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Characterization of genotype and beer fermentation properties of Norwegian Farmhouse Ale Yeasts

Truls C. Rasmussen

Chemical Engineering and Biotechnology
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Supervisor: Per Bruheim, IBT

Norwegian University of Science and Technology
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Foreword

This master thesis was delivered at the Department of Biotechnology at the faculty natural science and technology at Norwegian University of Science and Technology (NTNU) the 21st of February 2016.

I would like to thank my main supervisor Per Bruheim who has been a great inspiration and it has been a pleasure to work under his guidance. His supervision during the last couple of weeks before the deadline has been pivotal, and a very important motivator to continue pursuing to the best of my abilities. Words cannot express my gratitude. I would like to thank my co-supervisor Olav Vadstein for his research on the genetic analysis part of this thesis, without his help a very interesting element of this thesis would not exist. A special thank goes out to Ingrid Bakke and Zdenka Bartosova. Ingrid for her assistance in analyzing the genetic data and training in applicable software. Zdenka for her advice and help with Headspace GC-MS analysis.

Thanks to family and friends, especially my father and mother, without their continued support, I would never have pushed myself to where I am today.

Sammendrag

Åtte forskjellige kveikstammer fra voss, Stranda, Lærdal, Hornindal og Olden har blitt donert til IBT for analyse. Kveikstammene ble fenotypisk karakterisert og to ølvarianter ble brygget, en pale ale og en tradisjonell Honndalsøl. De sensoriske egenskapene ble testen på et utrent panel i form av en Duo-trio test. Konsentrasjonen av flyktige stoffer produsert under fermentering og på ferdig produkt ble bestemt ved å benytte Head space GC-MS. En gentisk analyse ble utført på kveikstammenes interne transkribert region for å bestemme det slektskap mellom kveikstammene. Alle kveikstammene ble bestemt til å være i slekten Saccharomyces bortsett fra én. To ble klassifisert til arten *S. boulardii*, to til arten *S. cerevisiae* og én til arten *S. bayanus / S. pastorianus*. En fenotypisk karakterisering av utvalgte stammer viste en variasjon i maksimal veksthastighet på over 20 %. En flokkuleringstest resulterte til en variasjon fdra full flukkolering, til ikke-flokkulering. Fermentering av kveikstammene viste at økt fermenteringstemperatur førte til en høyere konsentrasjon av estere og alkoholer bortsett fra langkjedete estere. Under fermenteringsforløpet sank konsentrasjonen av alkoholder, samt mange estere bortsett fra ethyl acetate, ethyl octanoate og ethyl decanoate. Et sensorisk panel kunne smake forskjellen på 3 av de 5 testede kveikstammene.

Abstract

Eight different Norwegian Farmhouse Ale Yeasts from Voss, Stranda, Lærdal, Hornindal and Olden were donated to IBT for analysis. The yeast were phenotypically characterized and two beer styles were produced under laboratory conditions, a pale ale and a traditional Honndalsøl. The sensory properties were assessed on an untrained panel as a Duo-trio test and the composition of volatile compounds produced during fermentation and after bottling at different fermentation temperatures was determined using dynamic Head space GC-MS. A genetic analysis of the yeasts internally transcribed spacer 1 and 2 region was performed to assess their species and genetic relationship between the strains. The Kveik strains were compared to reference yeast. All Kveik except one was classified as the genus Saccharomyces, two were classified as *S. boulardii*, two were classified as *S. cerevisiae*, one was classified as *S. bayanus / S. pastorianus*, two were unclassified specie of the Saccharomyces genus. Phenotypical characterization showed a variation of maximum growth rate with over 20 % of selected Kveik strains. Flocculation varies between fully flocculating and non-flocculating. As fermentation temperature increase, the concentration of esters and alcohols increase except longer chained esters. During the fermentation, the concentration of alcohols decrease, esters decrease over time as well except ethyl acetate, ethyl octanoate and ethyl decanoate. A sensory panel could distinguish between 3 of the 5 tested yeast strains.

