.

An alcolyzer was used to analyze the ale types before and after fermentation, and after maturation. The results from the measurements taken before (original gravity) and after fermentation (final gravity, alcohol, non-fermented substances and attenuation) are shown in Table 4.9, along with the standard deviation. The calculated p-values for attenuation and final gravity are listed in Table 4.10.

Table 4.9. *Resulting measurements from alcolyzer Anton Paar DM 4500 M, including yeast/kveik type, the use of einerlog and fermentation. The measurements were taken from wort (original gravity) and after fermentation, secondary fermentation and 2 weeks of cold storage (final gravity, alcohol (%ABV), non-fermented substances (Ea % w/w) and attenuation (ADF % w/w)). The values are averages of duplicates/triplicates/quadruplicates. The SD states the standard deviation for each value.*

Einerlog (+/-)	Yeast/kveik	Fermentation temperature (°C)	Original Gravity ± SD	Final Gravity ± SD	Alcohol ± SD (%ABV)	Ea ± SD (% w/w)	ADF ± SD (% w/w)
-	Gamlegrua	30	1.076^{b} ± 8	1.017^{b} ± 3	7.9^{b} ± 0.2	4.7^{b} ± 0.8	75.1^{b} ± 2.9
-		37	1.076^{b} ± 3	1.018^{b} ± 6	7.9^{b} ± 0.2	5.0^{b} ± 1.4	73.9^{b} ± 5.8
-	Gausemel	30	1.076^{b} ± 8	1.015^{b} ± 2	8.4^{b} ± 0.8	4.2^{b} ± 0.5	78.1^{b} ± 0.9
-		37	1.076^{b} ± 3	1.017^{b} ± 5	8.2^{b} ± 0.2	4.6^{b} ± 1.2	76.1^{b} ± 5.1
-	Ørjasæter	30	1.076^{b} ± 8	1.015^{b} ± 2	8.3^{b} ± 0.9	4.2^{b} ± 0.6	78.1^{b} ± 0.8
-		37	1.076^{b} ± 3	1.019^{b} ± 6	7.8^{b} ± 0.2	5.2^{b} ± 1.3	73.3^{b} ± 5.5
-	US-05	22	1.076^{b} ± 3	1.011^{c} ± 1	8.6^{c} ± 0.6	3.0^{c} ± 0.2	83.7^{c} ± 1.4
-		30	1.074^{b} ± 8	1.013^{c} ± 4	8.4^{c} ± 0.7	3.5^{c} ± 0.9	81.4^{c} ± 2.9
+		30	1.071^{d} ± 0	1.013^{b} ± 0	7.9^{b} ± 0.1	3.6^{b} ± 0.1	80.2^{b} ± 0.4
+	Gamlegrua	22	1.083^{d} ± 0	1.019^{b} ± 3	8.7^{b} ± 0.4	5.1^{b} ± 0.7	75.2^{b} ± 3.6
+		30	1.071^{d} ± 0	1.016^{a} ± 1	7.4^{a} ± 0.1	4.4^{a} ± 0.3	73.6^{a} ± 1.3

[a] average of duplicate. [b] average of triplicate. [c] average of quadruplicate. [d] singular value.

US-05 fermented at 22 °C had the lowest final gravity (1.011) and the lowest amount of non-fermented substances (3.0 %). The kveiks Ørjasæter (37 °C) and Gamlegrua with einerlog (22 °C) had the highest final gravity of the ales with 1.019. The highest level of alcohol was found in Gamlegrua with einerlog fermented at 22 °C with 8.7 % ABV, and the lowest alcohol level was found in Gamlegrua with einerlog fermented at 30 °C with 7.4 % ABV. The highest attenuation was found in US-05 fermented at 22 °C, and in general the US-05 yeast had a higher degree of attenuation than the three kveiks.

Table 4.10. *Statistical significance for fermentation days, attenuation and final gravity after maturation for all yeast/kveik cultures in the pilot-scale brewing experiment at different temperatures. A p-value < 0.05 indicates a significant statistical difference in the compared values. A 95% confidence interval is used. The statistical tools ANOVA and Tukey's method in RStudio were used to calculate the statistical significance. The p-values of comparisons found to be statistically significant are highlighted in bold letters.*

	Fermentation days (p-value)		**Attenuation (p-value)**		**Final gravity (p-value)**	
Comparison:	**Individual**	**Combined**	**Individual**	**Combined**	**Individual**	**Combined**
Gamlegrua w/einerlog vs. Gamlegrua	0.999997		0.9999794		0.9999342	
US-05 w/einerlog vs. Gausemel	0.8841903		0.7719913		0.7295133	
US-05 w/einerlog vs. Gamlegrua	0.9962542		0.182433		0.3553192	
Gausemel vs. Gamlegrua w/einerlog	0.9634502		0.6929673		0.9948561	
US-05 vs. Gamlegrua w/einerlog	0.0644779		**0.0016071**		0.0655397	
US-05 w/einerlog vs. Gamlegrua w/einerlog	0.9988901		0.1626318		0.4920910	
US-05 w/einerlog vs. Ørjasæter	0.8841903		0.4104776		0.5159979	
US-05 w/einerlog vs. US-05	0.072972	**0.026214**	0.8928991	**0.0003978**	0.9936016	**0.01301**
Ørjasæter vs. Gamlegrua w/einerlog	0.9634502		0.9729734		0.9999970	
Gausemel vs. Gamlegrua	0.9765728		0.7537192		0.9705546	
US-05 vs. Gamlegrua	0.059613		**0.0013747**		**0.0264034**	
Ørjasæter vs. Gausemel	1.0000000		0.9753536		0.9979890	
US-05 vs. Ørjasæter	0.2707456		**0.0075458**		0.0606988	
Ørjasæter vs. Gamlegrua	0.9765728		0.9890226		0.9993126	
US-05 vs. Gausemel	0.2707456		**0.0499569**		0.1549036	
22 °C vs. 30 °C	**0.0000134**		0.2863472		0.7176331	
30 °C vs. 37 °C	0.7897306	**0.000006627**	0.0923159	**0.01476**	0.0636694	**0.03153**
22 °C vs. 37 °C	**0.0000185**		**0.0130388**		**0.0461680**	

There was found to be significant differences in the number of fermentation days between the temperatures 30 °C and 22 °C, and between 37 °C and 22 °C. No significant difference was found between the fermentation temperatures 37 °C and 30 °C. There was found a significant difference in the amount of fermentation days between the different types of kveik/yeast using ANOVA, but no significant difference was found using Tukey's method.

The difference in attenuation between the cultures was found to be significant in the cases of US-05 vs. Gamlegrua, US-05 vs. Gamlegrua w/einerlog, US-05 vs. Gausemel and US-05 vs. Ørjasæter. The difference between the temperatures 37 °C and 22 °C was also found to be significant.

When analyzing the final gravity values of the cultures up against each other, a significant difference was found between US-05 and Gamlegrua. A significant difference was also found between the temperatures 37 °C and 22 °C.

The flocculation of two of the beers can be seen in Figure 4.30. The flocculation of US-05 at the bottom of the fermentation bucket that had been fermented at 22 °C is depicted to the left, and the flocculation of Gamlegrua that had been fermented at 37 °C can be seen to the right.

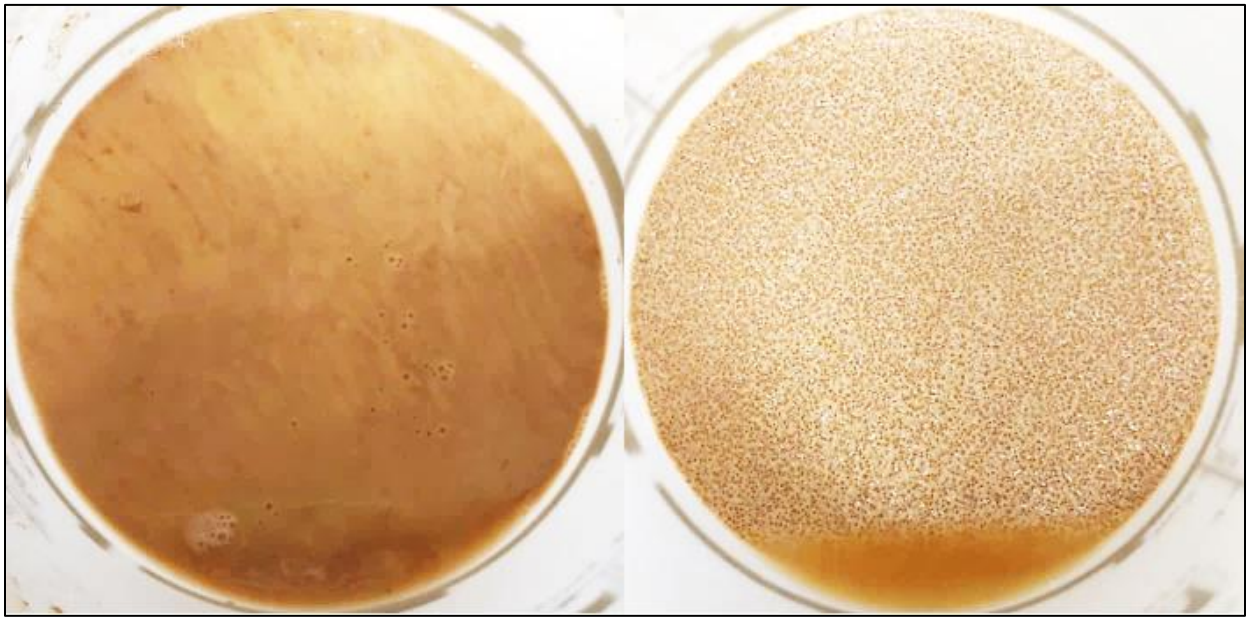

Figure 4.30. *Flocculation of SafAle US-05 fermented at 22 °C (to the left) and Gamlegrua fermented at 37 °C (to the right).*

The flocculation of the yeast in the beer that had been fermented using kveik was better than the flocculation in the US-05 fermented beer.

4.5.1 Volatile components

A selection of volatile components in all the different beer types in the pilot-scale brewing project were analyzed using HSGC at three stages of the process: the wort, the beer after fermentation and the finished product after two weeks of secondary fermentation and two weeks of cold storage. Only the results from the measurements of the finished products are mentioned, along with a comparison of the volatile components in the einerlog, the wort and the wort with einerlog. The results for the components with values above sensory threshold are summarized, illustrated and compared to one another in these sections. The first section compares the samples made with einerlog with the samples made without einerlog, where the same fermentation temperatures and the same cultures were used. The second section compares all samples produced without einerlog. The third section shows the statistical significance of the differences between the brews, where the cultures and the temperatures are compared to each other.

4.5.1.1 Brews with einerlog

The volatile components ethyl acetate, isoamyl acetate, ethyl hexanoate, 3-methyl-1-butanol, dimethyl sulphide, acetaldehyde, 2-methyl-propanal and diacetyl were measured at levels higher than their respective sensory threshold values. These components are illustrated in this section, along with their standard deviation values. The statistical significances were analyzed for each of the volatile components.

Figure 4.31 illustrates the results from the analysis of volatile components of wort with and without einerlog, along with results from the einerlog used in the brewing. Only the components with values above the sensory threshold for at least one of the three samples are included in the figure.

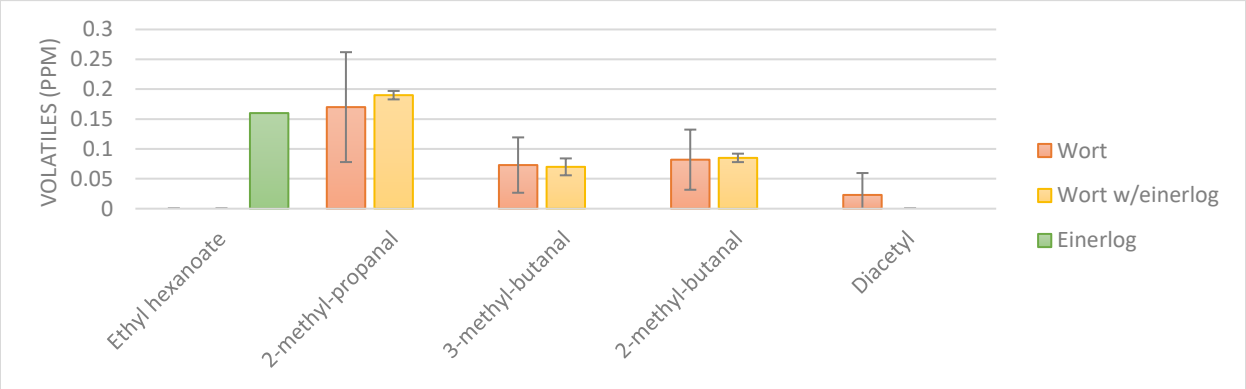

Figure 4.31. *Components from wort with and without einerlog, as well as from einerlog used in parts of the brewing experiment, with at least one value above sensory threshold for each of the respective components. The einerlog values are singular (n = 1), the wort w/einerlog values are averages of n = 2 and the values for wort without einerlog are averages of n = 6. The standard deviation is shown where it applies.*

The content of ethyl hexanoate in einerlog was 0.16 ppm which is above the threshold of 0.005 ppm. The worts with and without einerlog had contents of 2-methyl-propanal (threshold: 0.0023 ppm), 3-methyl-butanal (threshold: 0.032 ppm) and 2-methyl-butanal (threshold: 0.032 ppm) above the thresholds. The wort without einerlog had a content of diacetyl above the threshold of 0.017 ppm with 0.023 ppm.

4.5.1.1.1 Esters

The esters ethyl acetate, isoamyl acetate and ethyl hexanoate were found in levels above the sensory threshold levels for these components in all of the samples. The results are illustrated in Figure 4.32, Figure 4.33 and Figure 4.34.

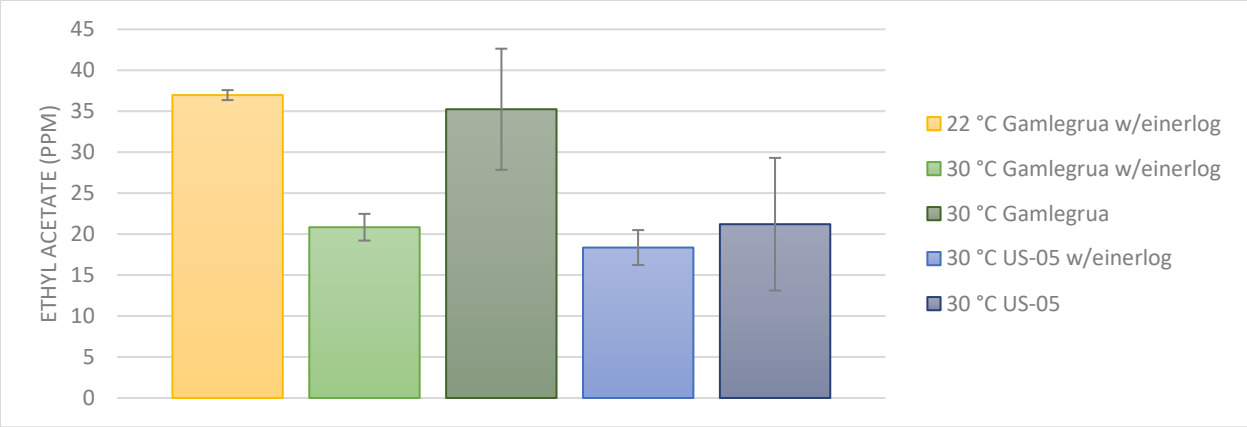

Figure 4.32. *Ethyl acetate results from HSGC analysis for brews with einerlog and their counterparts without einerlog, with standard deviation. The sensory threshold for ethyl acetate is 5 ppm. The values are averages of n = 3, except for Gamlegrua w/ einerlog at 22 °C (n = 2) and US-05 at 30 °C (n = 4).*

The lowest content of ethyl acetate was found in US-05 with einerlog fermented at 30 °C with 18.4 ppm and the highest content was found in Gamlegrua with einerlog fermented at 22 °C with 37.0 ppm. Brewing with einerlog at 30 °C resulted in a lower content of ethyl acetate for both Gamlegrua and US-05 compared to brewing without einerlog at the same temperature.

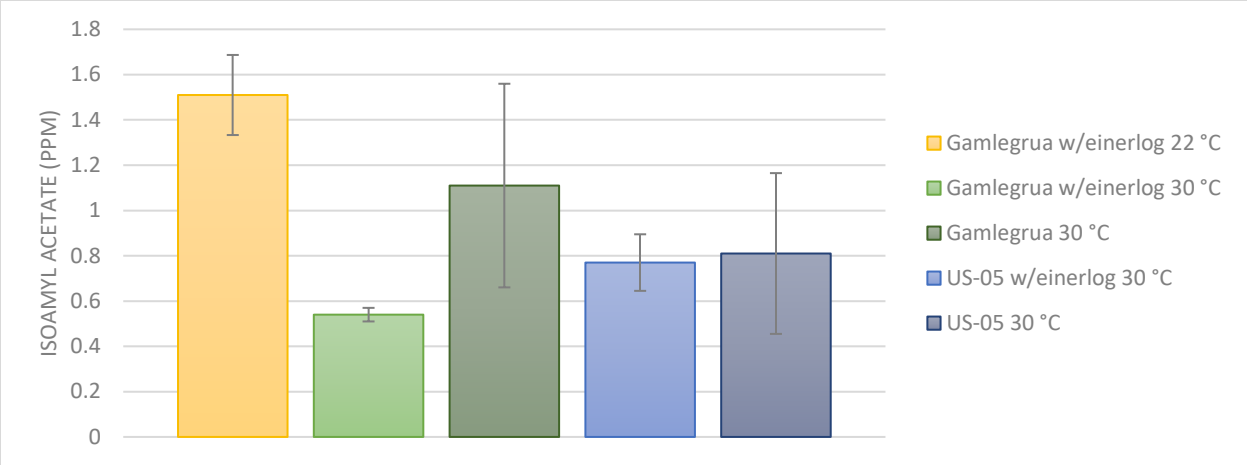

Figure 4.33. *Isoamyl acetate results from HSGC analysis for brews with einerlog and their counterparts without einerlog, with standard deviation. The sensory threshold for isoamyl acetate is 0.03 ppm. The values are averages of n = 3, except for Gamlegrua w/ einerlog at 22 °C (n = 2) and US-05 at 30 °C (n = 4).*

The lowest content of isoamyl acetate was found in Gamlegrua with einerlog fermented at 30 °C with 0.54 ppm, and the highest content was found in Gamlegrua with einerlog fermented at 22 °C with 1.51 ppm. Brewing with einerlog at 30 °C resulted in a considerably lower content of isoamyl acetate in Gamlegrua and a marginally lower content in US-05 compared to brewing without einerlog at 30 °C.

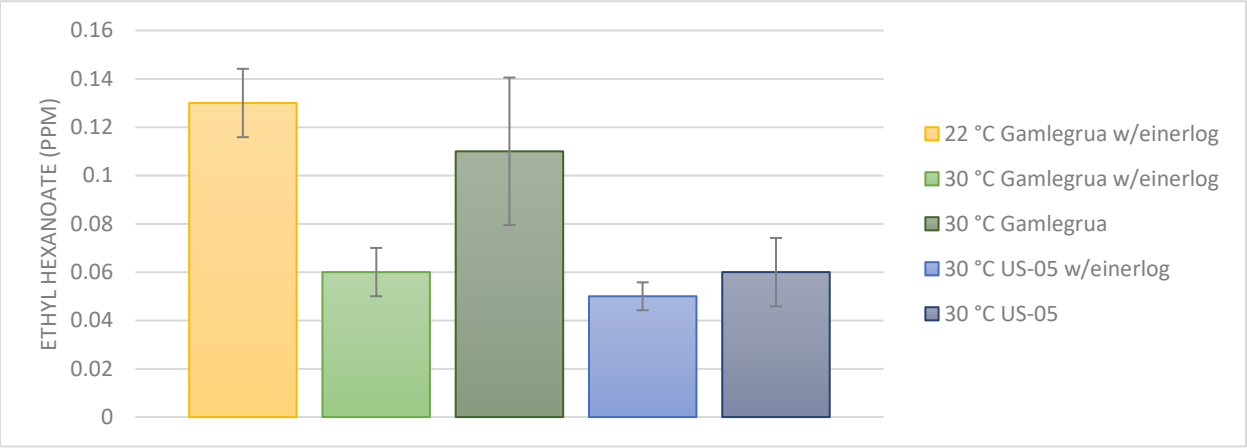

Figure 4.34. *Ethyl hexanoate results from HSGC analysis for brews with einerlog and their counterparts without einerlog, with standard deviation. The sensory threshold for ethyl hexanoate is 0.005 ppm. The values are averages of n = 3, except for Gamlegrua w/ einerlog at 22 °C (n = 2) and US-05 at 30 °C (n = 4).*

The lowest content of ethyl hexanoate was found in US-05 with einerlog fermented at 30 °C with 0.05 ppm and the highest content was found in Gamlegrua with einerlog fermented at 22 °C with 0.13 ppm. Brewing with einerlog at 30 °C resulted in a lower content of ethyl hexanoate for both Gamlegrua and US-05 compared to brewing without einerlog at the same temperature.

4.5.1.1.2 Ketones

The diacetyl results are illustrated in Figure 4.35.

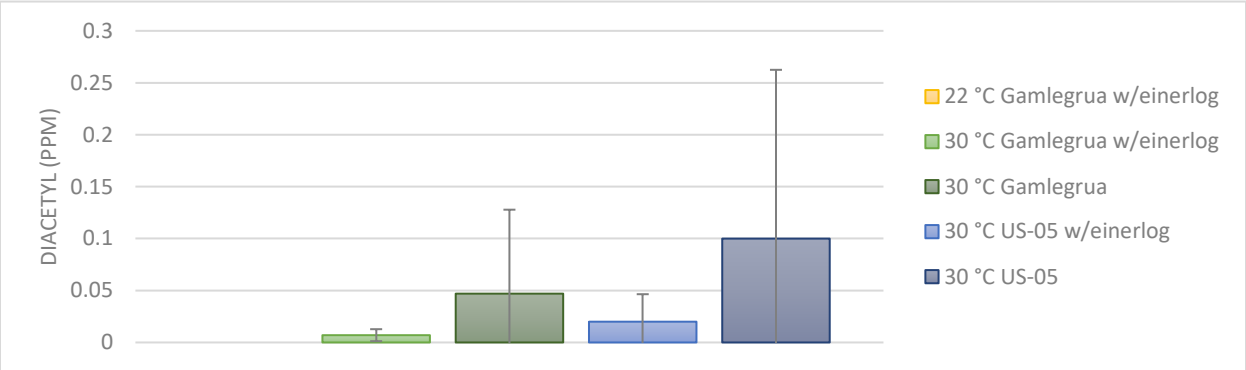

Figure 4.35. *Diacetyl results from HSGC analysis for brews with einerlog and their counterparts without einerlog, with standard deviation. The sensory threshold for diacetyl is 0.017 ppm. The values are averages of n = 3, except for Gamlegrua w/ einerlog at 22 °C (n = 2) and US-05 at 30 °C (n = 4).*

Three beer types at 30 °C, US-05 w/einerlog, Gamlegrua and US-05, had average contents of diacetyl measured to be higher than the sensory threshold of 0.017 ppm. The lowest content was found in Gamlegrua with einerlog fermented at 22 °C and the highest content was found in US-05 at 30 °C with 0.1 ppm. The beers without einerlog had higher values than the beers with einerlog.

4.5.1.1.3 Aldehydes

The acetaldehyde and 2-methyl-propanal results are illustrated in Figure 4.36 and Figure 4.37.

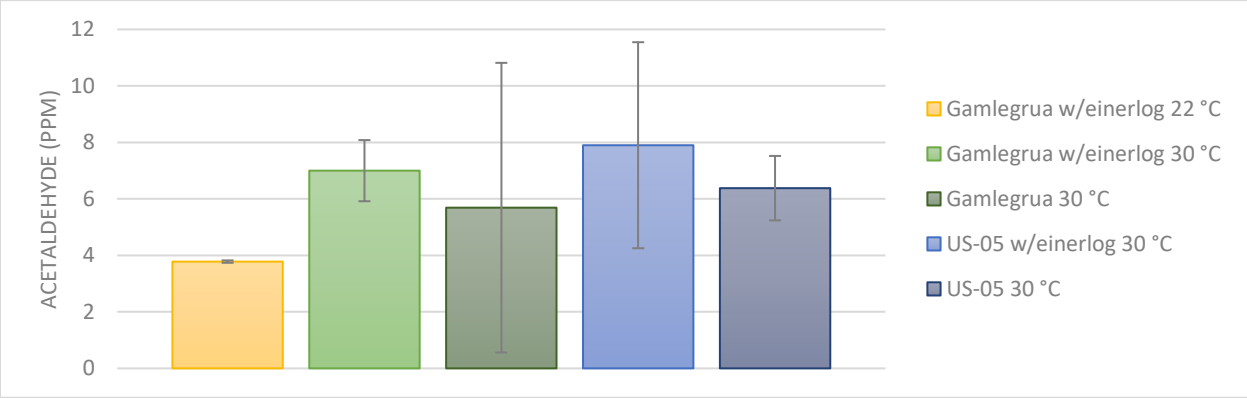

Figure 4.36. *Acetaldehyde results from HSGC analysis for brews with einerlog and their counterparts without einerlog, with standard deviation. The sensory threshold for acetaldehyde is 1.114 ppm. The values are averages of n = 3, except for Gamlegrua w/ einerlog at 22 °C (n = 2) and US-05 at 30 °C (n = 4).*

All five beers had average contents of acetaldehyde measured to be higher than the sensory threshold of 1.114 ppm. The lowest content was found in Gamlegrua with einerlog fermented at 22 °C with 3.8 ppm, and the highest content was found in US-05 with einerlog fermented at 30 °C with 7.9 ppm. The beers Gamlegrua without einerlog and US-05 with einerlog fermented at 30 °C had large standard deviations.

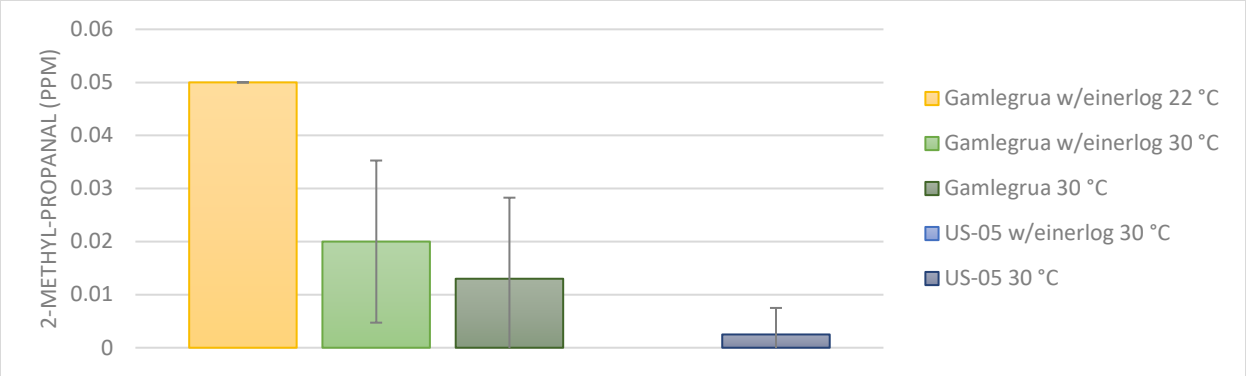

Figure 4.37. *2-methyl-propanal results from HSGC analysis for brews with einerlog and their counterparts without einerlog, with standard deviation. The sensory threshold of 2-methyl-propanal is 0.0023 ppm. The values are averages of n = 3, except for Gamlegrua w/ einerlog at 22 °C (n = 2) and US-05 at 30 °C (n = 4).*

Four out of the five beers had an average content of 2-methyl-propanal higher than the sensory threshold of 0.0023 ppm. The lowest content was found in US-05 with einerlog fermented at 30 °C and the highest content was found in Gamlegrua with einerlog fermented at 22 °C with 0.05 ppm. Gamlegrua had a higher content of 2-methyl-propanal than US-05, both with and without einerlog. The beers Gamlegrua with and without einerlog fermented at 30 °C had large standard deviations.

4.5.1.1.4 Higher alcohols

The results for 3-methyl-1-butanol content are illustrated in Figure 4.38.

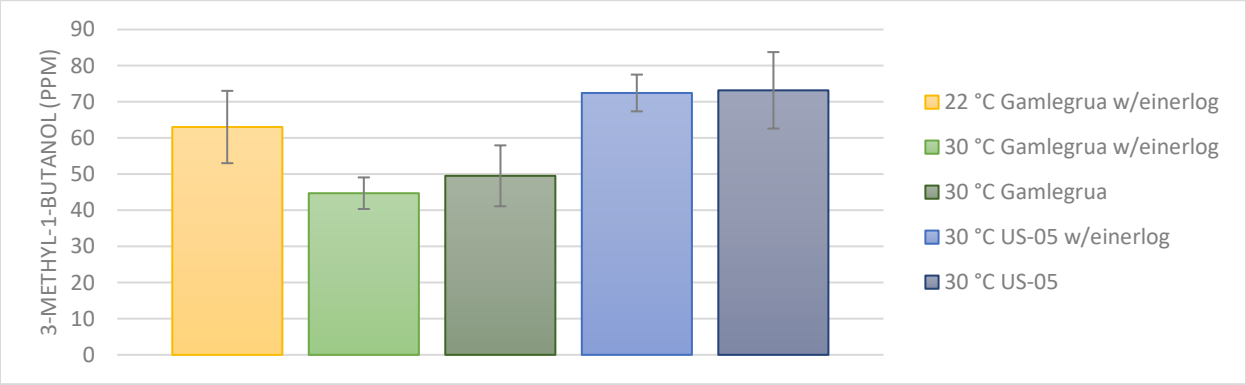

Figure 4.38. *3-methyl-1-butanol results from HSGC analysis for brews with einerlog and their counterparts without einerlog, with standard deviation. The sensory threshold for 3-methyl-1-butanol is 70 ppm. The values are averages of n = 3, except for Gamlegrua w/ einerlog at 22 °C (n = 2) and US-05 at 30 °C (n = 4).*

Two of the five beers, US-05 with and without einerlog, had average contents of 3-methyl-1-butanol measured to be higher than the sensory threshold of 70 ppm. The highest value was found in US-05 fermented at 30 °C with 73.2 ppm. The lowest value was found in Gamlegrua with einerlog fermented at 30 °C with 44.7 ppm. The contents of 3-methyl-1-butanol in US-05 were higher than in Gamlegrua.

4.5.1.1.5 Sulphur components

The results of dimethyl sulphide content are illustrated in Figure 4.39.

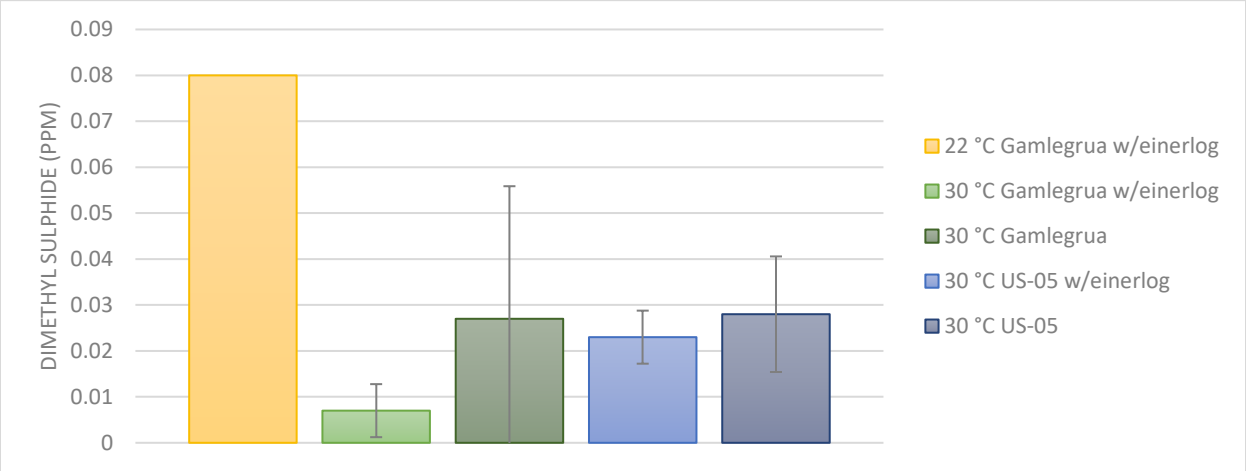

Figure 4.39. *Dimethyl sulphide (DMS) results from HSGC analysis for brews with einerlog and their counterparts without einerlog, with standard deviation. The sensory threshold of DMS is 0.06 ppm. The values are averages of n = 3, except for Gamlegrua w/ einerlog at 22 °C (n = 2) and US-05 at 30 °C (n = 4).*

One of the samples, Gamlegrua with einerlog fermented at 22 °C, had an average content of dimethyl sulphide above the sensory threshold of 0.06 ppm. This was the sample with the highest content of the compound with 0.08 ppm. The lowest content was found in Gamlegrua with einerlog fermented at 30 °C with 0.007 ppm.

4.5.1.2 Brews without einerlog

The volatile components ethyl acetate, isoamyl acetate, ethyl hexanoate, 3-methyl-1-butanol, acetaldehyde, 2-methyl-propanal and diacetyl were measured at levels higher than their respective sensory threshold values. These components are illustrated in this section.

The volatile components not illustrated in this section with contents at levels lower than their respective sensory threshold value are isobutyl acetate (0.04-0.09 ppm), ethyl heptanoate (0 ppm), ethyl octanoate (0.05-0.1 ppm), 1-propanol (15.8-32.8 ppm), 2-methyl-1-propanol (28.7-88.9 ppm), 2-methyl-1-butanol (16.6-23.0 ppm), 2-hexanol (0.3-1.2 ppm), dimethyl sulphide (0.007-0.03 ppm), 3-methyl-butanal (0-0.003 ppm), 2-methyl-butanal (0.007-0.02 ppm), hexanal (0 ppm) and acetone (0.3-0.6 ppm).

4.5.1.2.1 Esters

The esters ethyl acetate, isoamyl acetate and ethyl hexanoate were found in levels above the sensory thresholds for these components in all of the samples. The results are illustrated in Figure 4.40, Figure 4.41 and Figure 4.42.

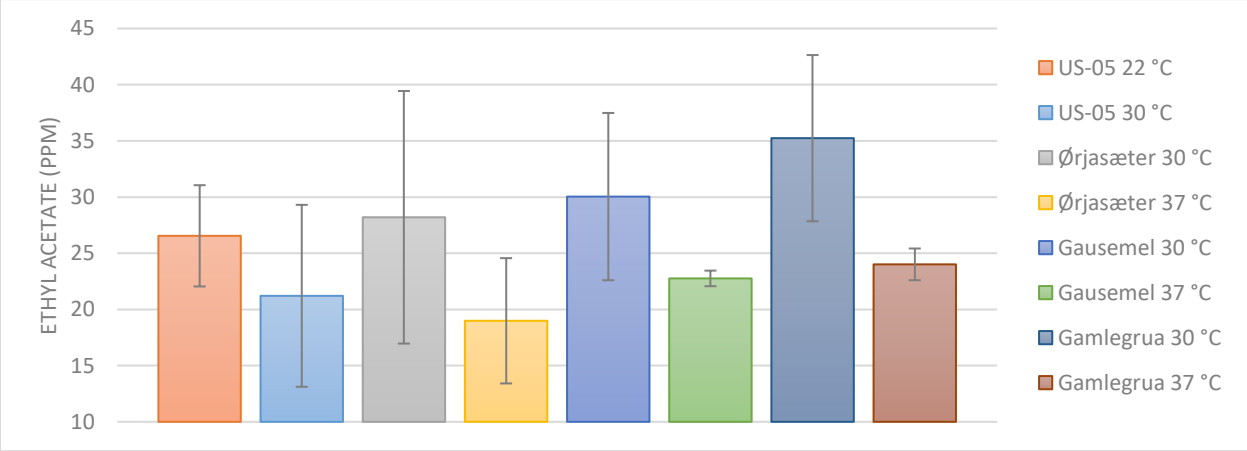

Figure 4.40. *Ethyl acetate results from HSGC analysis for brews without einerlog from the pilot-scale brewing, with standard deviation. The sensory threshold of ethyl acetate is 5 ppm. The values are averages of n = 3, except for US-05 at 22 °C and 30 °C (n = 4).*

The ethyl acetate content is consistently higher at the lower fermentation temperature for all of the beers. The highest content was found in Gamlegrua fermented at 30 °C and the lowest content was found in Ørjasæter fermented at 37 °C with 35.2 ppm and 19.0 ppm, respectively.

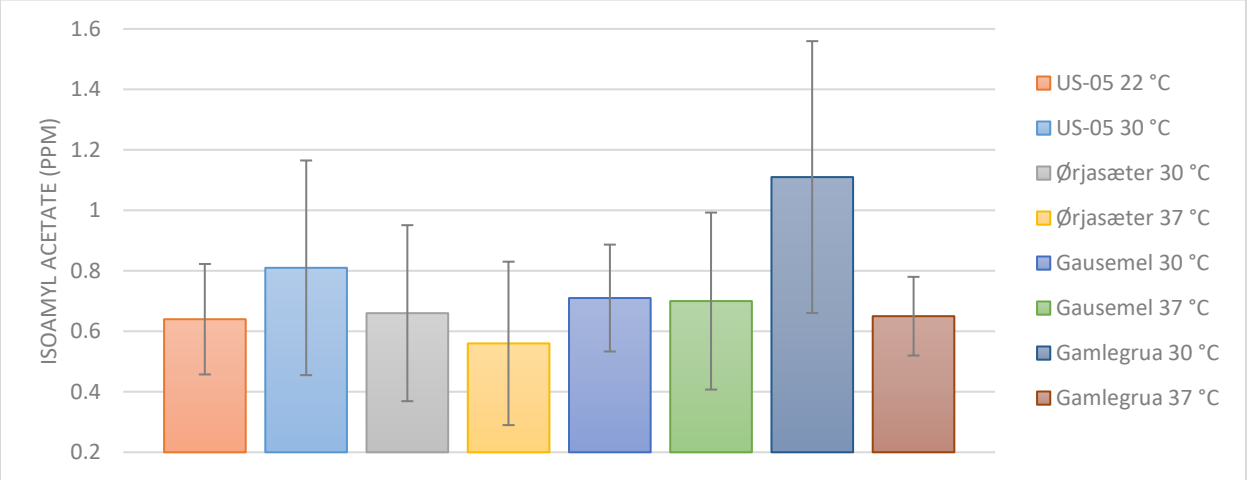

Figure 4.41. *Isoamyl acetate results from HSGC analysis for brews without einerlog from the pilot-scale brewing, with standard deviation. The sensory threshold of isoamyl acetate is 0.03 ppm. The values are averages of n = 3, except for US-05 at 22 °C and 30 °C (n = 4).*

The isoamyl acetate content was measured at the highest value in Gamlegrua fermented at 30 °C with 1.11 ppm and at the lowest value in Ørjasæter fermented at 37 °C with 0.56 ppm.

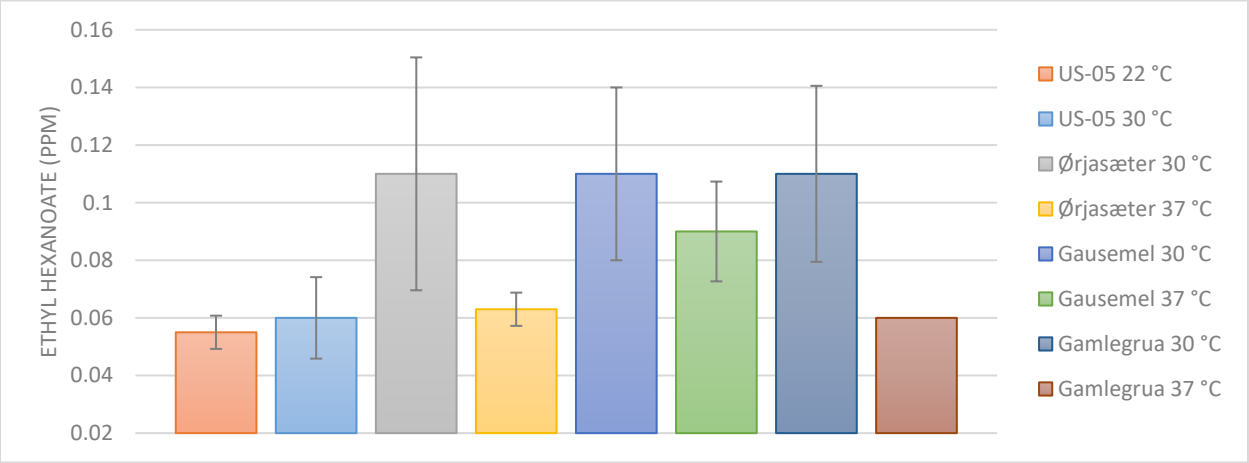

Figure 4.42. *Ethyl hexanoate results from HSGC analysis for brews without einerlog from the pilot-scale brewing, with standard deviation. The sensory threshold of ethyl hexanoate is 0.005 ppm. The values are averages of n = 3, except for US-05 at 22 °C and 30 °C (n = 4).*

The ethyl hexanoate content was consistently higher at 30 °C than at 37 °C for all three kveiks. As for the US-05 control, the content was slightly lower at 22 °C than at 30 °C. The highest values were measured in the kveiks Ørjasæter, Gausemel and Gamlegrua fermented at 30 °C with 0.11 ppm, and the lowest value was measured in US-05 fermented at 22 °C with 0.055 ppm.