Assignment text

The purpose of this thesis is to characterize the genotype and beer fermentation properties of traditional Norwegian Farmhouse Ale Yeasts. Eight different different Norwegian Farmhouse Ale Yeast from Voss, Stranda, Lærdal, Hornindal and Olden were donated to IBT for analysis. The genotype is characterized by genetic analysis of the internally transcribed spacer (ITS) region 1 and 2. The beer fermentation properties are analyzed by composition and concentration of volatile compounds from Headspace GC-MS analysis using 1-pentanol as internal standard, as well as a sensory evaluation of the final beer product.

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1 Introduction

Department for Biotechnology at Norwegian University of Science and Technology have in collaboration with Lars Marius Garshol come into possession traditional Norwegian farmhouse ale yeast from several municipalities. It is of interest to characterize these Kveik, the growth parameters, overall beer fermentation properties with especially the production of volatile compounds, and finally a sensory evaluation of beer end product (in comparison to a reference yeast). Establishing a taxonomic classification to determine their genus and species, as well as genetic similarity of each strain is also of high interest.

2 Theory

2.1 Brewing process

Beer is brewed by ethanol fermentation of carbohydrates from malted cereal grains. And the subsequent fermentation is carried out by yeast glycolytic enzymes. The starch are degraded to disaccharides and monosaccharides during mashing [1]. The process is divided into 5 steps:

1. Malting
2. Mashing
3. Boiling
4. Fermentation
5. Bottling, carbonation and maturation.

2.1.1 Malting

The process of malting involves three primary steps - steeping, germination and kilning [2], steeping involves placing the corn in hot water to let it soak in order to initiate germination. The barley corn consist of an embryo and starchy endosperm, the endosperm consist of two types of granules, packed in a matrix of protein and

a thin cell wall. The carbohydrates are inside the granules, and it is the embryo that control the breakdown of the endosperm. During the malting process, the moisture content is increased in the barley from 10 % to 44 % which triggers the synthesis and migration of enzymes into the endosperm [3]. The breakdown of the endosperm is called germination. The purpose of malting is to create the enzymes required to break down the protein matrix and cell wall surrounding the starch granules, prepare the starches for conversion, then stop this action until the malt is ready to be mashed [4]. The enzymes created during malting can hydrolyze the β-linkages of cellulose and other cell wall polysaccharides of the barley corn. Malt is a term used to abbreviate the sugars associated with brewing and maltose in particular. The main part of fermentable malt sugars are maltose, maltotriose, glucose, fructose and sucrose and constituents for roughly 80 % of wort sugars [5]. The non-fermentable fraction of sugars consist mainly of branched oligosaccharides (also called the dextrin fraction).

Malt is the carbohydrate source of beer. The malt composition determines ethanol concentration, color, malt taste, foam, mouth feeling [6]. The most common source for malted cereal grains is barley, other sources can be maize, sorghum and millet [7]. The ethanol concentration is dependent on the sugar concentration in the wort. The sugar concentration is measured with the Original Gravity of the wort, and is a measurement of the specific gravity of sugar compared to water. The gravity of beer is measured most commonly with a refractometer or a hydrometer. A hydrometer measures the difference in gravity between pure water and water with sugar dissolved in it by flotation [4]. Refractometers measure the degree of light changes direction. Refractormeter can only be used before fermentation [8].

The color, and malt taste is dependent on the non-enzymatic browning reaction caramelisation and Malliard reaction of sugars during kilning. Sugars heated to temperatures above 100 °C undergoes a complex series of reactions called caramelisation. This gives rise to flavor compounds in addition to brown pigments An aldose undergoes an reversible isomeration and the resulting hexose enediol under-

goes formation to hydroxymethyl furfular (HMF) by sequential dehydration. The Maillard reaction happens in presence of amino compounds and D-glucose[9] [10]. [6].