4.5.1.2.2 Ketones

The ketone diacetyl was found in levels above sensory threshold in two of the samples. The results are illustrated in Figure 4.43.

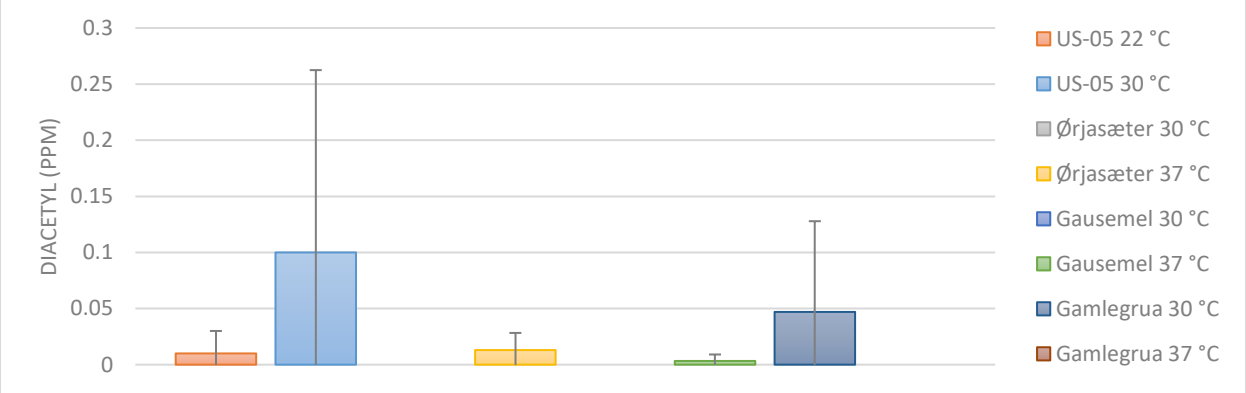

Figure 4.43. *Diacetyl results from HSGC analysis for brews without einerlog from the pilot-scale brewing, with standard deviation. The sensory threshold of diacetyl is 0.017 ppm. The values are averages of n = 3, except for US-05 at 22 °C and 30 °C (n = 4).*

The diacetyl content was measured at the highest level in US-05 fermented at 30 °C with 0.1 ppm. The component was not detected in Ørjasæter fermented at 30 °C, Gausemel fermented at 30 °C or in Gamlegrua fermented at 37 °C.

4.5.1.2.3 Aldehydes

The aldehydes 2-methyl-propanal and acetaldehyde were found in levels above the sensory thresholds of these components in all of the samples. The results are illustrated in Figure 4.44 and Figure 4.45.

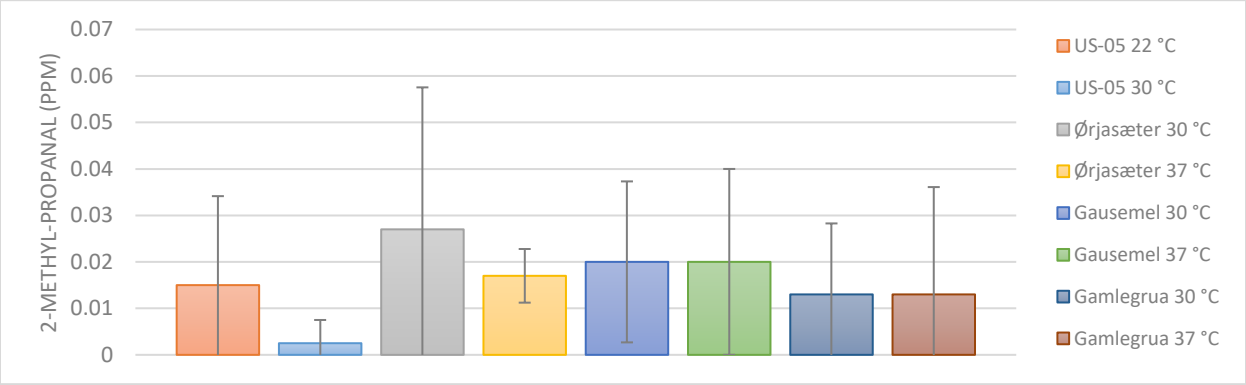

Figure 4.44. *2-methyl-propanal results from HSGC analysis for brews without einerlog from the pilot-scale brewing, with standard deviation. The sensory threshold of 2-methyl-propanal is 0.0023 ppm. The values are averages of n = 3, except for US-05 at 22 °C and 30 °C (n = 4).*

The 2-methyl-propanal content was measured at the highest value in Ørjasæter fermented at 30 °C with 0.027 ppm and at the lowest value in US-05 fermented at 30 °C with 0.0025 ppm. The standard deviations for the 2-methyl-propanal measurements are high in all of the beers.

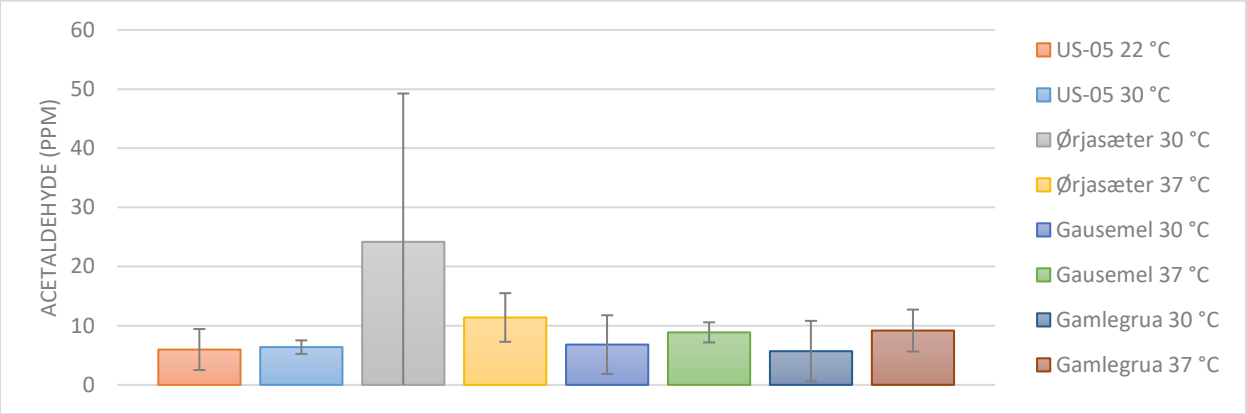

Figure 4.45. *Acetaldehyde results from HSGC analysis for brews without einerlog from the pilot-scale brewing, with standard deviation. The sensory threshold of acetaldehyde is 1.114 ppm. The values are averages of n = 3, except for US-05 at 22 °C and 30 °C (n = 4).*

The acetaldehyde content was measured at the highest value in Ørjasæter fermented at 30 °C with 24.2 ppm and at the lowest value in Gamlegrua fermented at 30 °C with 5.7 ppm. The standard deviation for the acetaldehyde measurements in Ørjasæter fermented at 30 °C is high.

4.5.1.2.4 Higher alcohols

The higher alcohol 3-methyl-1-butanol were found in levels above the sensory threshold of 70 ppm for this component in one of the samples. The results are illustrated in Figure 4.46.

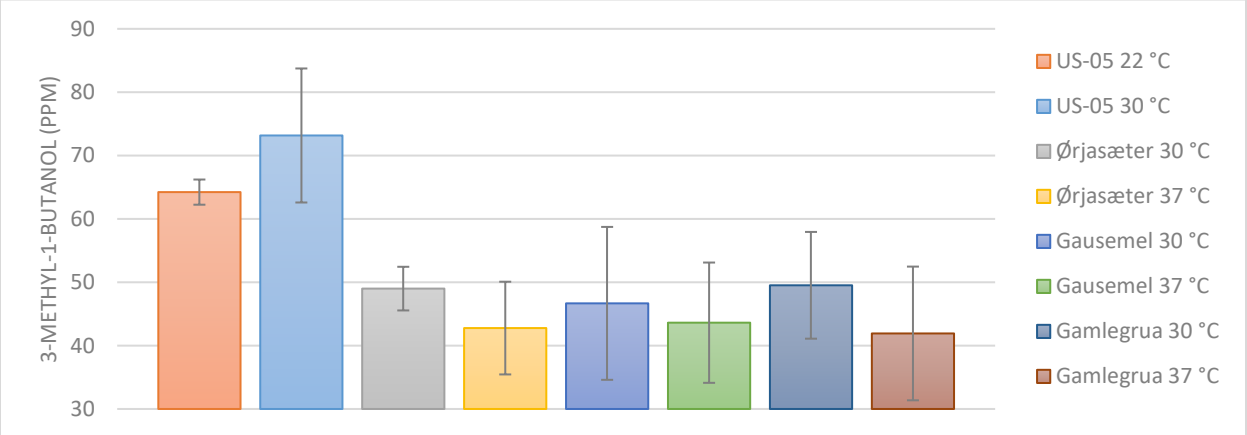

Figure 4.46. *3-methyl-1-butanol results from HSGC analysis for brews without einerlog from the pilot-scale brewing, with standard deviation. The sensory threshold of 3-methyl-1-butanol is 70 ppm. The values are averages of n = 3, except for US-05 at 22 °C and 30 °C (n = 4).*

The 3-methyl-1-butanol content was measured at the highest value in US-05 fermented at 30 °C with 73.2 ppm and at the lowest value in Gamlegrua at 37 °C with 41.9 ppm. The US-05 control had a higher content of this component than the kveiks on all temperatures. The kveiks fermented at 37 °C had a lower content of 3-methyl-1-butanol than the kveiks fermented at 30 °C.

4.5.2 Statistical significance

The resulting p-values for differences in the content of the volatile components, where at least one of the brews have values above the respective sensory threshold, are listed in Table 4.11.

Table 4.11. *Results from statistical analysis of HSGC results using type III ANOVA and Tukey's method in RStudio. A p-value < 0.05 indicates a significant statistical difference in the compared values. A 95% confidence interval was used. Only volatile components with levels above sensory threshold of at least one of the samples were analyzed. The p-values of comparisons found to be statistically significant are highlighted in bold letters.*

Volatile component	Ethyl acetate	Isoamyl acetate	Ethyl hexanoate	3-methyl-1-butanol		Dimethyl sulphide		Acetaldehyde	2-methyl-propanal	Diacetyl
Comparison:	**Combined p-value**	**Combined p-value**	**Combined p-value**	**Individual p-value**	**Combined p-value**	**Individual p-value**	**Combined p-value**	**Combined p-value**	**Combined p-value**	**Combined p-value**
Gamlegrua w/einerlog vs. Gamlegrua	0.3692	0.6253	0.06923	0.847055	**1.24E-05**	-	0.49805	0.12394	0.17093	0.6351
US-05 w/einerlog vs. Gausemel				**0.0021626**						
US-05 w/einerlog vs. Gamlegrua				**0.0027446**						
Gausemel vs. Gamlegrua w/einerlog				0.7949061						
US-05 vs. Gamlegrua w/einerlog				**0.0299441**						
US-05 w/einerlog vs. Gamlegrua w/einerlog				**0.0419248**						
US-05 w/einerlog vs. Ørjasæter				**0.0029442**						
US-05 w/einerlog vs. US-05				0.9887004						
Ørjasæter vs. Gamlegrua w/einerlog				0.8610487						
Gausemel vs. Gamlegrua				0.9999973						
US-05 vs. Gamlegrua				**0.000668**						
Ørjasæter vs. Gausemel				0.9999900						
US-05 vs. Ørjasæter				**0.0007338**						
Ørjasæter vs Gamlegrua				1.00000						
US-05 vs. Gausemel				**0.0004857**						
22 °C vs. 30 °C	0.1259	0.2362	0.6672	0.4312807	**0.004227**	**0.0287966**	**0.001903**	0.5342	0.259201	0.4823
30 °C vs. 37 °C				**0.0175269**		0.1325178				
22 °C vs. 37 °C				**0.0058568**		**0.0012608** [a]				

[a] Significant difference was found in values, but not showed in figures due to values for beers without einerlog being under sensory threshold.

There are no significant differences between the content of ethyl acetate, isoamyl acetate, ethyl hexanoate, acetaldehyde, 2-methyl-propanal and diacetyl in the samples when all cultures were compared to each other or when all fermentation temperatures were compared to each other.

Significant differences were found in the content of 3-methyl-1-butanol when the following cultures were compared: US-05 vs. the three kveiks, US-05 with einerlog vs. the three kveiks, US-05 with einerlog vs. Gamlegrua with einerlog and US-05 vs. Gamlegrua with einerlog. There were significant differences between the temperatures 30 °C and 37 °C, and between 22 °C and 37 °C.

No significant differences were found in sample content of dimethyl sulphide when the cultures were compared, but significant differences were found when comparing the temperatures 22 °C vs. 30 °C and 22 °C vs. 37 °C.

4.5.3 Phenolic analysis

The total phenolic content (TPC) in samples of wort with and without einerlog, as well as in samples after secondary fermentation and two weeks cold storage using different cultures of kveik and yeast at different fermentation temperatures, were analyzed. The results from this analysis can be found in Figure 4.47. The raw data can be found in appendix E-3 and E-11.

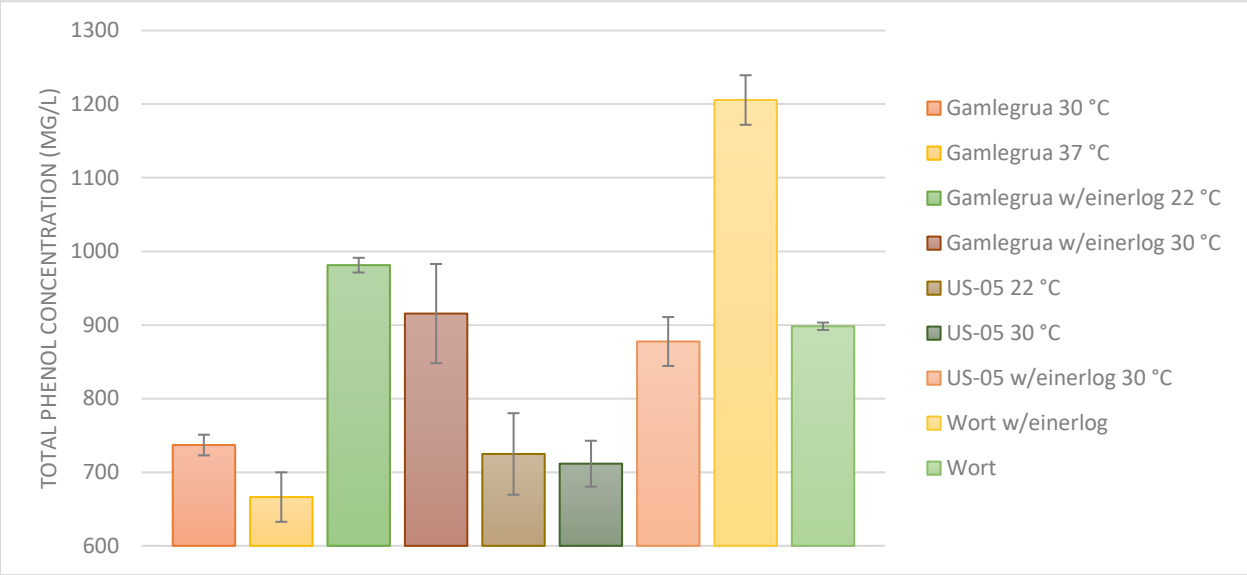

Figure 4.47. *The result from phenolic analysis from beer and wort with and without einerlog, fermented at different temperatures. Both of the values from Gamlegrua without einerlog, as well as Gamlegrua with einerlog at 22 °C and US-05 without einerlog at 30 °C, are done in triplicate. The results for Gamlegrua and US-05 with einerlog at 30 °C are averages from n = 3 done in triplicate, and the result for US-05 without einerlog at 22 °C is an average of n = 2 done in triplicate. Standard deviation for the results are shown in the figure.*

The highest TPC in the samples was found in the wort with einerlog with 1206 mg/L, and the highest TPC in beer was found in Gamlegrua with einerlog that had been fermented at 22 °C with 981 mg/L. The lowest amount of TPC in all the samples was found in Gamlegrua fermented at 37 °C (666 mg/L). The beers fermented with einerlog and at the lower temperatures contain a higher TPC than the beers without einerlog fermented at higher temperatures.

The phenol concentration in the wort without einerlog was measured from wort used in the growth and metabolism study, as well as in the preparation of cultures for the remainder of the study. The sugar concentration in this wort was approximately 10 °Plato, which is considerably lower than the wort used in the pilot-scale brewing trials with approximately 19 °Plato.

The results from the statistical analysis of significant differences in total phenolic content between the beers are listed in Table 4.12.

Table 4.12. *Statistical analysis results of phenolic analysis from type III ANOVA and Tukey's method in RStudio. A p-value < 0.05 indicates a significant statistical difference in the compared values. A 95% confidence interval was used. The p-values of comparisons found to be statistically significant are highlighted in bold letters.*

Comparison:	Individual p-value	Combined p-value
Gamlegrua w/einerlog vs. Gamlegrua	**0.0000000**	**0.00000000001263**
US-05 vs. Gamlegrua	0.8961830	
US-05 w/einerlog vs. Gamlegrua	**0.0000012**	
US-05 vs. Gamlegrua w/einerlog	**0.0000000**	
US-05 w/einerlog vs. Gamlegrua w/einerlog	0.0931126	
US-05 w/einerlog vs. US-05	**0.0000013**	
30 °C vs. 22 °C	0.5253150	**0.01562**
37 °C vs. 22 °C	0.0982127	
37 °C vs. 30 °C	**0.0130575**	

When the fermentation temperatures were compared to one another, a significant difference was found in the comparison 37 °C vs. 30 °C. Four of the comparisons between yeast/kveik types were statistically significant: the results from Gamlegrua w/einerlog vs. Gamlegrua, US-05 w/einerlog vs. Gamlegrua, US-05 vs. Gamlegrua w/einerlog and US-05 w/einerlog vs. US-05.

4.6 Sensory analysis

The result from a sensory analysis performed by five qualified judges of the finished ales is listed in Table 4.13.

Table 4.13. *Result from sensory analysis. The results are a summary of the statements given by all five judges. At least two of the judges had to mention a characteristic for it to be included in the summary. The level present of a characteristic is indicated by the codes: not detected (n.d.), a low amount (-), a medium amount (+) and a large amount (++). In cases where there were divided opinions, the opinion of the majority determined the final outcome.*

Characteristic		Without einerlog								With einerlog		
		30 °C				37 °C			22 °C	30 °C	22 °C	30 °C
		Gamlegrua	Gausemel	Ørjasæter	US-05	Gamlegrua	Gausemel	Ørjasæter	US-05	US-05	Gamlegrua	Gamlegrua
Aroma	Malt	-	-	-	+	+	+	-	-	-	-	-
	Hops	-	-	-	-	-	-	-	-	-	-	-
	Yeast	++	+	-	+	+	+	+	+	+	+	+
	Intensity	+	+	-	+	+	+	-	-	+	+	+
	Citrus	n.d.	n.d.	n.d.	+	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Fruit	++	++	+	-	++	+	n.d.	++	++	++	++
	Phenols	+	n.d.	-	+	+	n.d.	++	++	+	++	++
	Floral	++	n.d.	++	n.d.	++	n.d.	+	n.d.	n.d.	n.d.	n.d.
	Green apples	n.d.	-	n.d.	n.d.	n.d.	+	n.d.	n.d.	n.d.	n.d.	n.d.
	Caramel	n.d.	+	n.d.	n.d.	n.d.	+	n.d.	++	+	n.d.	n.d.
	Toffee	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	+	n.d.	n.d.
	Marzipan	n.d.	n.d.	++	n.d.	+	n.d.	-	-	n.d.	-	n.d.
	Spirit	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	-	-	n.d.
Visual	Golden	++	++	++	++	++	+	++	+	+	n.d.	+
	Amber	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	-	++	n.d.
	Haze	++	++	n.d.	n.d.	++	++	++	++	++	++	++
	Clear	n.d.	n.d.	+	++	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Foam	-	-	-	-	-	+	++	+	-	-	-
Flavor	Bitter	-	-	-	-	-	-	+	+	+	+	+
	Sweet	+	+	+	+	+	-	+	+	-	-	-
	Acidity	-	-	-	-	-	+	+	-	-	-	-
	Hops	-	-	-	-	-	-	-	-	-	-	-
	Yeast	+	+	-	+	+	+	+	-	+	+	-
	Malt	-	+	-	-	+	+	-	-	-	-	-
	Green apples	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	++	n.d.	n.d.	-	n.d.
	Fruit	++	++	+	n.d.	++	+	n.d.	++	++	++	+
	Floral	n.d.	n.d.	++	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	++
	Phenols	+	-	n.d.	+	++	+	++	+	++	++	++
	Citrus	n.d.	n.d.	n.d.	++	+	-	-	-	n.d.	n.d.	n.d.
Mouthfeel	Body	+	+	+	+	+	+	+	+	+	++	+
	Carbonation	-	+	+	+	-	+	++	++	+	++	+
	Length	+	+	-	+	+	+	++	+	+	++	+
	Astringency	n.d.	n.d.	n.d.	n.d.	-	n.d.	n.d.	-	-	n.d.	n.d.

The characteristics that is considered as “phenols” in this study are spice, pepper, roasted and clove. For a characteristic to be added in the table, at least two of the judges had to mention the attribute. In cases where the judges do not agree on the level of a characteristic that is present in an ale, the opinion of the majority determines the final result.

The results indicate that US-05, Gamlegrua and Ørjasæter are phenolic, more so than Gausemel. The phenolic aroma and flavor appears to become stronger as the fermentation temperature increased. The brews with einerlog have a very strong phenolic aroma and flavor.

Ørjasæter and Gamlegrua have a floral aroma when fermented at both 30 °C and 37 °C, and Ørjasæter and Gamlegrua with einerlog had strong floral flavors when fermented at 30 °C. Ørjasæter had a strong green apple flavor at 37 °C, and less aroma and flavor of yeast than the other kveiks when fermented at 30 °C.

Gausemel have an aroma of green apples and caramel at both 30 °C and 37 °C.

US-05 with einerlog have a caramel and toffee aroma at 30 °C. The counterpart without einerlog at the same temperature had an aroma and flavor of citrus. The US-05 fermented beer at 22 °C had strong flavor of citrus, a strong aroma of caramel, as well as a fruitier aroma than US-05 fermented at 30 °C.

The kveiks fermented at 37 °C had a citrus flavor. Gausemel and Ørjasæter fermented at 37 °C were acidic in flavor. Gamlegrua and Gausemel have a fruitier aroma and flavor than Ørjasæter when fermenting at both 30 °C and 37 °C.

The brews with einerlog had a very fruity aroma and flavor. Gamlegrua with einerlog fermented at 22 °C had a flavor of green apple.

5 Discussion

5.1 Why were the kveiks Ørjasæter, Gamlegrua, Gausemel and Otterdal chosen?

The kveik in this study have different ideal pitching temperatures. For the in-depth studies, kveiks with ideal pitching temperatures of 30 ± 1 °C were chosen. This was done so that the attributes of different kveik with the same ideal pitching temperature could be compared to one another, instead of comparing kveik with different ideal pitching temperatures.

The Gamlegrua kveik and the Ørjasæter kveik were recently added to the kveik yeast registry (Garshol, 2020). They were therefore both considered to be good choices for this study, as considerable information could be added to the existing knowledge of these kveiks. The stated pitching temperature of Ørjasæter was initially 31 °C, so it would fit very well with the set requirements. The pitching temperature was discovered to be 33 °C at the end of the study (Garshol, 2020), but the study is based on the originally stated pitching temperature.

Otterdal was chosen to be part of some of the in-depth studies, as there was no publicly available information about this kveik apart from its origin and pitching temperature. This kveik was not chosen to be a part of the pilot-scale brewing or the growth and metabolism study due to the result from the isolation and sequencing, where Otterdal and Ørjasæter shared a lot of characteristics. It was therefore deemed more interesting to include the other three kveiks from these trials in the remainder of the in-depth studies.

Gausemel has been mixed with Stalljen several times, which led to the conclusion to just include one of these kveiks in the deeper levels of this study. In the end, Gausemel was chosen.

The kveiks Sigmund, Ebbegarden and Tormodgarden were excluded in the in-depth studies due to large amounts of publicly available information about these kveiks. The kveiks Midtbust and Espe were not chosen for the growth and metabolism study or the pilot-scale brewing due to pitching temperatures that did not coincide with the 30±1 °C desired for this study.

5.2 Antibacterial effect of einerlog

A small study performed in 1979 showed no antibacterial effect of einerlog against a strain of *E. coli* and a strain of *Streptococcus lactis* (Høeg, 1981). This study, on the other hand, showed an antibacterial effect of extracts of juniper twigs and needles, and to a small extent of unripe berries, against the LABs *Lb. plantarum, Lb. brevis* and *Lb. buchneri*. Ripe juniper berries showed no antibacterial qualities against the selected microorganisms in this study. The juniper twigs were more antibacterial than juniper needles. The growth of *Lb. plantarum* was inhibited at a lower concentration of juniper needle and twig extract than the other two LAB. The extract of unripe juniper berries only inhibited the growth of *Lb. buchneri* at the highest concentration (1 g/mL) used in this experiment, and it did not inhibit growth of the other two LAB.

The LAB strains used in this study were chosen because they are robust, and because they have been used to produce sour beer. The stress tolerance of these three LAB strains was evaluated in a recent study (Dysvik et al., 2020b). *Lb. brevis* is the most robust and *Lb. plantarum* had the lowest stress tolerance, which coincides with the inhibition by juniper twigs and needles on bacterial growth observed in this study.

As this was just a small preliminary study for one of the main focuses of this study, the pilot-scale brewing, the components of the einerlog were not studied further to figure out what was responsible for the antibacterial effect found in this study, or what the concentration of these components were.

5.3 Sanger sequencing and microbiota assay

Plating of the kveiks showed almost identical cell numbers on PCA and MRS as on the yeast media YM and Rose-Bengal agar, suggesting that yeasts are the dominating organisms in the kveiks. The YM and Rose-Bengal agar contains components that inhibit bacterial growth, lactic acid that results in a pH value of 4 and chloramphenicol, respectively. Both low pH and chloramphenicol are inhibiting to bacterial growth (Welthagen & Viljoen, 1997).

Sanger sequencing is a cultivation-based method that is dependent on the growth of the organism. In the kveiks Ørjasæter and Otterdal, the yeast *S. cerevisiae* was the only organism that was isolated and sequenced. The yeast *S. cerevisiae* was also found in Gausemel and Gamlegrua, but the bacteria *Lb. plantarum* was also isolated and identified from these kveiks, as well as

Acetobacter malorum from Gausemel. This could imply that there is a dominance of yeast in two of the four kveiks, but as only a selection of the chosen colonies have been sequenced and these colonies are also just a small part of the total number of colonies that were cultivated, it is difficult to say anything with certainty. Some of the yeast-isolates should have been included in a growth and metabolism study to observe differences and similarities between the isolates. The results from these studies could then have been compared to the growth of kveik.

The results from the microbiota assay, a method that is not cultivation-based, diverged from the previously known knowledge of the ten kveiks (Garshol, 2020). The kveik Sigmund, who was thought to contain three strains of *S. cerevisiae* and no bacteria, contained nine sequence variants of *S. cerevisiae* and many types of bacteria, among others *Lactococcus lactis*, *Acetobacter* and two sequence variants of *Lb. backii*. Tormodgarden and Stalljen were thought to contain no bacteria, but several bacterial strains were found in these samples. Both Stalljen and Tormodgarden contains, amongst other bacteria, *Lb. brevis* and *Lactococcus lactis*, and the kveik Tormodgarden also contained *Acetobacter*. A higher number of sequence variants of *S. cerevisiae* than number strains previously known to be in the kveiks were found in Midtbust, Gausemel, Espe and Stalljen. The literature states that Midtbust and Gausemel contains 6 strains of *S. cerevisiae*, Espe contains 4 strains and Stalljen contains 5 strains (Garshol, 2020). This study identified 15, 28, 23 and 21 sequence variants of *S. cerevisiae* in the respective kveiks. Tormodgarden was the only kveik in this study where a lesser amount of sequence variants of *S. cerevisiae* were found than what was stated in literature (Garshol, 2020). The literature stated that in consisted of 10 variants of *S. cerevisiae*, but this study only identified 8 variants of the yeast in this kveik. The kveik from Voss, Sigmund, consisted of different sequence variants of *S. cerevisiae* than the other nine kveiks in this study. This was expected due to the geographic distance between the origins of this kveik and the other kveiks. The kveiks Tormodgarden and Gamlegrua, originating from Sykkylven and Hornindal respectively, consisted of largely the same sequence variants of *S. cerevisiae*. This was unexpected as Gamlegrua was made from several local Hornindal kveiks mixed together in 1995, and Tormodgarden originates from Skodje (Garshol, 2020).

When comparing the results for the kveiks from the 16S rRNA bacterial microbiota assay, it becomes clear that Stalljen, Otterdal, Gamlegrua and Gausemel share a lot of the same bacterial microbiota; the main bacterial species found in these four kveiks are four sequence variants of *Lb.*

brevis. These kveiks all originate in the Hornindal municipality. The kveik Espe also originate from Hornindal, but the results from the bacterial microbiota assay for this kveik differs from the other kveiks analyzed in this study from that area. Espe contains large amounts of two sequence variants of *Acetobacter*, which accounts for approximately 98% of the bacterial microbiota in this kveik. This kveik is quite similar to Tormodgarden, as they both contain large amounts of the same sequence variant of *Acetobacter*. In comparison, Ørjasæter contains approximately 45% *Acetobacter*, 20% *Lb. brevis* and 10% *Lb. backii*. Midtbust contains *Acetobacter* and three sequence varieties of *Lb. backii*, where the two bacteria types are present in similar amounts.

Since the microbiota assay is not cultivation-based, the nature of the growth and metabolism is not known for the organisms identified using this method. The organisms can have genes coding for a certain metabolism, but this cannot be determined without doing growth and metabolism studies.

The addition of cycloheximide to growth agar makes the media selective for wild yeasts and bacteria, as brewing yeasts are sensitive to the antibiotic (Boulton & Quain, 2001). Isolates number 127 and 136 from MRS + cycloheximide agar, as well as isolate 137 from MRS agar, isolated from Gamlegrua kveik showed no PCR product after gel electrophoresis. The reason for this could be that the bacteria are Gram positive and have not lysed during the preparation of these samples. It is also possible that the primer did not bind to the template DNA. Therefore, these isolates were not sent to sequencing. The result of this was that no isolates of bacteria from these two agar types isolated from Gamlegrua were sequenced and identified.

An interspecies *Saccharomyces cerevisiae* and *Saccharomyces uvarum* hybrid was isolated from a kveik culture, Muri, in 2018 (Krogerus et al., 2018). However, this hybrid was discovered to be a contaminant and not a part of a kveik culture (Krogerus, 2019).

5.4 Alcohol content

In the growth and metabolism study, the kveiks Gausemel, Gamlegrua and Ørjasæter (3.6 %) had produced less alcohol on day 7 than the control ale yeast US-05 (4.1 %) when the samples were incubated at 22 °C. The kveiks (3.9 - 4.1 %) had produced more than the control (3.7 %) when the samples were incubated at 37 °C. At 30 °C the differences were less defined, but the US-05 samples (4.1 %) had higher alcohol contents than the kveiks (3.8 - 4.0 %). There were significant differences when comparing the fermentation temperatures 30 °C and 37 °C to the fermentation temperature 22 °C. This was expected due to the difference in optimal fermentation temperature

for the kveiks vs. the control. The optimal fermentation temperature for the US-05 control ale yeast is 18-28 °C (Fermentis, 2018), which explains why there was observed a drop in alcohol content when the control sample was incubated at 37 °C. The high alcohol contents of the kveiks at 37 °C was expected, as the ideal pitching temperature of the kveiks studied in the growth and metabolism experiment was 30 ± 1 °C, and kveiks have previously been found to be more thermotolerant than industrial, domesticated beer yeasts (Preiss et al., 2018).

In the pilot-scale brewing, the differences between the measured values after maturation are less consistent. Gamlegrua with einerlog fermented at 22 °C had an alcohol content of 8.7 %, which is higher than the other brews made using Gamlegrua kveik (7.4 % and 7.9%) and marginally higher than the alcohol content of US-05 fermented at 22 °C (8.6 %). The Gamlegrua brews with einerlog fermented at 22 °C were, however, made using a wort with a higher original gravity (1.083) than the other beers (1.071 - 1.076), and the attenuation for these brews are almost identical to the Gamlegrua brews fermented at 30 °C. It is therefore difficult to compare the results from these beers with the other results and reach a strong conclusion.

Gausemel and Ørjasæter had a higher alcohol by volume content in the pilot-scale brewing when fermented at 30 °C, with 8.4 % and 8.3 % respectively, than at 37 °C, with 8.2 % and 7.8 % respectively. This does not coincide with the results from the growth experiment. This could, however, be a result of using a wort with a lower sugar content in the growth experiment and the difference in fermentation time, as the growth experiment only lasted for 7 days, even as the measured gravity of the growth experiment samples does indicate a complete fermentation. The exceptions to this are the kveiks at 22 °C and the US-05 control at 37 °C, which have higher gravities and lower alcohol by volume percentages than the other samples in the growth experiment. This can be explained from the difference in optimal fermentation temperatures for the kveiks vs. the control yeast.

5.5 Pilot-scale brewing

Brew number eight, which consisted of Gamlegrua with einerlog fermented at 22 °C, was brewed using other equipment than the other brews. This must be considered when comparing and analyzing the results for the beers from this brewing session with the other beers, as it may be a contributing cause of variation in aroma components and sensory characteristics, even when the

processes were performed as similarly as possible. The yield in °Plato was approximately the same, but it cannot be ruled out that differences occurred due to the equipment used during the brewing.

To reach an original gravity in the wort of approximately 1.080 proved to be difficult as the digital refractometer used in the study showed inconsistent readings, which made it problematic to adjust the gravity of the wort successfully. This resulted in original gravities that diverged from the goal and varied from 1.071 to 1.083. These divergences could potentially affect the results of the study, such as the content of aromatic components, alcohol percentage and fermentation time.

The fermentation time of the brews fermented at 22 °C was found to be significantly different from the brews fermented at 30 °C and 37 °C. This was expected, as the increase in the fermentation temperature leads to an increased yeast activity (Kucharczyk & Tuszynski, 2018). The ANOVA test performed on the fermentation days to examine the difference between the cultures showed that at least one of the cultures was significantly different from the others. The Tukey test did, however, not show any significant differences between the cultures, which leads to the possibility that a type II error occurred: a false negative, where the H_0 hypothesis was not rejected when it should have been. The Tukey post hoc test adjusts the p values to avoid doing a type I error (to reject a H_0 hypothesis when it is actually not possible), which could lead to a type II error, which is possibly what happened in this case.

Many variables influence the composition of beer, such as pitching rate, fermentation temperature and wort composition (Webersinke et al., 2018). When the pitching rate is increased four-fold, the ester synthesis is reduced (Peddie, 1990). Traditional brewers in Norway under-pitch the kveik, to get a more prominent yeast character in the beer (Garshol, 2017). The kveiks in the pilot-scale brewing were under-pitched in this study, to replicate the brewing methods used traditionally in Norway and to achieve a high content of volatile compounds. A study has showed that the connection of low pitch rates and aroma production is not clear for all kveiks, but there are clear trends for some kveiks (Preiss & Netto, 2019b). This claim cannot be further substantiated, or contradicted, by this study, as the pitching rates used for all kveiks in the pilot-scale brewing were identical, and the pitching rates for all cultures in the growth and metabolism experiment were very similar (5.7 – 5.8 log CFU/mL).

Flocculation is a symbol of yeast domestication (Preiss et al., 2018). The flocculation of the yeasts in the pilot-scale brewing was observed in several cases to be better in the kveiks Gausemel,

Ørjasæter and Gamlegrua than in the control ale yeast US-05. Some kveiks do not flocculate as efficiently as others, possibly due to the procedure the farmhouse brewers use to harvest the yeast (Preiss et al., 2018). All three kveiks in the pilot-scale brewing of this study were observed to have good flocculation. This was, however, only observed visually and not tested as a part of the study.

5.6 Growth and metabolism study

The cell counts for the three kveiks in the timespan used in this study (0-7 days) were very similar. This was expected, as the ideal pitching temperatures for the three kveiks are similar and they should therefore have similar temperature preferences during fermentation. The goal for the inoculation level was 6.0 log CFU per mL, but the average inoculation were 5.7 - 5.8 log CFU/mL for the kveiks and 6.2 log CFU/mL for the US-05 yeast. The control ale yeast SafAle US-05 diverged from the kveiks, showing a slow-growing cell count from day 0 to day 7 at 37 °C and a drastic drop in cell count at 30 °C from day 2 to day 7. The cell counts observed on day 2 and day 7 were lower for US-05 than for the kveiks, and the differences between the control and the kveiks on day 7 were found to be statistically different.

In the case of pH development during fermentation, the cultures were divided into two groups depending on their observed pH values on day 7: Ørjasæter and Gamlegrua with pH values of 4.1 – 4.3 vs. US-05 and Gausemel with pH values of 3.8 – 4.0. The pH values observed for the cultures within each group on day 7 were similar, and they were significantly different from the cultures in the other group statistically.

5.7 Sugar content

The °Plato of the samples at 0, 2 and 7 days showed that the samples incubated at 22 °C had a higher sugar content at day 2 than the samples incubated at 30 °C and 37 °C. The exception to this is the US-05 samples incubated at 37 °C , which had a very high content of sugar. The cell count on day 2 for US-05 at 37 °C was approximately 0.6 log below the US-05 cell counts at 22 °C and 30 °C on the same day, which could explain the large content of sugar in the sample on day 2. The sugar content values were very similar on day 7 for all cultures and temperatures. However, a significant difference was found when comparing the fermentation temperatures 22 °C and 30 °C on day 7.

In the pilot-scale brewing, the final gravity of the brews with the kveik Gamlegrua and the control US-05 were found to be significantly different, as did the fermentation temperatures 22 °C and 37 °C. No significant differences were found when the sugar content Gamlegrua and US-05 on day 7 were compared against each other in the growth and metabolism study.

There are no clear lines to be drawn when comparing sugar content values at different fermentation temperatures between the results of the growth and metabolism study and of the pilot-scale brewing. The resulting values are different due to the original gravity of the worts used. Both experiments showed significant differences when comparing the fermentation temperature 22 °C with one of the other temperatures (30 °C and 37 °C).

5.8 Volatile components

5.8.1 Esters

The content of the esters ethyl acetate, isoamyl acetate and ethyl hexanoate in beer brewed with einerlog was consistently lower compared to the beers brewed without einerlog, when fermented at 30 °C. This was observed for both Gamlegrua and the control US-05. However, the content of esters in Gamlegrua brewed with einerlog fermented at 22 °C was higher than in the beers fermented at 30 °C that had been brewed with and without einerlog. The beers brewed without einerlog showed the same tendencies, with higher compound contents at the lower fermentation temperature for each culture, in the contents of ethyl acetate (all cultures), isoamyl acetate (Ørjasæter and Gamlegrua) and ethyl hexanoate (Ørjasæter, Gamlegrua and Gausemel). Overall, the kveik Gamlegrua fermented at 30 °C had the highest content of these three esters. The results were unexpected, as an increase in fermentation temperature should result in an increase in ester production (Pires et al., 2014). Literature states that when the temperature is increased by 15 °C, from 10 °C to 25 °C, the ester concentration can increase by up to 75%. The effect of temperature varies with both type of ester and the temperature range (Peddie, 1990).

The results from the growth and metabolism study show that the US-05 control had low ester values compared to the kveiks. It is known that kveiks produce a range of fruity esters, but it is unclear in literature how the ester production of kveik compares to industrial beer yeasts (Preiss et al., 2018). The content of the three esters ethyl acetate, isoamyl acetate and ethyl hexanoate were high at 22 °C and decreased with an increase in fermentation temperature, except for Ørjasæter, who had higher contents of the esters ethyl acetate and isoamyl acetate at 30 °C than at 22 °C. The

brews with kveik in the pilot-scale brewing were not fermented at 22 °C, except for Gamlegrua with einerlog. If the high ester concentration in the result from Gamlegrua with einerlog fermented at 22 °C is representative for the esters produced by other kveiks when fermented at 22 °C, the observations from the growth and metabolism study could potentially be comparable to the results from the pilot-scale brewing.

5.8.2 Ketones

The beers brewed with einerlog had consistently lower contents of diacetyl compared to their counterparts without einerlog. The beers fermented with US-05 and Gamlegrua at 30 °C had the highest contents of diacetyl in the pilot scale-brewing with 0.1 ppm and 0.05 ppm, respectively. Literature states that an increase in fermentation temperature reduces the levels of diacetyl in the beer (Kucharczyk & Tuszynski, 2018). No consistent results from the sensory analysis substantiates this, as at least two out of the five judges had to make the same observation on a beer for it to be taken into consideration. In the growth experiment, US-05 had a high content of diacetyl after two days of fermentation, but that content had declined on day 7. The contents of diacetyl in the growth and metabolism experiment for the two beers with the highest content in the pilot-scale brewing had been reduced to zero on day 7.