2.1.2 Mashing

Malt is mixed with water and then mashed at a temperature around $60 - 70\ ^{\circ}C$. The mashing allows the enzymes formed in the malting process to degrade the polysaccharides to form maltose, glucose, and other simple sugars, which are soluble in the aqueous medium [1]. During the malting process, debranching, β-glucanase and proteolytic enzymes have prepared the endospermic starches for easy access and conversion [4]. Increasing the temperature to $60 - 70\ ^{\circ}C$ activates the amylases, α-, and β-amylase, which hydrolyse alpha glycosidic bonds in starch into glucose and maltose [11]. Mouth feeling appear as is dependent on the mashing of beer, higher temperature mashing gives a richer beer due to the activity of α-amylase while lower temperature mashing gives a dryer beer. α-amylase cuts glycosidic bonds in random places in starch, yielding maltriose (three units of glucose), maltose (two units of glucose) or glucose [12]. β-amylase catalyse the hydrolysis of non-reducing ends, cleaves off two glucose units, yielding maltose [13]. The different enzymes working in the mash are shown in Figure 1

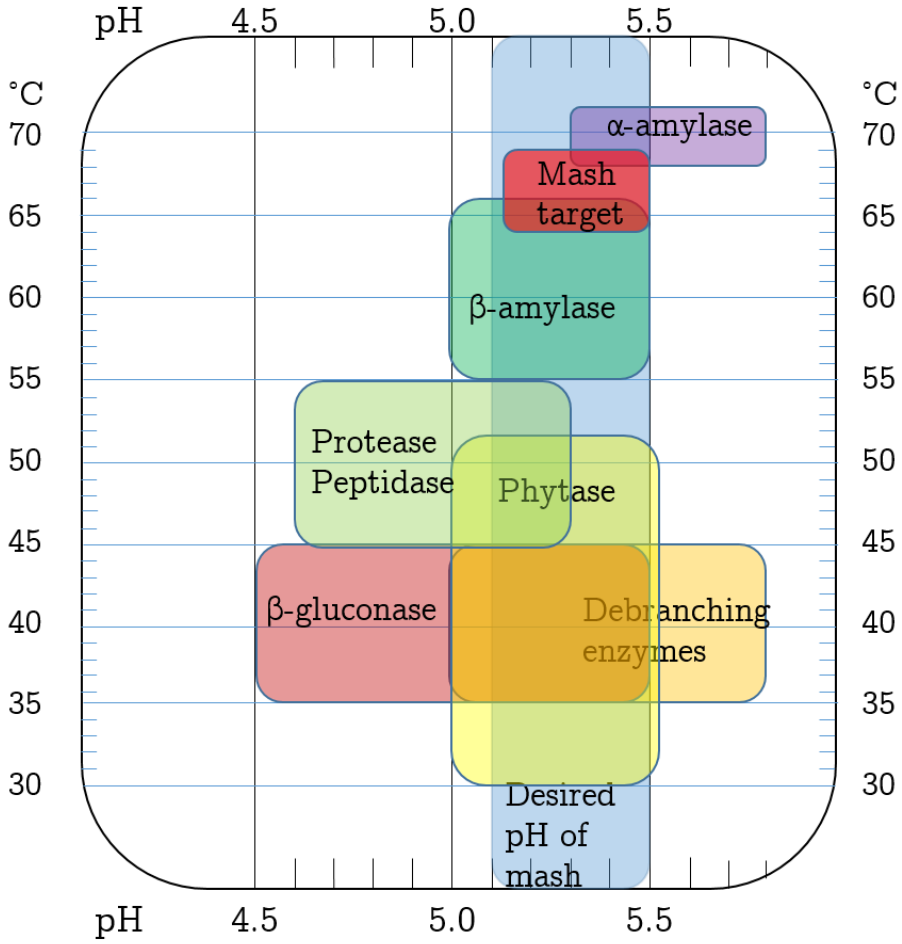

Figure 1: Overview of enzymatic activities in the mash with their temperature optimum and pH range

The desired pH and temperature target for the mash is shown on Figure 1, the activity of various enzymes are also shown. pH is controlled by adding $NaHCO_3$ and $CaCO_3$ to the desired range around 5.0 to 5.5 [4].

Water is 90 % of the beer and is a major reason why the beer taste the way it does. Adjusting the mineral content in water is important. The yeast desires a calcium content at 50 mg L^{-1}, adjusting the minerals alters the pH in the mash and changing the mineral content have a significant taste contribution to the style of beer [14]. Desired minerals to balance include also sodium, chloride, sulfate, magnesium, and zinc. Sodium and chloride act to round out and accentuate the sweetness of the beer, while sulfate makes the hop bitterness more crisp [4]. Magne-

sium and zinc are primary for the yeast together with calcium as many enzymatic reactions requires them. Table 1 list the concentration of minerals to in the tap water from Jonsvatnet in Trondheim [15].

Table 1: List over concentration of minerals and elements from Jonsvatnet in Trondheim

Mineral	Concentration [$\mathrm{mg\,L^{-1}}$]
Calsium, Ca	22,46
Chloride, Cl	7,3
Sodium Na	4,4
Sulphate, SO_4	5,0

2.1.3 Boiling

The head of the beer, foam is formed by nucleation sites between hydrophobic proteins and dissolved CO_2 as a results of the boiling process. Higher bitterness will increase the iso-α acid concentration, which helps foam stability [16]. Boiling the wort with hops (*Humulus lupulus*) help disinfect the final product. The addition of hops are also used to give bitterness and flavor in form of humulone (alpha acid) and lupulone (beta acid). The source of bitterness is primarily the alpha acids found within the lupulin glands of the hop cone (Figure 2). These compounds have little to no bittering value in their natural form , but upon heating, an isomerization reaction takes place which converts the alpha acids into bitter-tasting iso-alpha acids. This isomerization reaction is one of the main reasons for boiling wort, and the time required for the reaction to take place is one of the main factors determining the duration of kettle boil portion of the brewing process [17].

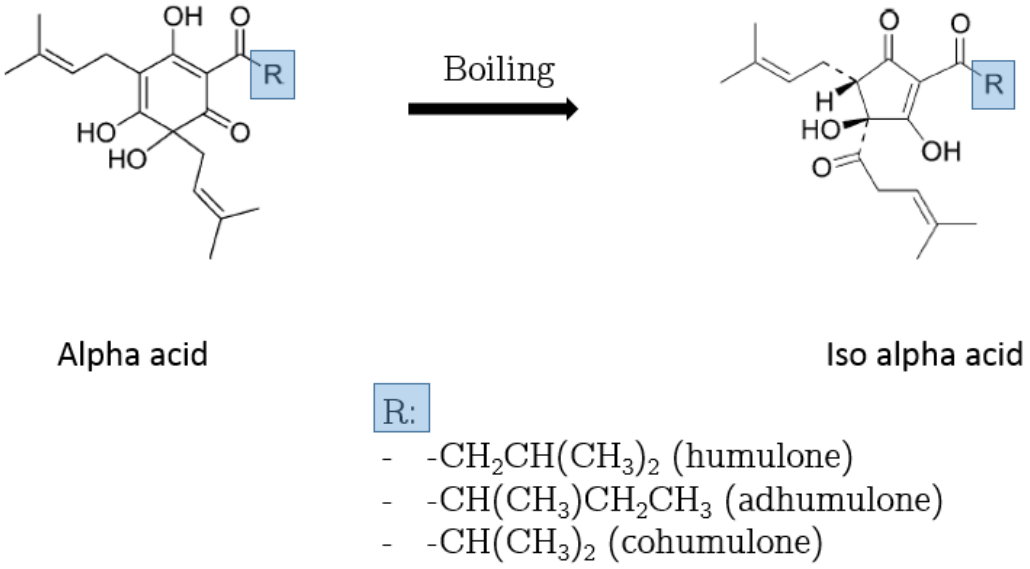

Figure 2: Structure of alpha acid with denoted R variants which is isomerized to iso alpha acid during the boiling of wort.