Diacetyl levels above sensory threshold values results in a beer with a buttery flavor (Pires et al., 2014). This flavor is undesirable in many beer styles and it is regarded as a defect. In some beer styles, such as Bohemian Pilsner and some English ales, levels of diacetyl above sensory threshold is acceptable. Microbial contamination of bacteria such as *Lactobacillus* spp. or *Pediococcus* spp. could also be indicated by a buttery flavor in the beer (Krogerus & Gibson, 2013). All three kveiks that were included in the growth and metabolism study, and the pilot-scale brewing, contains *Lactobacillus* spp., though Gausemel and Gamlegrua contains larger numbers of these bacteria than Ørjasæter. This could potentially explain the slightly higher values of diacetyl on day 2 of the growth experiment for Gamlegrua and Gausemel compared to Ørjasæter. However, the kveik Gamlegrua is observed to contain higher values of diacetyl than the other two kveiks in the pilot-scale brewing, which makes the theory that the *Lactobacillus* spp. contributes significantly to the diacetyl content of the beers in this study unlikely.

5.8.3 Aldehydes

The beers brewed with kveik, with or without einerlog, had higher contents of 2-methyl-propanal than the beers brewed with US-05 fermented at 30 °C. The content of the compound in US-05 fermented at 22 °C was higher than Gamlegrua fermented at 30 °C and 37 °C.

The acetaldehyde content of the beers that had been brewed with einerlog and fermented at 30 °C was 7.0 ppm for Gamlegrua and 7.9 ppm for US-05, which is higher than the content of this compound in their counterparts without einerlog with the values 5.7 ppm for Gamlegrua and 6.4 ppm for US-05. Gamlegrua brewed with einerlog and fermented at 22 °C had a lower content of acetaldehyde than the other beers brewed with einerlog, with 3.8 ppm. The acetaldehyde content in the Gamlegrua (92.7 ppm) and Gausemel (56.4 ppm) samples fermented on 37 °C on day 7 in the growth and metabolism experiment were high and the values for the other samples were low. In the brews without einerlog in the pilot-scale brewing, the beers fermented with Ørjasæter had high contents of acetaldehyde (11.4 ppm at 37 °C and 24.2 ppm at 30 °C). There are therefore very big differences in the content of this compound in the results from the growth and metabolism experiment and the pilot-scale brewing.

The HSGC analysis of volatile components show that ale fermented with Ørjasæter kveik has a high level of acetaldehyde. This coincides with the sensory analysis, which states that the Ørjasæter ale fermented at 37 °C had a green apple flavor. The only brew with einerlog stated to have a flavor of green apple in the sensory analysis was Gamlegrua fermented at 22 °C. This is unexpected, as this beer had a lower content of acetaldehyde than the other einerlog brews, but the content is still above threshold. Gausemel fermented at both 30 °C and 37 °C were stated to have green apple aroma in the sensory analysis, which is supported by the fact that these values are above sensory threshold in the HSGC analysis.

5.8.4 Higher alcohols

The levels of the higher alcohols 1-propanol and 2-methyl-1-propanol in the growth and metabolism study were consistently higher in US-05 than in the three kveiks Gamlegrua, Gausemel and Ørjasæter.

In the pilot-scale brewing, 3-methyl-1-butanol was found in high levels in US-05 at 22 °C (64.2 ppm), 30 °C (73.2 ppm) and 30 °C with einerlog (72.4 ppm), and in Gamlegrua with einerlog fermented at 22 °C (63.0 ppm). This was higher than both the content found in the other beers

fermented with kveik and the other beer made with einerlog, which was in the range 41.9 - 49.5 ppm. The results from the growth and metabolism study showed little differences between the cultures and an increasing content of the compound from day 0 to day 7.

The control US-05, with and without einerlog, was found to be significantly different from the other cultures when it comes to 3-methyl-1-butanol. The beers fermenting at 37 °C had a content of 3-methyl-1-butanol found to be significantly different from the beers with fermentation temperatures 22 °C and 30 °C. These significant differences can be explained by the high levels measured in the pilot-scale brewing of the component observed in US-05 at the fermentation temperatures 22 °C and 30 °C, and in Gamlegrua with einerlog fermented at 22 °C.

Literature states that higher alcohol formation is temperature dependent, where an increase in fermentation temperature from 10 °C to 12 °C causes an increase in higher alcohol levels (Pires et al., 2014). This coincides with the US-05 observations, but not the observations of the kveiks, where the highest contents of 3-methyl-1-butanol are observed at the lower fermentation temperatures. The literature does, however, mention temperatures much lower than the fermentation temperatures used in this study.

5.8.5 Sulphur components

The beer brewed with einerlog, fermented using Gamlegrua at 22 °C, was the only beer in the pilot-scale brewing that had a content of DMS over sensory threshold. This could explain why the beers with the fermentation temperature 22 °C were found to be significantly different from the beers at temperatures 30 °C and 37 °C. The results from the growth and metabolism study were contents way below sensory threshold for all cultures and temperatures. The sensory analysis of the beer with levels of DMS above the sensory threshold according to the HSGC analysis resulted in no consistent result, and the DMS content in this beer could therefore not be assumed to affect the sensory aspects of the beer.

5.9 Phenols

A HPLC-analysis should have been used to analyze the content of some of the phenols that are commonly found in beer, such as phenolic acids, flavonoids and tannins (Humia et al., 2019). This would have resulted in a more nuanced information regarding the phenol content of the beers.

The results from the phenolic analysis shows that the ales with einerlog fermented with Gamlegrua (915.5 mg/L at 30 °C and 981 mg/L at 22 °C) are more phenolic than the ales with einerlog fermented with US-05 (877.6 mg/L). The ales with einerlog have a higher TPC than the ales without einerlog fermented with US-05 and Gamlegrua (666.3 - 736.9 mg/L). A statistical analysis shows that the TPC of the beers with einerlog are significantly different from the TPC of the beers without einerlog. In addition to this, a higher fermentation temperature seems to decrease the TCP content of the ale. This is confirmed by the statistically significant difference found when comparing the fermentation temperatures 37 °C and 30 °C.

It is undesirable in pilsner beers to contain excessive amounts of the phenolic compound 4-vinylguiacol, but the phenolic flavor is desirable in beers such as Belgian white beers and German Weizen beers (Vanbeneden et al., 2008).

Interestingly, the phenol aroma and flavor described in the sensory analysis increases in strength when the fermentation temperature increased, which contradicts the results from the TPC analysis. All three brews with einerlog were found to be phenolic in both aroma and flavor, which coincides with the results from the TPC analysis.

The sugar content of the wort sample without einerlog used to measure the TPC could potentially have affected the total phenol concentration of the sample. This caused the wort sample without einerlog to be more diluted than the worts used in the pilot-scale brewing, and probably resulted in a lower value of total phenol content for this sample than would have been observed if a wort sample from the pilot-scale brewing would have been analyzed.

5.10 Statistical strength

Due to the fact that the experiments conducted in this study were only done in duplicate, triplicate or quadruplicate the statistical strength of the results is weak, and it is difficult to make conclusions based on the results of these experiments. Two to four observations are often not enough to notice significant statistical differences, and only the most extreme differences in results are noticed using such a low amount of observations. Therefore, there could be significant differences between values that are now deemed as insignificant and these differences would be discovered if a larger amount of observations were made.

It is, however, common to do these types of studies in triplicate. The pilot-scale brewing experiment was very complex, with many factors affecting the growth and metabolism of the yeast/kveik used in the brewing process. The growth and metabolism study was performed in a more controlled way, which is indicated by the low standard deviations for the observations done in this study compared to the standard deviations observed in the pilot-scale brewing.

5.11 Planning of experiments

The nature of this study made it very difficult to plan the entirety of the experiments that were conducted at the start of the project. This was challenging, and several parts of the study could have been done differently. Experience from the study revealed new information that was not considered at the beginning of the project. The process of isolation and sequencing was very time demanding, and it would have been better to spend this time conducting a more extensive growth and metabolism study containing either more types of kveik or additional fermentation temperatures, to gain more information about the kveiks and their attributes. The pilot-scale brewing could also have been done in a more elaborate way, if there would have been more time to dispose. The isolation, Sanger sequencing and identification parts of this study showed that the yeast in the kveik was *S. cerevisiae*, which confirmed findings in other literature. Both yeast and bacteria isolates were obtained from kveik, which could be used separately or multiple isolates together in growth experiments and those results could then be compared with the original kveik. The microbiota assay that was conducted ended up yielding a lot more information than could be extracted from the isolation and identification that was done separately.

5.12 Sensory analysis

The sensory analysis was performed in a way that gave the judges very loose reins. The judges were not calibrated, the time and the duration of the analysis was left up to the individual judge, and there was no rules set for the temperature of the beer and order of the beer tastings. These factors resulted in sensory analyses with a lot of diverging observations, which made it difficult to make any conclusions in regard to the sensory character of each beer from the sensory analysis alone. Some of the results have been considered in cases where they could substantiate or contradict the results from other analyses.

6 Conclusion

This study showed that extracts of juniper twigs and needles, and unripe juniper berries to a smaller extent, are antibacterial against LAB in the concentrations used in this study. The antibacterial effect was especially evident against *Lb. plantarum*, which is the least stress tolerant *Lactobacillus* strain included in this study.

The ten kveiks included in this study contained sequence variants of *Saccharomyces cerevisiae*, as well as sequence variants of several bacteria, such as *Acetobacter* spp., *Lactobacillus brevis*, *Lactobacillus backii* and *Lactococcus lactis*. The kveik Sigmund from Voss contained sequence variants of *S. cerevisiae* not found in the other nine kveiks. Gamlegrua and Tormodgarden have similar *S. cerevisiae* sequence variants, and the majority of the yeast in the kveiks Midtbust, Stalljen, Gausemel, Ebbegarden, Otterdal, Espe and Ørjasæter show the same sequence variants of *S. cerevisiae* . The kveiks Stalljen, Otterdal, Gamlegrua and Gausemel from Hornindal contain a lot of the same bacterial sequence variants.

Higher fermentation temperatures (30 °C and 37 °C) resulted in a higher alcohol content for the kveik in the growth and metabolism study than at a low fermentation temperature (22 °C). The pH values on day 7 of the growth and metabolism experiment showed similarities between Ørjasæter and Gamlegrua, and between Gausemel and US-05. The final gravity of Gamlegrua and US-05 was found to be significantly different in the pilot-scale brewing. The addition of einerlog to the brews fermented at 30 °C resulted in a lower content of the ketone diacetyl, and the esters ethyl acetate, isoamyl acetate and ethyl hexanoate in the finished beer. Higher ester contents were inversely connected to the fermentation temperatures, with high ester contents at low fermentation temperatures. US-05 was observed to have a lower content of esters and a higher content of diacetyl and the higher alcohols 3-methyl-1-butanol, 1-propanol and 2-methyl-1-propanol than the kveiks.

The ales with einerlog fermented with Gamlegrua are more phenolic than the ales with einerlog fermented with US-05, and the ales with einerlog have a higher total phenol content than the ales without einerlog. A higher fermentation temperature decreases the total phenolic content of the ale.

6.1 Future projects

Old Norwegian traditions in beer brewing includes the use of juniper, a large number of herbs and spices local to the Nordic climate, in addition to the special brewing process described earlier and the use of kveik adapted to a typical traditional brewing process. It would be of interest to study each of these topics in depth. It could be interesting to investigate the effect of traditional additives in beer apart from juniper, such other types of Norwegian plants and herbs, e.g. bog myrtle (*Myrica gale*), chaga (*Inonotus obliquus*), meadowsweet (*Filipendula ulmaria*), black elder (*Sambucus nigra*) and yarrow (*Achillea millefolium*). Both the possible antimicrobial effects and the sensory aspects could be intriguing research topics.

In addition to this, investigating the use of kveik in raw ale would be very interesting, possibly also in a study that compares the resulting ale from a modern brewing process and a traditional brewing process that results in a raw ale product.

Brewing with varying pitch-rates, as well as a larger range of pitching temperatures than what was done in this study, could be intriguing, where the effect these factors would have on the flavor profile and the fermentation as a whole could be investigated.

This study resulted in the isolation of 156 yeasts and bacteria from 4 kveiks. These isolates could be studied individually in growth assays and pilot-scale brewing, to properly understand the metabolism and growth of these single-components of kveik. The isolation of yeasts and bacteria from other kveiks, which could in turn be compared to the isolates from this study, could be interesting.

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Appendices

Appendix A – Growth and metabolism experiment

A-1 pH values and °Plato values – raw data (n = 3)

	Temperature	Yeast	Day 0			Day 2			Day 7		
°Plato	22 °C	US-05	9.4	10.2	9.7	6.9	7.2	7.8	4.5	4.8	4.6
		Gamlegrua	9.4	10.1	8.6	6.3	7.1	5.5	4.9	5.2	4.2
		Ørjasæter	9.4	10.3	9.1	6.7	7.3	6.5	4.8	5.3	4.8
		Gausemel	9.5	9.7	9.5	6.5	6.7	6.7	5.0	5.1	4.8
	30 °C	US-05	9.4	10.2	9.7	5.7	5.8	5.9	4.4	4.7	4.5
		Gamlegrua	9.4	10.1	8.6	5.1	5.7	4.6	4.5	4.9	3.9
		Ørjasæter	9.4	10.3	9.1	5.6	6.1	5.2	4.4	4.8	4.0
		Gausemel	9.5	9.7	9.5	5.6	5.6	5.4	4.4	4.8	4.5
	37 °C	US-05	9.4	10.2	9.7	8.3	8.5	8.7	4.8	4.8	8.3
		Gamlegrua	9.4	10.1	8.6	4.8	5.3	4.1	4.5	4.8	3.9
		Ørjasæter	9.4	10.3	9.1	4.8	5.5	4.5	4.5	4.8	4.1
		Gausemel	9.5	9.7	9.5	4.9	4.8	4.8	4.6	4.9	4.3
pH	22 °C	US-05	5.46	5.53	5.39	4.22	4.22	4.32	3.96	4.01	3.93
		Gamlegrua	5.47	5.51	5.35	4.37	4.45	4.33	4.26	4.39	4.22
		Ørjasæter	5.48	5.47	5.35	4.42	4.45	4.35	4.14	4.22	4.01
		Gausemel	5.45	5.45	5.33	4.34	4.40	4.34	4.10	3.86	3.91
	30 °C	US-05	5.46	5.53	5.39	3.87	3.83	3.86	3.87	4.01	3.82
		Gamlegrua	5.47	5.51	5.35	4.18	4.35	4.15	4.14	4.22	4.06
		Ørjasæter	5.48	5.47	5.35	4.22	4.24	4.05	4.10	4.23	4.00
		Gausemel	5.45	5.45	5.33	4.17	4.10	4.01	3.92	3.78	3.60
	37 °C	US-05	5.46	5.53	5.39	4.26	4.14	4.18	3.84	3.83	4.01
		Gamlegrua	5.47	5.51	5.35	4.26	4.21	4.16	4.34	4.36	4.30
		Ørjasæter	5.48	5.47	5.35	4.09	4.17	4.03	4.24	4.29	4.03
		Gausemel	5.45	5.45	5.33	4.20	4.19	4.14	3.92	4.16	3.84

A-2 log CFU per mL – growth experiment – raw data

Temperature	Yeast	Growth (log CFU/mL) Day 0			Growth (log CFU/mL) Day 2			Growth (log CFU/mL) Day 7		
22 °C	US-05	6.20	6.18	6.06	6.99	6.93	6.89	6.86	6.53	6.63
	Gamlegrua	5.92	5.43	5.73	7.46	7.57	7.57	7.57	7.29	7.36
	Ørjasæter	5.85	5.70	5.66	7.57	7.44	7.51	7.36	7.43	7.48
	Gausemel	5.90	5.71	5.80	7.60	7.46	7.63	7.51	7.59	7.56
30 °C	US-05	6.20	6.18	6.06	6.91	6.84	6.76	6.03	5.57	5.88
	Gamlegrua	5.92	5.43	5.73	7.59	7.53	7.48	7.41	7.44	7.37
	Ørjasæter	5.85	5.70	5.66	7.44	7.59	7.32	7.57	7.44	6.81
	Gausemel	5.90	5.71	5.80	7.60	7.55	7.68	7.47	7.44	7.42
37 °C	US-05	6.20	6.18	6.06	6.38	6.10	6.22	6.69	6.79	4.00
	Gamlegrua	5.92	5.43	5.73	7.15	7.42	7.21	6.93	6.88	7.17
	Ørjasæter	5.85	5.70	5.66	7.33	7.16	7.34	6.71	6.76	7.04
	Gausemel	5.90	5.71	5.80	7.34	7.37	7.57	6.61	6.91	7.58

A-3 Alcohol content, °Plato and gravity – growth experiment – raw data

Temp., °C	Yeast	Alcohol (% v/v)			°Plato			Gravity		
22	Gausemel	3.60	3.77	3.32	2.36	2.39	2.87	1.007	1.008	1.009
	Gamlegrua	3.65	3.90	3.12	2.38	2.69	2.25	1.007	1.009	1.007
	Ørjasæter	3.72	3.89	3.08	2.25	2.81	2.94	1.007	1.009	1.010
	US-05	*	4.28	3.88	*	1.89	2.46	*	1.006	1.008
30	Gausemel	3.95	4.09	3.61	1.90	1.99	2.13	1.005	1.006	1.006
	Gamlegrua	3.95	4.14	3.39	1.94	2.14	1.72	1.005	1.006	1.005
	Ørjasæter	3.98	4.30	3.71	1.92	2.03	1.71	1.005	1.006	1.005
	US-05	3.91	4.25	4.13	1.74	1.85	1.84	1.005	1.005	1.005
37	Gausemel	*	4.16	3.93	*	1.94	1.80	*	1.006	1.005
	Gamlegrua	4.06	4.22	3.51	1.88	1.99	1.62	1.005	1.006	1.005
	Ørjasæter	4.03	4.35	3.73	1.84	2.05	1.73	1.005	1.006	1.005
	US-05	3.41	3.99	1.21	2.67	2.46	7.66	1.009	1.008	1.029

* Not measured.

A-4 Result from HSGC analysis – Gamlegrua – Growth experiment – Raw data

Yeast	Gamlegrua																				
Temperature	-			22 °C						30 °C						37 °C					
Time, days	0			2			7			2			7			2			7		
Esters	ppm			ppm			ppm			ppm			ppm			ppm			ppm		
Ethyl acetate	0	0	0	3.16	2.06	2.22	7.29	7.8	6.66	6.22	3.72	4.23	8.36	8.32	3.51	5.15	5.78	3.11	4.15	2.45	2.56
Isoamyl acetate	0	0	0	0.16	0.11	0.11	0.18	0.23	0.24	0.3	0.12	0.16	0.19	0.18	0.04	0.13	0.16	0.06	0.03	0	0.01
Isobutyl acetate	0	0	0	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.01	0	0.01	0.01	0	0	0	0
Ethyl hexanoate	0	0	0	0.06	0.04	0.04	0.06	0.08	0.05	0.07	0.05	0.05	0.04	0.03	0.02	0.02	0.02	0.01	0.01	0	0
Ethyl heptanoate	0	0	0	0	0	0	0	0	0	0	0.01	0	0	0	0	0	0	0	0	0	0
Ethyl octanoate	0	0	0.01	0.02	0	0.01	0.03	0.03	0.03	0.03	0.02	0.02	0.05	0.05	0.04	0.02	0	0.01	0.02	0.02	0.02
Higher alcohols																					
1-propanol	0.23	0.28	0.2	6.06	5.88	5.27	10.4	8.38	9.4	10.78	7.93	7.69	12.13	10.33	8.85	8.01	9.61	7.31	9.64	8.03	9.56
2-methyl-1-propanol	0.13	0.11	0.15	5.73	5.7	5.24	11.06	10.04	8.9	11.62	9.44	9.17	17.03	14.76	12.76	12.23	12.88	11.11	16.2	13.18	14.47
3-methyl-1-butanol	0.32	0.18	0.32	15.22	15.56	13.51	25.96	23.45	24.43	22.29	19.38	17.99	28.17	24.82	21.5	19.95	21.54	18.55	24.35	20.01	20.52
2-methyl-1-butanol	0.07	0.06	0.08	5.15	5.03	4.61	9.53	8.98	7.89	9.57	8.53	7.99	13.02	11.29	9.93	9.37	9.72	8.62	11.59	9.2	10.02
2-hexanol	0	0	0	0.11	0.07	0.07	0.18	0.29	0.17	0.14	0.09	0.11	0.15	0.15	0.05	0.06	0.06	0.03	0.04	0.01	0.02
Sulphur components																					
Dimethyl sulphide	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aldehydes																					
Acetaldehyde	1.84	1.97	2.29	22.19	26.07	18.93	12.52	8.55	11.95	16.69	17.24	16.79	16.07	15.43	13.5	13.73	14.82	10.91	77.26	46.48	154.23
2-methyl-propanal	0.37	0.58	0.45	0.08	0.05	0.05	0.03	0.02	0.03	0.18	0.08	0.09	0.05	0.03	0.04	0.16	0.12	0.12	0.27	0.15	0.35
3-methyl-butanal	0.17	0.3	0.21	0.02	0.02	0.01	0	0.01	0.01	0.02	0.02	0.02	0	0	0	0.01	0.01	0.01	0.01	0	0
2-methyl-butanal	0.16	0.26	0.2	0.03	0	0.02	0	0	0.01	0.05	0.03	0.04	0	0	0	0.04	0.03	0.03	0	0.01	0.01
Hexanal	0	0.01	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ketones																					
Acetone	1.32	1.77	1.73	1.42	1.31	1.43	0.66	0.52	1.37	1.42	1.25	1.49	0.59	0.58	0.41	1.24	1.36	1.3	1	0.71	1.04
Diacetyl	0	0.17	0.12	0.17	0.25	0.22	0	0	0.2	0.43	0.42	0.31	0	0	0	0.33	0.44	0.28	0	0	0

A-5 Result from HSGC analysis – Gausemel – Growth experiment – Raw data

Yeast	Gausemel																				
Temperature	-			22 °C						30 °C						37 °C					
Time, days	0			2			7			2			7			2			7		
Esters	ppm			ppm			ppm			ppm			ppm			ppm			ppm		
Ethyl acetate	0	0	0	3.16	2.69	2.51	10.78	7.14	8.25	4.42	4.95	4.84	7.97	8.32	7.94	5.02	3.92	4.83	5.12	6.58	4.03
Isoamyl acetate	0	0	0	0.18	0.13	0.12	0.37	0.04	0.15	0.16	0.14	0.14	0.06	0.02	0	0.14	0.07	0.12	0.07	0.11	0.04
Isobutyl acetate	0	0	0	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0	0.01	0.01	0.01	0.01	0.01	0
Ethyl hexanoate	0	0	0	0.06	0.05	0.05	0.11	0.02	0.05	0.05	0.04	0.04	0.02	0.01	0	0.02	0.01	0.02	0.01	0.01	0.01
Ethyl heptanoate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ethyl octanoate	0	0	0.01	0.02	0	0.02	0.04	0.02	0.03	0.02	0.02	0.02	0.04	0.03	0.02	0.02	0	0.02	0.03	0.03	0.03
Higher alcohols																					
1-propanol	0.22	0.38	0.33	7.26	7.47	6.62	10.96	9.13	10.52	9.48	10.41	11.36	10.67	9.94	7.79	10.37	12.23	8.06	9.29	11.02	11.08
2-methyl-1-propanol	0.18	0.29	0.15	6.77	7.48	7.1	14.26	10.31	11.25	11.09	12.56	12.87	15.14	13.12	10.43	16.46	15.85	13.3	18	18.43	18.13
3-methyl-1-butanol	0.28	0.97	0.34	15.68	17.7	16.17	27.29	23.7	26.48	19.88	22.39	22.49	23.49	22.52	18	23.28	23.26	20.87	25.06	25.22	24.68
2-methyl-1-butanol	0.08	0.18	0.09	5.54	5.92	5.6	11.21	8.56	9.17	8.94	9.58	9.79	11.31	9.77	7.96	11.6	10.73	9.52	12.38	11.73	11.92
2-hexanol	0	0	0	0.09	0.08	0.08	0.32	0.09	0.15	0.1	0.09	0.08	0.08	0.07	0	0.06	0.03	0.06	0.06	0.09	0.04
Sulphur components																					
Dimethyl sulphide	0	0.01	0.01	0	0	0	0.01	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aldehydes																					
Acetaldehyde	1.6	3.49	2.27	26.71	17.85	18.21	4.31	15.67	13.6	12.28	3.78	16.39	6.39	6.17	8.83	13.27	15.05	11.88	51.94	17.15	100.24
2-methyl-propanal	0.4	0.55	0.57	0.15	0.07	0.07	0.02	0	0.02	0.08	0.03	0.09	0	0	0	0.21	0.11	0.12	0.23	0.11	0.31
3-methyl-butanal	0.19	0.25	0.27	0.03	0.01	0.02	0.01	0	0	0.01	0	0.02	0	0	0	0.01	0.01	0.01	0.01	0	0.01
2-methyl-butanal	0.18	0.23	0.25	0.05	0	0.02	0	0	0	0.03	0	0.03	0	0	0	0.05	0.03	0.03	0	0	0.01
Hexanal	0	0	0.01	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ketones																					
Acetone	1.46	1.93	1.8	1.51	1.59	1.52	0.29	1.13	1.23	1.27	1.42	1.38	0.62	0.83	0.43	1.39	1.35	1.38	1.12	1.19	1.13
Diacetyl	0	0.19	0.11	0.19	0.29	0.27	0.28	0.13	0.51	0.33	0.43	0.45	0	0	0.02	0.63	0.6	0.64	0	0	0

A-6 Result from HSGC analysis – Ørjasæter – Growth experiment – Raw data

Yeast	Ørjasæter																				
Temperature	-			22 °C						30 °C						37 °C					
Time, days	0			2			7			2			7			2			7		
Esters	ppm			ppm			ppm			ppm			ppm			ppm			ppm		
Ethyl acetate	0	0	0	3.19	2.53	2.36	8.41	9.13	6.34	6	4.47	4.74	12.71	6.18	6.56	4.67	4.09	3.69	6.49	4.24	2.67
Isoamyl acetate	0	0	0	0.13	0.09	0.1	0.15	0.2	0.11	0.24	0.11	0.15	0.39	0.07	0.13	0.09	0.09	0.08	0.14	0.04	0.01
Isobutyl acetate	0	0	0	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.03	0.01	0.01	0.01	0.01	0.01	0.01	0	0
Ethyl hexanoate	0	0	0	0.05	0.05	0.04	0.08	0.07	0.04	0.08	0.04	0.04	0.07	0.03	0.03	0.02	0.02	0.02	0.02	0.01	0
Ethyl heptanoate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ethyl octanoate	0	0	0.01	0.02	0	0.01	0.04	0.04	0.02	0.03	0.02	0.02	0.04	0.04	0.04	0.02	0	0.01	0.03	0.03	0.02
Higher alcohols																					
1-propanol	0.23	0	0	7.47	7.3	6.18	13.36	11.41	11.5	11.27	11.02	13.04	14.82	14.2	15.93	12.52	8.75	8.68	9.43	11.03	13.29
2-methyl-1-propanol	0.15	0.16	0.11	7.74	7.53	7.06	16.19	13.68	12.25	13.16	12.34	14.17	21.66	18.98	21.81	18.09	13.28	13.44	19.89	19.18	20.59
3-methyl-1-butanol	0.19	0.41	0.15	16.82	18.32	15.62	29.35	26.24	24.71	21.6	21.4	22.67	30.31	28.35	31.21	23.25	19.77	18.48	25.11	25.61	24.62
2-methyl-1-butanol	0.06	0.08	0.03	5.99	6.21	5.4	12.03	10.67	9.34	9.7	9.2	9.6	14.03	12.83	13.8	11.56	8.77	8.78	12.59	12.25	12.39
2-hexanol	0	0	0	0.09	0.08	0.07	0.24	0.26	0.13	0.15	0.09	0.09	0.24	0.07	0.09	0.05	0.04	0.04	0.09	0.03	0.01
Sulphur components																					
Dimethyl sulphide	0	0.01	0.01	0	0	0	0.01	0	0	0	0	0	0.01	0	0	0	0	0	0	0	0
Aldehydes																					
Acetaldehyde	1.43	2.33	1.43	22	22.69	8.89	7.71	8.71	14.29	12.22	19.04	18.32	22.56	11.57	16.73	15.79	13.06	10.42	3.91	25.91	20.18
2-methyl-propanal	0.39	0.6	0.46	0.14	0.09	0.04	0.04	0.03	0.04	0.14	0.1	0.13	0.04	0.02	0.03	0.22	0.14	0.13	0.02	0.07	0.06
3-methyl-butanal	0.2	0.29	0.24	0.02	0.02	0.01	0.01	0.01	0.01	0.02	0.02	0.02	0	0	0	0.01	0.02	0.01	0	0	0
2-methyl-butanal	0.18	0.26	0.22	0.04	0.03	0.02	0	0.01	0.02	0.04	0.03	0.03	0	0	0	0.04	0.03	0.03	0	0	0
Hexanal	0	0.01	0.01	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ketones																					
Acetone	1.48	1.72	1.67	1.58	1.46	1.6	1.21	1.25	1.25	1.52	1.3	1.37	1.26	0.92	1.08	1.3	1.13	1.2	1.19	1.06	0.91
Diacetyl	0	0.14	0.1	0.16	0.19	0.12	0.13	0.08	0.34	0.23	0.24	0.38	0	0	0	0.21	0.16	0.06	0	0	0

A-7 Result from HSGC analysis – US-05 – Growth experiment – Raw data

Yeast	US-05																				
Temperature	-			22 °C						30 °C						37 °C					
Time, days	0			2			7			2			7			2			7		
Esters	ppm			ppm			ppm			ppm			ppm			ppm			ppm		
Ethyl acetate	0	0	0	1.01	0.58	0.43	3.63	4.43	3.53	2.38	1.95	1.71	2.64	4	2.05	0.33	0.26	0.11	2.53	2.42	0.65
Isoamyl acetate	0	0	0	0.02	0	0	0.07	0.1	0.11	0.08	0.05	0.05	0.03	0.1	0.03	0.01	0.01	0	0.04	0.05	0.04
Isobutyl acetate	0	0	0	0	0	0	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0	0.02	0.02	0.01
Ethyl hexanoate	0	0	0	0.01	0.01	0.01	0.02	0.02	0.04	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0	0	0.01	0.01	0
Ethyl heptanoate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ethyl octanoate	0	0	0	0.02	0	0.02	0.03	0.03	0.05	0.02	0	0.02	0.03	0.03	0.04	0	0	0.01	0.02	0.02	0.02
Higher alcohols																					
1-propanol	0	0	0	6.62	5.69	4.99	11.78	12.35	10.92	11.95	13	11.51	15.53	16.96	16.48	3.38	3.54	3.26	16.85	17.39	3.84
2-methyl-1-propanol	0	0	0.04	9.67	7.78	7.57	27.58	25.77	22.71	25.32	23.54	21.98	47.55	42.25	42.51	19.16	19.18	17.12	40.2	40.96	24.75
3-methyl-1-butanol	0	0	0	14.95	12.76	12.84	28.16	28.87	28.67	22.92	21.56	21.01	33.79	33.8	33.42	12.91	12.98	12	27.17	28.6	15.87
2-methyl-1-butanol	0	0	0.02	4.41	3.66	3.37	10.29	10.31	10.42	7.84	7.2	6.87	12.57	12.25	11.78	3.44	3.57	3.17	12.87	13.15	4.57
2-hexanol	0	0	0	0.03	0.02	0.01	0.16	0.2	0.19	0.07	0.05	0.05	0.05	0.1	0.04	0.01	0	0	0.03	0.03	0.02
Sulphur components																					
Dimethyl sulphide	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aldehydes																					
Acetaldehyde	1.11	1.45	1.56	17.8	22.12	11.64	25.91	17.87	6.73	13.95	14.84	11.17	7.54	11.08	11.73	1.88	2.87	2.18	4.85	3.18	0.5
2-methyl-propanal	0.44	0.42	0.55	0.27	0.17	0.11	0.05	0.04	0.04	0.4	0.28	0.23	0.04	0.03	0.02	0.17	0.18	0.15	0.3	0.09	0
3-methyl-butanal	0.16	0.15	0.21	0.04	0.03	0.03	0.01	0.01	0.01	0.03	0.02	0.02	0	0.01	0	0.01	0	0	0.01	0	0
2-methyl-butanal	0.15	0.13	0.19	0.04	0.03	0.02	0	0	0	0.03	0	0.02	0	0	0.01	0	0	0.01	0.03	0	0
Hexanal	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ketones																					
Acetone	1.55	1.53	1.63	1.58	1.25	1.27	1.29	1.28	1.26	1.41	1.16	1.2	0.77	0.96	0.73	1.54	0.93	1.21	0.92	0.96	0.96
Diacetyl	0	0.11	0.18	1.96	1.53	1.45	0.11	0	0.11	0.6	0.65	0.45	0	0	0	0.71	0.65	0.84	0.19	0	0

Appendix B – Antimicrobial effect of einerlog

B-1 Microtiter plate Assay with einerlog and LAB – raw data – OD_{600}

Day	Extract type	Bacteria	Concentration of extract (g/mL)											
			0.5	0.25	0.125	0.0625	0.0313	0.0156	0.00781	0.00391	0.00195	0.000977	0.000489	0
0	Twig	*Lb. plant.*	3.481	3.053	2.165	1.883	1.131	0.463	0.204	0.175	0.164	0.16	0.157	0.157
		Lb. brevis	3.837	3.001	2.17	1.927	0.95	0.296	0.171	0.142	0.135	0.129	0.128	0.124
		Lb. buchn.	3.698	2.971	2.08	1.864	0.839	0.303	0.178	0.151	0.141	0.136	0.135	0.132
	Needle	*Lb. plant.*	2.523	1.744	1.702	0.489	0.203	0.171	0.164	0.162	0.159	0.159	0.157	0.156
		Lb. brevis	2.387	1.734	1.761	0.522	0.198	0.136	0.13	0.128	0.127	0.128	0.128	0.126
		Lb. buchn.	2.269	1.621	1.792	0.555	0.254	0.15	0.138	0.134	0.134	0.132	0.133	0.13
	Unripe berries	*Lb. plant.*	0.517	0.217	0.18	0.166	0.158	0.158	0.153	0.153	0.153	0.153	0.152	0.151
		Lb. brevis	0.432	0.199	0.159	0.142	0.131	0.129	0.126	0.124	0.122	0.125	0.124	0.123
		Lb. buchn.	0.408	0.198	0.159	0.142	0.136	0.135	0.13	0.13	0.13	0.13	0.129	0.129
	Ripe berries	*Lb. plant.*	0.293	0.224	0.182	0.171	0.161	0.167	0.157	0.155	0.154	0.157	0.153	0.152
		Lb. brevis	0.213	0.199	0.164	0.142	0.135	0.133	0.129	0.127	0.124	0.127	0.128	0.123
		Lb. buchn.	0.266	0.197	0.164	0.145	0.143	0.14	0.132	0.134	0.132	0.131	0.13	0.13
1	Twig	*Lb. plant.*	3.725	3.217	2.619	2.453	2.021	1.853	1.729	1.7	1.695	1.679	1.679	1.667
		Lb. brevis	3.888	3.142	2.512	2.283	1.322	0.619	0.492	0.449	0.472	0.496	0.473	0.505
		Lb. buchn.	3.761	3.142	2.547	2.352	1.664	1.252	1.06	1.046	1.093	1.067	1.031	0.829
	Needle	*Lb. plant.*	2.747	2.577	1.856	1.861	1.709	1.678	1.694	1.704	1.69	1.716	1.686	1.648
		Lb. brevis	2.526	2.127	0.881	0.831	0.531	0.497	0.471	0.482	0.49	0.467	0.507	0.495
		Lb. buchn.	2.469	2.277	1.345	1.325	1.096	0.998	0.959	0.951	0.998	0.968	0.984	0.918
	Unripe berries	*Lb. plant.*	2.016	1.819	1.697	1.707	1.705	1.678	1.67	1.669	1.653	1.683	1.689	1.685
		Lb. brevis	0.821	0.496	0.449	0.485	0.493	0.495	0.479	0.478	0.464	0.464	0.466	0.499
		Lb. buchn.	0.783	0.89	0.89	0.854	0.905	0.875	0.863	0.854	0.832	0.836	0.856	0.84
	Ripe berries	*Lb. plant.*	1.84	1.728	1.714	1.693	1.704	1.715	1.693	1.7	1.717	1.722	1.701	1.67
		Lb. brevis	0.617	0.624	0.501	0.475	0.482	0.466	0.434	0.458	0.472	0.511	0.485	0.496
		Lb. buchn.	1.243	1.035	1.068	1.066	0.989	0.909	0.903	0.9	0.974	0.979	0.988	0.962

Appendix C – Identification, isolation and sanger sequencing of strains

C-1 Ørjasæter – raw data

Media	Dilution											
	10^{-2}		10^{-3}		10^{-4}		10^{-5}		10^{-6}		10^{-7}	
PCA	**	**	**	**	**	**	>1000	>1000	425	390	**	**
PCA + cycloheximide	*1	*1	0	0	*1	*1	*1	0	0	0	**	**
MRS	**	**	**	**	**	**	>1000	>1000	277	328	**	**
MRS + cycloheximide	0	0	0	0	0	0	0	0	0	0	**	**
YM	**	**	**	**	**	**	>1000	>1000	408	355	34	41
Rose-Bengal	**	**	**	**	**	**	>1000	>1000	320	284	34	51

* Contamination.

** Not applicable.

C-2 Otterdal – raw data

Media	Dilution									
	10^{-1}		10^{-2}		10^{-5}		10^{-6}		10^{-7}	
PCA	*	*	*	*	659	618	78	72	13	11
MRS	*	*	*	*	728	384	78	74	10	3
YM	*	*	*	*	630	639	80	67	6	10
Rose-Bengal	*	*	*	*	561	590	80	88	11	6
MRS + cycloheximide	0	0	0	0	*	*	*	*	*	*
PCA + cycloheximide	*	*	0	0	*	*	*	*	*	*

* Not applicable.

C-3 Gausemel – raw data

Media	Dilution									
	10^{-1}		10^{-2}		10^{-5}		10^{-6}		10^{-7}	
PCA	*	*	*	*	496	491	67	59	3	7
MRS	*	*	*	*	490	464	63	65	6	9
YM	*	*	*	*	451	408	76	51	6	5
Rose-Bengal	*	*	*	*	489	531	81	66	10	9
MRS + cycloheximide	8	4	2	0	*	*	*	*	*	*
PCA + cycloheximide	*	*	0	2	*	*	*	*	*	*

* Not applicable.

C-4 Gamlegrua – raw data

Media	Dilution									
	10^{-1}		10^{-2}		10^{-5}		10^{-6}		10^{-7}	
PCA	*	*	*	*	468	437	55	65	7	11
MRS	*	*	*	*	394	263	63	46	4	9
YM	*	*	*	*	461	473	54	62	5	5
Rose-Bengal	*	*	*	*	427	483	63	53	12	6
MRS + cycloheximide	>1000	>1000	1060	1162	*	*	*	*	*	*
PCA + cycloheximide	*	*	1039	957	*	*	*	*	*	*

* Not applicable.

C-5 Results from NanoDrop™ – raw data

Primers	Sample	DNA concentration (ng/µL)	$A_{260/280}$	$A_{260/230}$
NL1 & NL4	1	8.3	2.16	1.46
	2	27.7	1.85	1.75
	3	16.8	1.92	1.99
	4	18.2	1.89	1.05
	9	26.1	1.98	1.39
	12	22.6	1.94	1.39
	24	6.4	2.21	1.08
	25	27.9	1.92	1.51
	29	27.2	1.92	1.49
	34	29.5	1.90	1.91
	39	17.2	2.05	1.20
	57	24.4	1.97	1.42
	60	13.6	1.96	1.49
	63	21.4	1.97	1.74
	67	23.6	1.92	1.98
	70	16.5	1.88	2.00
	72	11.1	1.90	1.28
	75	15.2	1.97	1.61
	78	28.1	1.92	1.98
	80	16.0	1.98	1.68
	84	18.2	1.91	1.97
	89	17.7	2.05	1.62
	91	22.4	1.95	1.74
	94	18.9	1.98	1.80
	97	21.6	1.92	1.80
	102	24.4	1.94	1.89
	107	16.2	1.97	1.96
	111	36.4	1.85	1.90
	121	29.6	1.97	1.72
	124	17.0	1.98	1.98
	140	22.1	1.97	1.98
	143	24.4	1.96	1.80
	146	14.8	1.96	1.57
	147	18.9	1.91	1.91
	150	20.6	1.94	1.69
	153	26.7	1.90	1.92
	155	28.6	1.92	1.82
ITS1 & ITS4	9	22.1	1.95	1.51
	12	24.3	1.93	1.48
	25	24.8	1.99	1.49
	29	23.5	1.93	1.35
	39	14.7	2.02	1.18
	57	17.6	2.04	1.45
1F & 5R	50	16.7	1.97	1.71
	54	7.5	2.37	1.04
	149	17.2	1.99	2.00
	118	27.1	1.92	1.92
	119	21.1	2.02	1.91
	120	18.7	2.02	1.91

C-6 Example of a result from gel electrophoresis with NL1 and NL4 primers

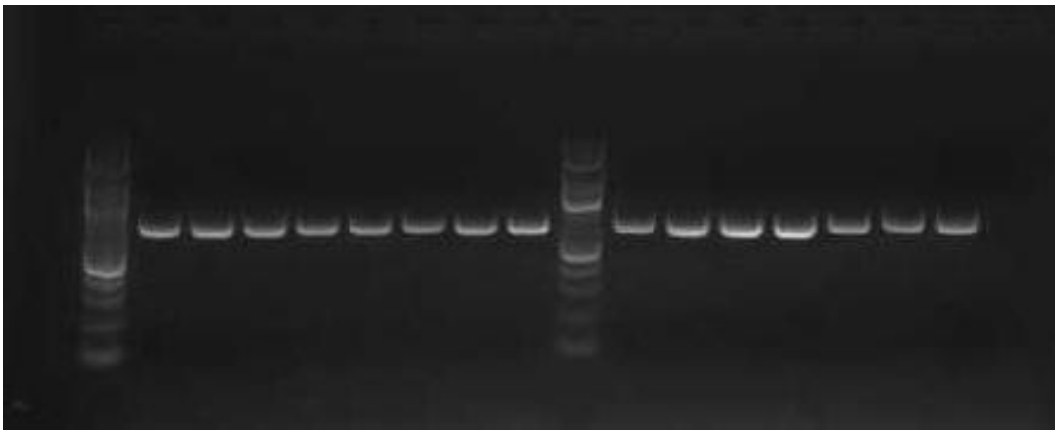


Figure 4.2. *Result from gel electrophoresis. The ladders are placed in wells number 1 and 10. The samples depicted, from left to right, are isolates 78, 80, 84, 89, 91, 94, 97, 102, 107, 111, 121, 124, 140, 143 and 146. The primers used are NL1 and NL4, resulting in products with sizes of approximately 800 bp.*

Appendix D - Microbiota

D-1 Results from microbiota assay – NanoDrop measurements

Results from the DNA extraction done prior to the microbiota analysis, measured using Nanodrop.