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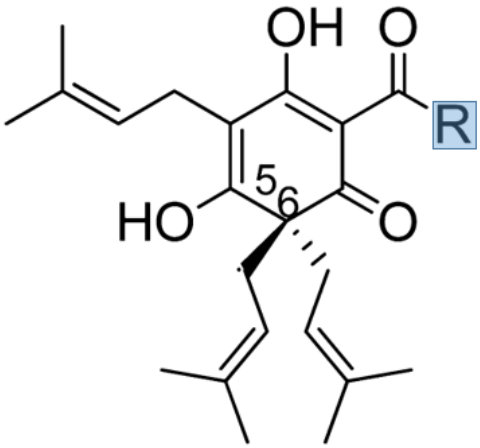
Beta acids are not isomerized due to the lack of tertiary alcohol function at C-6 (Figure 3), making the beta-acids less acidic and water-soluble than the respective isomerized alpha- acids. Therefore, lupulones are essential to control the growth of *Lactobacillus* during fermentation and known to be partially transformed by oxidation and proton-catalyzed cyclization to more soluble and sensory active compounds during wort boiling [19]. The bitterness from beta acid is harsher compared to alpha acids [4].



R:

- $-CH_2CH(CH_3)_2$ (lupulones)
- $-CH(CH_3)CH_2CH_3$ (adlupulones)
- $-CH(CH_3)_2$ (colupulones)

Figure 3: Structure of beta acid with denoted R variants which is oxidized during fermentation.

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2.1.4 Fermentation

Fermentation is the metabolism in absence of external electron acceptors [18]. Ethanol fermentation of glucose in yeast results in CO_2 and ethanol production while the intracellular red-ox balance is maintained.

Beer yeast In the aerobic wort the yeast grows and reproduces very rapidly, using energy obtained from available sugars. No ethanol forms during this stage. Yeast is supplied with oxygen, oxidizes the pyruvate formed by glycolysis to CO_2 and H_2O via the citric acid cycle. When all the dissolved oxygen in the wort has been consumed, the yeast cells switch to anaerobic metabolism, and from this point they ferment the sugars into ethanol and CO_2 [1]. The most common beer yeast are *S. cerevisiae* (ale yeast) and *S. pastorianus* (lager yeast). The

latter previously known as *S. carlsbergensis* known being named after the work of Emil Christian Hanse at the Carlsberg brewery in Denmark, wheras it is now named after Louis Pasteur. Other compounds in the wort, e.g amino acids, are also metabolized by yeast. These products are important contributors to the beer aroma and taste. Figure 4 shows a simple schematic that summarize main metabolic pathways during beer fermentation.