Kveik, number & name	Concentration, ng/ml	260/280	260/230
1. Sigmund	2.9	1.82	2.20
2. Midtbust	3.1	1.90	-0.84
3. Stalljen	9.1	2.05	0.6
4. Gausemel	6.0	1.93	0.76
5. Ebbegarden	2.2	2.34	0.12
6. Tormodgarden	3.9	1.86	0.07
7. Gamlegrua	6.4	1.99	-9.11
8. Otterdal	12.6	1.86	5.00
9. Espe	5.9	2.15	1.82
10. Ørjasæter	3.0	2.47	0.41

D-2 Results from 16S rRNA microbiota assay

	Sigmund	Midtbust	Stalljen	Gausemel	Ebbe-garden	Tormod-garden	Gamle-grua	Otterdal	Espe	Ørja-sæter
Acetobacter	1691	38781	630	873	1024	30132	768	617	70117	1709
Lb. brevis	14	5	15207	35665	1084	2499	31897	26306	1012	631
Lb. brevis	0	0	3696	7735	235	669	6954	5265	240	110
Lb. brevis	0	0	3706	6821	256	723	6483	5871	240	108
Lb. backii	154	19390	0	0	0	0	0	0	0	175
Lb. backii	126	18824	0	0	7	0	0	0	0	167
Acetobacter	169	0	98	127	158	239	118	91	15249	170
Lb. brevis	0	0	1373	4126	81	99	2648	796	100	0
Lb. backii	72	8737	0	0	0	0	0	0	0	92
Lactococcus lactis	1071	47	597	1051	1458	1305	136	446	589	38
Lb. brevis	0	0	226	1101	0	0	1458	419	0	0
Lb. brevis	0	0	1139	1149	33	41	74	14	31	0
Other	19564	1012	8469	5512	15310	2039	7220	367	459	798

D-3 Result from ITS rRNA microbiota assay

	Sigmund	Midtbust	Stalljen	Gausemel	Ebbegarden	Tormodgarden	Gamlegrua	Otterdal	Espe	Ørjasæter
S. cerevisiae	0	28638	37813	37133	26284	0	0	30707	34405	41448
S. cerevisiae	0	0	0	0	0	33599	35896	0	0	0
S. cerevisiae	0	1074	1737	1653	1142	0	0	1177	7306	3374
S. cerevisiae	15534	0	0	0	0	0	0	0	0	0
S. cerevisiae	8924	0	0	0	0	0	0	0	0	0
S. cerevisiae	0	0	0	0	0	1992	2601	0	0	0
S. cerevisiae	0	532	510	493	339	0	418	381	447	506
S. cerevisiae	0	0	688	227	486	418	0	552	0	772
S. cerevisiae	0	0	585	533	396	0	0	706	492	0
S. cerevisiae	0	0	0	418	0	0	0	391	955	825
S. cerevisiae	1669	0	0	0	0	0	0	0	0	0
S. cerevisiae	0	233	234	218	154	0	230	0	238	218
S. cerevisiae	0	0	0	178	197	0	0	164	198	334
S. cerevisiae	0	0	0	455	0	0	602	0	0	0
S. cerevisiae	0	0	0	0	0	0	593	0	0	377
S. cerevisiae	0	0	0	0	0	0	0	0	730	0
S. cerevisiae	0	114	103	113	0	0	0	105	166	115
S. cerevisiae	0	91	67	70	94	0	0	70	115	67
S. cerevisiae	0	251	238	0	0	0	0	0	0	0
S. cerevisiae	0	0	0	0	0	0	0	251	234	0
S. cerevisiae	0	0	0	109	0	0	0	0	152	189
S. cerevisiae	0	94	0	0	0	0	0	125	0	157
S. cerevisiae	108	0	0	0	0	243	0	0	0	0
S. cerevisiae	171	176	0	0	0	0	0	0	0	0
S. cerevisiae	303	0	0	0	0	0	0	0	0	0
S. cerevisiae	0	55	56	66	0	0	0	0	47	64
S. cerevisiae	0	0	0	0	0	0	255	0	0	0
S. cerevisiae	0	86	57	54	0	0	0	0	54	0
S. cerevisiae	0	0	0	0	0	111	140	0	0	0
S. cerevisiae	0	0	69	31	31	0	0	0	59	51
S. cerevisiae	238	0	0	0	0	0	0	0	0	0
S. cerevisiae	0	0	46	67	0	0	50	0	0	66
S. cerevisiae	0	0	0	0	0	0	0	44	92	85
S. cerevisiae	0	44	43	56	47	0	0	0	0	28
S. cerevisiae	0	0	0	66	0	0	0	0	151	0
S. cerevisiae	215	0	0	0	0	0	0	0	0	0
S. cerevisiae	0	0	35	32	33	0	0	0	66	48
S. cerevisiae	0	0	0	93	117	0	0	0	0	0
S. cerevisiae	0	0	0	0	0	186	0	0	0	0
S. cerevisiae	0	41	28	41	26	0	0	41	0	0
S. cerevisiae	0	0	0	0	67	0	0	59	48	0
S. cerevisiae	0	33	39	11	0	0	10	0	52	11
S. cerevisiae	0	0	33	46	0	0	29	0	0	41
S. cerevisiae	0	0	48	32	23	0	0	0	0	37
S. cerevisiae	0	34	6	27	25	0	22	0	0	25
S. cerevisiae	137	0	0	0	0	0	0	0	0	0
S. cerevisiae	0	0	0	0	0	97	40	0	0	0
S. cerevisiae	0	0	0	0	0	0	136	0	0	0
S. cerevisiae	0	0	0	0	0	0	0	0	123	0
S. cerevisiae	0	0	31	0	0	0	0	0	0	81
S. cerevisiae	0	0	0	28	0	0	0	0	47	36
S. cerevisiae	0	0	0	29	0	0	0	0	42	34
S. cerevisiae	0	0	0	0	0	0	104	0	0	0
S. cerevisiae	0	0	0	100	0	0	0	0	0	0
S. cerevisiae	0	0	0	0	0	46	54	0	0	0

Appendix E – Brewing

E-1 Raw data from all eight brews.

Beer		Wort	After fermentation			After two weeks of maturation in cold storage						
Brew	Yeast/kveik	Original Gravity	Gravity	Alc. (% ABV)	°Plato	Final Gravity	Alc. (%ABV)	°Plato	CO_2	Color (EBC)	Ea (% w/w)	ADF (% w/w)
1	Gamlegrua	1.080	1.018	7.6		1.018	7.9	5.1	1.7	14.1	4.9	74.5
1	Gausemel	1.080	1.015	8.3		1.016	8.8	4.7	1.7	16.0	4.5	77.8
1	Ørjasæter	1.080	1.015	8.2		1.016	8.7	4.7	1.6	15.5	4.5	77.6
1	US-05	1.080	1.015	8.2		1.014	9.0	4.2	2.2	14.3	3.9	80.6
2	Gamlegrua	1.074	1.016	7.7		1.015	8.0	4.5	1.6	18.1	4.3	77.0
2	Gausemel	1.074	1.015	8.0		1.014	8.4	4.1		21.4	3.8	79.7
2	Ørjasæter	1.074	1.019	7.3		1.017	7.9	4.9		28.9	4.6	75.4
2	US-05	1.074	1.010	8.1	3.2	1.010	8.5	3.1	2.1	13.6	2.8	84.5
2	US-05	1.074	1.010	8.5	3.2	1.010	9.0	3.1	2.1	14.1	2.9	85.0
3	Gamlegrua	1.071	1.015	7.1		1.015	7.5	4.2	1.8	28.5	4.0	72.1
3	Gamlegrua	1.071	1.018	6.9		1.017	7.4	4.8		17.6	4.6	74.6
3	Gamlegrua	1.071	1.019	6.9		1.017	7.3	4.9		17.0	4.6	74.0
3	US-05	1.071	1.013	7.5		1.013	8.0	3.8		16.5	3.5	80.5
3	US-05	1.071	1.013	7.4		1.013	7.9	3.8		15.0	3.6	80.2
3	US-05	1.071	1.014	7.4		1.013	7.9	2.8		15.8	3.6	79.8
4	Gamlegrua	1.067	1.013	7.3	4.0	1.014	7.6	4.0	1.7	22.2	3.8	78.3
4	Gausemel	1.067	1.013	7.2	3.9	1.013	7.5	3.8	1.5	23.5	3.6	79.2
4	Ørjasæter	1.067	1.014	6.7	3.9	1.013	7.2	3.8	2.3	30.5	3.5	79.0
4	US-05	1.067	1.010	6.9	3.1	1.009	7.6	2.9	2.6	13.1	2.5	84.7
4	US-05	1.067	1.012	7.3	3.5	1.011	8.0	3.4	2.2	12.8	3.1	82.3
5	Gamlegrua	1.074	1.015	7.7	4.3	1.015	7.9	4.3	1.5	16.0	4.2	77.5
5	Gausemel	1.074	1.015	7.7	4.2	1.015	8.1	4.4	2.2	17.8	4.1	78.2
5	Ørjasæter	1.074	1.015	7.7	4.4	1.015	8.0	4.4	1.8	18.4	4.2	77.4
5	US-05	1.074	1.011	7.4	3.3	1.011	7.8	3.3	1.6	17.1	3.1	81.9
6	Gamlegrua	1.081	1.020	7.8	5.6	1.020	8.1	5.6	1.5	23.1	5.4	72.6
6	Gausemel	1.081	1.017	8.6	4.8	1.017	8.9	4.8	1.7	21.6	4.6	77.4
6	Ørjasæter	1.081	1.016	8.7	4.6	1.016	9.0	4.7	1.4	19.2	4.5	77.8
6	US-05	1.081	1.017	8.5	4.7	1.017	9.0	4.7	1.6	15.8	4.5	77.9
7	Gamlegrua	1.080	1.026	7.0	7.1	1.025	7.7	6.8	2.2	15.9	6.6	67.3
7	Gausemel	1.080	1.023	7.5	6.3	1.023	8.0	6.2	1.9	18.1	6.0	70.3
7	Ørjasæter	1.080	1.027	6.8	7.4	1.026	7.7	7.0	2.6	17.1	6.7	67.0
7	US-05	1.080	1.012	8.6	3.5	1.012	9.0	3.5	1.7	16.9	3.3	83.3
8	Gamlegrua	1.083	1.017	8.7	4.8	1.017	9.0	4.8	1.8	21.3	4.6	77.7
8	Gamlegrua	1.083	1.021	8.2	5.8	1.021	8.4	5.2	1.6	22.5	5.6	72.6

E-2 An overview of the fermentation time for all pilot-scale brews

	Yeast / kveik	Temperature (°C)	Days of fermentation			
			Parallel 1	Parallel 2	Parallel 3	Parallel 4
W/einer	US-05	30	6	6	6	-
Without einerlog	Gamlegrua	30	6	6	6	-
		22	9	9	-	-
		30	6	7	10	-
		37	8	7	7	-
	Gausemel	30	13	7	14	-
		37	8	7	7	-
	Ørjasæter	30	13	7	14	-
		37	8	7	7	-
	US-05	22	22	22	20	22
		30	6	7	7	10

E-3 Phenolic analysis – raw data

Kveik/yeast	Additives	Fermentation temperature (°C)	GAE mg/L			Average (GAE mg/L)	Standard deviation
			1st	2nd	3rd		
Gamlegrua	-	37	674	696	630	666	34
US-05	-	22	792	790	734	772	33
Gamlegrua	Einerlog	30	864	850	834	849	15
Gamlegrua	Einerlog	30	891	904	908	901	9
Gamlegrua	Einerlog	30	968	996	1028	997	30
US-05	Einerlog	30	868	878	930	892	33
US-05	Einerlog	30	840	896	924	886	43
US-05	Einerlog	30	869	861	836	855	17
Gamlegrua	-	30	752	736	724	737	14
US-05	-	30	676	730	730	712	31
US-05	-	22	675	677	683	678	4
Gamlegrua	Einerlog	22	974	978	993	981	10
Wort	Einerlog	-	1180	1244	1194	1206	34
Wort	-	-	894	898	904	898	5

E-4 Result from HSGC – Einerlog, and wort with and without einerlog - Brewing

	Wort						Wort w/einerlog		Einerlog
Esters	ppm						ppm		ppm
Ethyl acetate	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Isoamyl acetate	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Isobutyl acetate	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ethyl hexanoate	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.16
Ethyl heptanoate	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ethyl octanoate	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.00
Higher alcohols									
1-propanol	0.00	0.20	0.21	0.00	0.00	0.00	0.20	0.20	0.00
2-methyl-1-propanol	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	0.00
3-methyl-1-butanol	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2-methyl-1-butanol	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2-hexanol	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sulphur components									
Dimethyl sulphide	0.01	0.01	0.07	0.04	0.04	0.16	0.02	0.01	0.00
Aldehydes									
Acetaldehyde	0.15	0.21	0.59	0.44	0.37	0.49	0.58	0.50	0.40
2-methyl-propanal	0.13	0.12	0.33	0.19	0.18	0.06	0.18	0.19	0.00
3-methyl-butanal	0.06	0.06	0.14	0.09	0.09	0.00	0.06	0.08	0.00
2-methyl-butanal	0.06	0.06	0.17	0.09	0.09	0.02	0.08	0.09	0.00
Hexanal	0.01	0.01	0.02	0.01	0.01	0.00	0.01	0.00	0.00
Ketones									
Acetone	0.18	0.19	0.41	0.30	0.24	0.21	0.25	0.11	0.06
Diacetyl	0.00	0.00	0.00	0.00	0.06	0.08	0.00	0.00	0.00

E-5 Result from HSGC – Gamlegrua – Brewing – After fermentation and after maturation

Yeast	Gamlegrua											
Temperature	30 °C						37 °C					
	After fermentation			After maturation			After fermentation			After maturation		
Esters	ppm			ppm			ppm			ppm		
Ethyl acetate	36.44	29.95	34.81	43.69	29.97	32.05	24.93	25.60	25.78	23.62	22.83	25.57
Isoamyl acetate	0.86	1.17	0.82	1.60	1.00	0.72	0.71	1.11	0.59	0.64	0.78	0.52
Isobutyl acetate	0.05	0.06	0.06	0.09	0.06	0.05	0.05	0.06	0.04	0.04	0.06	0.04
Ethyl hexanoate	0.11	0.07	0.13	0.14	0.08	0.12	0.04	0.03	0.06	0.06	0.06	0.06
Ethyl heptanoate	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ethyl octanoate	0.11	0.06	0.11	0.12	0.08	0.10	0.04	0.02	0.07	0.05	0.05	0.06
Higher alcohols												
1-propanol	22.77	18.58	15.16	18.72	18.93	14.53	13.86	23.39	13.83	14.32	22.58	13.40
2-methyl-1-propanol	30.23	34.19	32.52	32.28	34.72	31.58	28.11	31.14	26.69	29.39	29.89	26.78
3-methyl-1-butanol	39.09	55.10	40.62	52.77	55.83	39.95	38.61	55.59	31.92	40.50	53.11	32.15
2-methyl-1-butanol	17.58	20.87	16.74	20.76	21.30	16.43	15.70	20.08	13.65	16.63	19.32	13.88
2-hexanol	0.74	0.54	0.74	0.90	0.53	0.66	0.40	0.37	0.44	0.40	0.37	0.43
Sulphur components												
Dimethyl sulphide	0.05	0.02	0.01	0.06	0.01	0.01	0.00	0.01	0.01	0.00	0.01	0.01
Aldehydes												
Acetaldehyde	5.61	4.41	13.29	1.79	3.79	11.50	6.48	6.39	8.65	8.67	5.92	12.96
2-methyl-propanal	0.09	0.09	0.10	0.01	0.00	0.03	0.09	0.09	0.08	0.00	0.00	0.04
3-methyl-butanal	0.01	0.01	0.01	0.00	0.00	0.00	0.01	0.01	0.01	0.00	0.00	0.00
2-methyl-butanal	0.00	0.02	0.03	0.03	0.02	0.01	0.00	0.00	0.02	0.02	0.03	0.01
Hexanal	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ketones												
Acetone	0.42	0.50	0.52	0.26	0.51	0.53	0.44	0.60	0.48	0.41	0.49	0.53
Diacetyl	0.00	0.18	0.00	0.00	0.14	0.00	0.00	0.00	0.00	0.00	0.00	0.00

E-6 Result from HSGC – Gausemel – Brewing – After fermentation and after maturation

Yeast	Gausemel											
Temperature	30 °C						37 °C					
	After fermentation			After maturation			After fermentation			After maturation		
Esters	ppm			ppm			ppm			ppm		
Ethyl acetate	40.47	24.22	28.49	38.34	23.96	27.82	17.78	23.76	24.61	21.96	23.08	23.23
Isoamyl acetate	1.82	0.97	0.54	0.83	0.80	0.51	0.48	1.05	0.57	0.62	1.02	0.45
Isobutyl acetate	0.09	0.05	0.04	0.05	0.05	0.04	0.03	0.06	0.04	0.04	0.06	0.04
Ethyl hexanoate	0.15	0.07	0.13	0.11	0.08	0.14	0.03	0.03	0.06	0.10	0.10	0.07
Ethyl heptanoate	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ethyl octanoate	0.11	0.06	0.12	0.09	0.09	0.13	0.03	0.03	0.08	0.07	0.06	0.07
Higher alcohols												
1-propanol	18.71	22.34	15.26	23.60	22.21	16.59	12.37	21.65	15.49	13.72	22.64	15.35
2-methyl-1-propanol	31.73	37.35	31.26	31.94	36.31	33.49	28.53	32.59	31.27	31.21	33.66	31.14
3-methyl-1-butanol	51.28	62.00	35.41	41.23	60.50	38.28	36.95	52.40	35.61	40.48	54.28	36.09
2-methyl-1-butanol	20.53	22.32	14.96	18.60	21.78	16.26	15.43	20.04	14.93	17.07	20.81	14.90
2-hexanol	0.87	0.44	0.65	0.73	0.44	0.64	0.28	0.33	0.43	0.44	0.54	0.39
Sulphur components												
Dimethyl sulphide	0.04	0.01	0.01	0.04	0.01	0.01	0.00	0.01	0.01	0.01	0.01	0.01
Aldehydes												
Acetaldehyde	4.02	4.89	12.49	6.98	1.77	11.68	7.01	5.85	8.20	7.43	10.74	8.44
2-methyl-propanal	0.06	0.06	0.10	0.03	0.00	0.03	0.09	0.15	0.09	0.00	0.04	0.02
3-methyl-butanal	0.01	0.00	0.01	0.00	0.00	0.00	0.01	0.01	0.01	0.00	0.00	0.00
2-methyl-butanal	0.00	0.04	0.00	0.00	0.02	0.00	0.00	0.00	0.02	0.00	0.03	0.00
Hexanal	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ketones												
Acetone	0.45	0.50	0.40	0.53	0.36	0.00	0.49	0.49	0.52	0.53	0.55	0.54
Diacetyl	0.15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00

E-7 Result from HSGC – Ørjasæter – Brewing – After fermentation and after maturation

Yeast	Ørjasæter											
Temperature	30 °C						37 °C					
	After fermentation			After maturation			After fermentation			After maturation		
Esters	ppm			ppm			ppm			ppm		
Ethyl acetate	35.23	15.63	28.52	40.01	17.64	26.95	10.1	23.52	22.87	12.61	21.43	22.93
Isoamyl acetate	0.89	0.42	0.60	0.99	0.44	0.55	0.21	0.95	0.61	0.30	0.84	0.55
Isobutyl acetate	0.05	0.02	0.04	0.06	0.03	0.04	0.02	0.06	0.04	0.02	0.05	0.04
Ethyl hexanoate	0.10	0.06	0.14	0.13	0.06	0.13	0.02	0.03	0.05	0.06	0.06	0.07
Ethyl heptanoate	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ethyl octanoate	0.10	0.04	0.11	0.10	0.05	0.11	0.02	0.03	0.07	0.04	0.05	0.06
Higher alcohols												
1-propanol	20.62	15.70	18.31	21.27	15.99	17.76	9.30	21.62	14.68	10.77	21.96	14.74
2-methyl-1-propanol	37.19	31.85	39.70	39.11	31.81	37.71	27.42	36.50	33.32	31.00	36.52	33.48
3-methyl-1-butanol	44.49	52.59	51.90	46.91	52.98	47.12	34.66	49.81	37.51	39.9	51.09	37.33
2-methyl-1-butanol	20.61	19.46	22.47	22.11	19.35	19.90	13.99	20.88	15.78	16.22	21.25	16.01
2-hexanol	0.65	0.28	0.56	0.75	0.32	0.52	0.14	0.32	0.38	0.23	0.34	0.38
Sulphur components												
Dimethyl sulphide	0.05	0.01	0.01	0.05	0.01	0.01	0.00	0.01	0.01	0.00	0.01	0.01
Aldehydes												
Acetaldehyde	8.43	16.32	147.79	11.21	8.24	53.08	7.75	7.02	12.20	10.92	15.72	7.54
2-methyl-propanal	0.10	0.14	0.37	0.02	0.00	0.06	0.09	0.06	0.10	0.02	0.01	0.02
3-methyl-butanal	0.01	0.02	0.02	0.00	0.00	0.00	0.01	0.00	0.01	0.00	0.00	0.00
2-methyl-butanal	0.03	0.04	0.05	0.02	0.01	0.00	0.00	0.00	0.02	0.00	0.02	0.00
Hexanal	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ketones												
Acetone	0.34	0.51	0.69	0.64	0.51	0.68	0.43	0.44	0.45	0.56	0.74	0.60
Diacetyl	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.14	0.01	0.03	0.00

E-8 Result from HSGC – US-05 – Brewing – After fermentation and after maturation

Yeast	US-05															
Temperature	30 °C								22 °C							
	After fermentation				After maturation				After fermentation				After maturation			
Esters	ppm				ppm				ppm				ppm			
Ethyl acetate	24.88	10.74	18.09	28.74	26.81	11.51	17.66	28.86	31.71	29.92	20.19	28.18	27.61	27.82	20.12	30.65
Isoamyl acetate	1.09	0.33	0.94	1.20	0.97	0.31	0.82	1.13	0.90	0.88	0.47	0.85	0.66	0.74	0.37	0.77
Isobutyl acetate	0.09	0.07	0.11	0.11	0.09	0.07	0.10	0.11	0.04	0.04	0.05	0.04	0.03	0.04	0.05	0.04
Ethyl hexanoate	0.07	0.03	0.05	0.07	0.07	0.04	0.06	0.07	0.07	0.07	0.05	0.07	0.05	0.06	0.05	0.06
Ethyl heptanoate	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ethyl octanoate	0.11	0.04	0.07	0.11	0.10	0.06	0.07	0.11	0.13	0.11	0.11	0.11	0.09	0.10	0.09	0.08
Higher alcohols																
1-propanol	27.74	37.17	37.74	25.79	27.65	37.10	36.63	26.88	32.87	31.74	33.86	31.11	32.80	32.73	35.21	30.59
2-methyl-1-propanol	61.42	120.52	103.16	71.99	62.22	118.61	100.64	74.15	47.28	44.91	62.80	48.85	47.53	46.23	63.96	49.61
3-methyl-1-butanol	59.13	77.91	82.54	66.67	60.16	81.28	82.28	68.97	65.36	64.70	60.94	63.23	65.12	66.51	62.00	63.30
2-methyl-1-butanol	18.32	26.90	26.06	19.56	18.78	26.80	25.78	20.46	22.17	22.24	22.35	23.21	22.17	22.65	22.16	23.62
2-hexanol	0.93	0.53	0.78	0.88	0.85	0.54	0.73	0.86	1.33	1.32	1.01	1.42	1.07	1.13	0.94	1.46
Sulphur components																
Dimethyl sulphide	0.02	0.02	0.03	0.03	0.03	0.01	0.03	0.04	0.06	0.04	0.02	0.02	0.03	0.03	0.02	0.02
Aldehydes																
Acetaldehyde	3.98	10.14	5.48	3.27	4.72	6.78	6.67	7.33	17.55	17.66	47.14	14.93	6.27	7.82	1.00	8.77
2-methyl-propanal	0.04	0.08	0.04	0.04	0.01	0.00	0.00	0.00	0.06	0.07	0.12	0.04	0.02	0.00	0.00	0.04
3-methyl-butanal	0.01	0.02	0.01	0.01	0.00	0.00	0.00	0.00	0.02	0.02	0.02	0.01	0.00	0.00	0.00	0.01
2-methyl-butanal	0.00	0.01	0.02	0.00	0.00	0.02	0.02	0.02	0.02	0.01	0.02	0.01	0.02	0.01	0.00	0.00
Hexanal	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ketones																
Acetone	0.26	0.55	0.39	0.47	0.41	0.54	0.56	0.69	0.42	0.49	0.53	0.40	0.53	0.53	0.28	0.59
Diacetyl	0.12	0.47	0.12	0.16	0.06	0.00	0.00	0.34	0.00	0.06	0.01	0.08	0.04	0.00	0.00	0.00

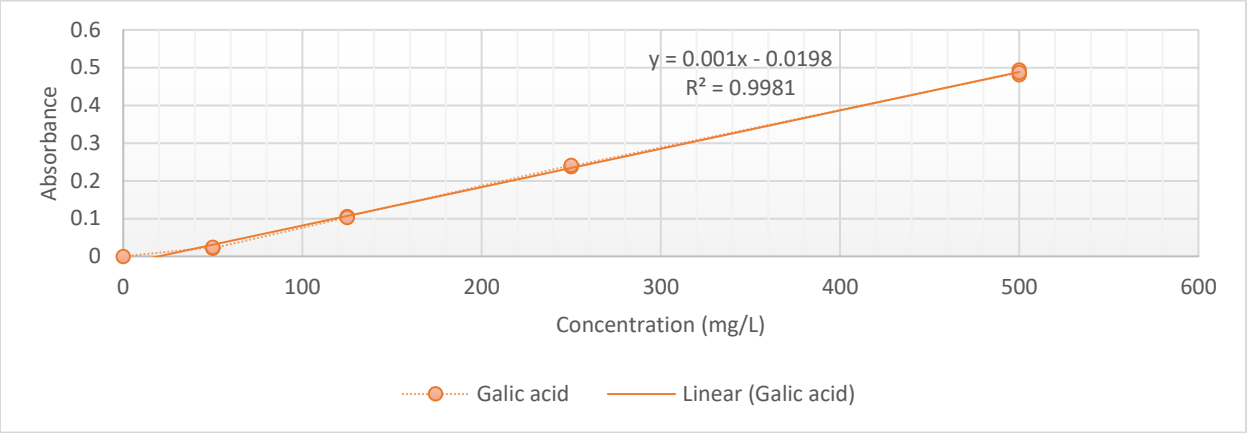
E-9 Result from HSGC – Brewing with einerlog – After fermentation and after maturation

	Gamlegrua						US-05						Gamlegrua			
	30 °C						30 °C						22 °C			
	After fermentation			After maturation			After fermentation			After maturation			After fermentation		After maturation	
Esters	ppm			ppm			ppm			ppm			ppm		ppm	
Ethyl acetate	25.70	25.42	23.04	19.26	22.52	20.74	15.41	17.44	19.55	16.98	17.28	20.81	38.59	36.85	37.40	36.54
Isoamyl acetate	0.99	0.85	0.70	0.54	0.57	0.51	0.65	0.83	0.95	0.67	0.73	0.91	2.17	1.72	1.63	1.38
Isobutyl acetate	0.05	0.05	0.05	0.03	0.04	0.04	0.07	0.08	0.09	0.07	0.07	0.08	0.09	0.08	0.08	0.08
Ethyl hexanoate	0.07	0.08	0.07	0.05	0.06	0.07	0.04	0.05	0.06	0.05	0.05	0.06	0.13	0.16	0.12	0.14
Ethyl heptanoate	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ethyl octanoate	0.05	0.05	0.05	0.05	0.06	0.06	0.04	0.03	0.02	0.05	0.07	0.07	0.09	0.12	0.08	0.12
Higher alcohols																
1-propanol	17.38	20.73	19.08	17.71	20.41	18.65	31.38	30.22	28.51	34.73	32.63	30.79	28.13	27.38	33.44	29.56
2-methyl-1-propanol	31.29	30.07	29.90	30.21	29.02	28.85	79.00	75.26	66.30	85.00	78.53	70.58	35.19	27.69	37.88	28.25
3-methyl-1-butanol	51.07	44.16	41.91	49.60	43.36	41.18	70.84	67.77	62.63	77.74	71.97	67.60	65.10	55.24	70.09	55.94
2-methyl-1-butanol	20.33	19.00	18.75	19.86	18.35	18.11	22.03	20.81	19.39	23.78	22.20	20.78	24.76	19.47	26.06	19.59
2-hexanol	0.47	0.46	0.41	0.32	0.37	0.35	0.58	0.68	0.80	0.61	0.66	0.82	0.80	0.85	0.76	0.82
Sulphur components																
Dimethyl sulphide	0.01	0.01	0.01	0.00	0.01	0.01	0.01	0.02	0.02	0.02	0.02	0.03	0.11	0.08	0.08	0.08
Aldehydes																
Acetaldehyde	3.53	2.48	3.61	5.96	6.93	8.12	3.81	3.32	3.70	10.27	9.73	3.70	16.78	16.47	3.81	3.75
2-methyl-propanal	0.07	0.07	0.07	0.00	0.02	0.03	0.04	0.03	0.04	0.00	0.00	0.00	0.07	0.10	0.05	0.05
3-methyl-butanal	0.01	0.01	0.01	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.00	0.02	0.02	0.00	0.00
2-methyl-butanal	0.03	0.00	0.00	0.02	0.02	0.02	0.00	0.03	0.00	0.02	0.00	0.02	0.02	0.02	0.02	0.02
Hexanal	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ketones																
Acetone	0.54	0.33	0.32	0.33	0.37	0.36	0.33	0.37	0.42	0.46	0.48	0.34	0.46	0.55	0.69	0.51
Diacetyl	0.13	0.00	0.00	0.01	0.00	0.01	0.00	0.00	0.10	0.00	0.05	0.01	0.28	0.11	0.00	0.00

E-10 °Brix – °Plato – Specific Gravity: Conversion Table

Brix	Plato	SG
0.0	0.0000	1.0000
0.2	0.1970	1.0008
0.4	0.3970	1.0016
0.6	0.5970	1.0024
0.8	0.7970	1.0031
1.0	0.9970	1.0039
1.2	1.1970	1.0047
1.4	1.3971	1.0054
1.6	1.5971	1.0062
1.8	1.7971	1.0070
2.0	1.9972	1.0078
2.2	2.1972	1.0086
2.4	2.3973	1.0094
2.6	2.5973	1.0101
2.8	2.7974	1.0109
3.0	2.9975	1.0117
3.2	3.1975	1.0125
3.4	3.3976	1.0133
3.6	3.5977	1.0141
3.8	3.7977	1.0149
4.0	3.9978	1.0157
4.2	4.1979	1.0165
4.4	4.3980	1.0173
4.6	4.5981	1.0181
4.8	4.7982	1.0189
5.0	4.9983	1.0197
5.2	5.1984	1.0205
5.4	5.3985	1.0213
5.6	5.5986	1.0221
5.8	5.7987	1.0229
6.0	5.9988	1.0237
6.2	6.1989	1.0245
6.4	6.3990	1.0253
6.6	6.5991	1.0261
6.8	6.7992	1.0269
7.0	6.9994	1.0277
7.2	7.1995	1.0285
7.4	7.3996	1.0294
7.6	7.5997	1.0302
7.8	7.7998	1.0310
8.0	7.9999	1.0318
8.2	8.2000	1.0326
8.4	8.4002	1.0334
8.6	8.6003	1.0343
8.8	8.8004	1.0351
9.0	9.0005	1.0359
9.2	9.2006	1.0367
9.4	9.4007	1.0376
9.6	9.6009	1.0384
9.8	9.801	1.0392
10.0	10.0011	1.0400
10.2	10.2012	1.0409
10.4	10.4013	1.0417
10.6	10.6014	1.0425
10.8	10.8015	1.0434
11.0	11.0016	1.0442
11.2	11.2017	1.0450
11.4	11.4018	1.0459
11.6	11.6019	1.0467
11.8	11.8020	1.0475
12.0	12.0021	1.0484
12.2	12.2022	1.0492
12.4	12.4023	1.0501
12.6	12.6024	1.0509
12.8	12.8025	1.0518
13.0	13.0026	1.0526
13.2	13.2027	1.0534
13.4	13.4027	1.0543
13.6	13.6028	1.0551
13.8	13.8029	1.0560
14.0	14.0030	1.0568
14.2	14.2030	1.0577
14.4	14.4031	1.0586
14.6	14.6031	1.0594
14.8	14.8032	1.0603
15.0	15.0032	1.0611
15.2	15.2033	1.0620
15.4	15.4033	1.0628
15.6	15.6033	1.0637
15.8	15.8034	1.0646
16.0	16.0034	1.0654
16.2	16.2034	1.0663
16.4	16.4034	1.0672
16.6	16.6034	1.0680
16.8	16.8034	1.0689
17.0	17.0034	1.0698
17.2	17.2034	1.0706
17.4	17.4034	1.0715
17.6	17.6034	1.0724
17.8	17.8034	1.0733
18.0	18.0033	1.0741
18.2	18.2033	1.0750
18.4	18.4033	1.0759
18.6	18.6032	1.0768
18.8	18.8032	1.0777
19.0	19.0031	1.0785
19.2	19.2030	1.0794
19.4	19.4030	1.0803
19.6	19.6029	1.0812
19.8	19.8028	1.0821
20.0	20.0027	1.0830
20.2	20.2026	1.0839
20.4	20.4025	1.0848
20.6	20.6024	1.0857
20.8	20.8023	1.0866
21.0	21.0021	1.0875
21.2	21.2020	1.0884
21.4	21.4018	1.0892
21.6	21.6017	1.0901
21.8	21.8015	1.0911
22.0	22.0014	1.0920
22.2	22.2012	1.0929
22.4	22.4010	1.0938
22.6	22.6008	1.0947
22.8	22.8006	1.0956
23.0	23.0004	1.0965
23.2	23.2002	1.0974
23.4	23.4000	1.0983
23.6	23.5997	1.0992
23.8	23.7995	1.1001
24.0	23.9992	1.1011
24.2	24.1990	1.1020
24.4	24.3987	1.1029
24.6	24.5984	1.1038
24.8	24.7982	1.1047
25.0	24.9979	1.1057
25.2	25.1976	1.1066
25.4	25.3972	1.1075
25.6	25.5969	1.1084
25.8	25.7966	1.1094
26.0	25.9963	1.1103
26.2	26.1959	1.1112
26.4	26.3956	1.1122
26.6	26.5952	1.1131
26.8	26.7948	1.1140
27.0	26.9944	1.1150
27.2	27.1940	1.1159
27.4	27.3936	1.1168
27.6	27.5932	1.1178
27.8	27.7928	1.1187
28.0	27.9924	1.1197
28.2	28.1919	1.1206
28.4	28.3915	1.1216
28.6	28.5910	1.1225
28.8	28.7905	1.1235
29.0	28.9901	1.1244
29.2	29.1896	1.1254
29.4	29.3891	1.1263
29.6	29.5886	1.1273
29.8	29.7880	1.1282
30.0	29.9875	1.1292
30.2	30.1870	1.1302
30.4	30.3864	1.1311
30.6	30.5859	1.1321
30.8	30.7853	1.1330
31.0	30.9847	1.1340
31.2	31.1841	1.1350
31.4	31.3835	1.1359
31.6	31.5829	1.1369
31.8	31.7823	1.1379
32.0	31.9817	1.1389
32.2	32.1810	1.1398
32.4	32.3804	1.1408
32.6	32.5797	1.1418
32.8	32.7791	1.1428
33.0	32.9784	1.1437
33.2	33.1777	1.1447
33.4	33.3770	1.1457
33.6	33.5763	1.1467
33.8	33.7756	1.1477
34.0	33.9749	1.1487
34.2	34.1741	1.1497
34.4	34.3734	1.1507
34.6	34.5727	1.1516
34.8	34.7719	1.1526
35.0	34.9711	1.1536
35.2	35.1703	1.1546
35.4	35.3695	1.1556
35.6	35.5687	1.1566
35.8	35.7679	1.1576
36.0	35.9671	1.1586
36.2	36.1663	1.1596
36.4	36.3655	1.1606
36.6	36.5646	1.1617
36.8	36.7638	1.1627
37.0	36.9629	1.1637
37.2	37.1620	1.1647
37.4	37.3612	1.1657
37.6	37.5603	1.1667
37.8	37.7594	1.1677
38.0	37.9585	1.1688
38.2	38.1576	1.1698
38.4	38.3566	1.1708
38.6	38.5557	1.1718
38.8	38.7548	1.1728
39.0	38.9538	1.1739
39.2	39.1529	1.1749
39.4	39.3519	1.1759
39.6	39.5509	1.1770
39.8	39.7500	1.1780
40.0	39.9490	1.1790

E-11 Phenolic content – standard curve made using galic acid with R-value



Appendix F – Sensory evaluation

F-1 Sensory evaluation form

Evaluation of beer		Comment
LOOKS		
Clarity	Clear – Unclear – Hazy	
Color	Straw – Yellow – Gold – Amber – Deep amber	
	Copper – Deep copper – Brown – Dark brown	
	Brown-black – Black – Opaque black	
Foam	Light – Medium – Vigorous	
AROMA		
Faults	Clean – Contaminated (Contaminated: oxidized – diacetyl – DMS – other)	
Intensity	Light + ÷ Medium + ÷ Vigorous	
Malt	Light + ÷ Medium + ÷ Vigorous	
Hops	Light + ÷ Medium + ÷ Vigorous	
Yeast	Light + ÷ Medium + ÷ Vigorous	
Aroma characteristics	Fruity – Floral – Spice – Roasted – Burnt – Other	
FLAVOR		
Sweetness	Light + ÷ Medium + ÷ Vigorous	
Acidity	Light + ÷ Medium + ÷ Vigorous	
Bitterness	Light + ÷ Medium + ÷ Vigorous	
Malt	Light + ÷ Medium + ÷ Vigorous	
Hops	Light + ÷ Medium + ÷ Vigorous	
Yeast	Light + ÷ Medium + ÷ Vigorous	
Body/Mouthfeel	Light + ÷ Medium + ÷ Vigorous	
Concentration	Light + ÷ Medium + ÷ Vigorous	
Additives	Fruit – Nuts – Spice – Oak/Barrel – Wild yeast – Other	
Alcohol level	Low (< 5%) – Medium (5,1÷7,5%) – High (> 7,6%)	
Length	Short + ÷ Medium + ÷ Long	
Flavor characteristics	Fruity – Floral – Spice – Roasted – Burnt – Other	
CONCLUSION		
Quality	Bad – Acceptable – Good – Very good – Exceptional	

Appendix G – Statistical analysis

G-1 ANOVA type III

```
Anova Table (Type III tests)

Response: Verdi
             Sum Sq Df F value    Pr(>F)
(Intercept) 1802.67  1 147.441 2.567e-13 ***
Temperatur   439.01  2  17.953 6.627e-06 ***
Residuals    379.02 31
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

G-2 Tukey post hoc

```
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = lm(Verdi ~ Temperatur, data = Bryggedager))

$Temperatur
              diff         lwr        upr     p adj
30-22  -9.0701754 -13.100234 -5.040117 0.0000134
37-22 -10.0000000 -14.535681 -5.464319 0.0000185
37-30  -0.9298246  -4.412193  2.552544 0.7897306
```

Norges miljø- og biovitenskapelige universitet
Noregs miljø- og biovitskapelege universitet
Norwegian University of Life Sciences

Postboks 5003
NO-1432 Ås
Norway