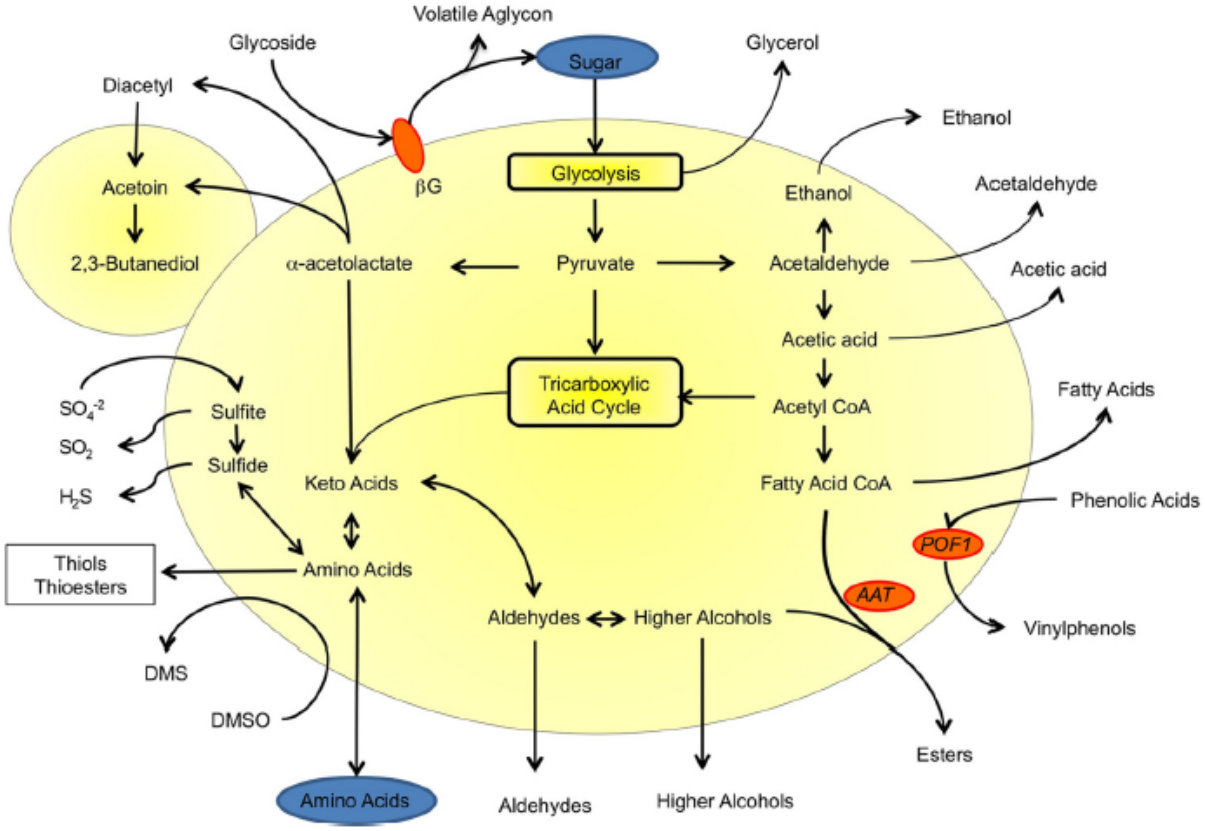


Figure 4: Overview over metabolic pathways influencing beer flavor in *S. cerevisiae*

During fermentation, yeast excretes molecules in addition to ethanol and carbon dioxide, some of these are shown in Figure 4. There are many diverse brewing yeasts available [20], and it is suggested that the majority of these yeasts do not differ widely in their genes that they will not produce unique flavor components [2]. There is one exeption to this, the production of Hefeweizen in Germany use a yeast that have a gene that encodes the enzyme ferulic acid decarboxylase which converts ferulate from the cell wall of the barley corn to 4-vinylguaiacol

[21]. All yeast strains produce glycerol, vicinal diketones, alcohols, esters, fatty acids, organic acids and sulphur containing substances. The concentration of these compounds and depended on yeast strains, but also depended on fermentation conditions which includes pitching rate, temperature, oxygen availability, duration of fermentation and storage [22].

Kveik are traditional Norwegian farmhouse ale yeasts, which is used in traditional homebrewing. The traditional homebrewers make stjørdalsøl, konnjøl, maltøl and other old styles [23]. Bread yeast and beer yeast was the same thing. People would keep yeast from fermentation, and reuse it for baking and later brewing. The preferences to only keep top fermenting yeast or bottom fermenting yeast was generally independent of geographic location, one farmer would keep the top fermenting, their neighbor would keep the bottom fermenting yeast. In order to preserve the yeast throughout the year, the farmer would drill holes in a wooden log, and dip it into the fermentation vessel to catch yeast, rolled in flour, dried and repeated. The yeast log would be hung up to dry [24].

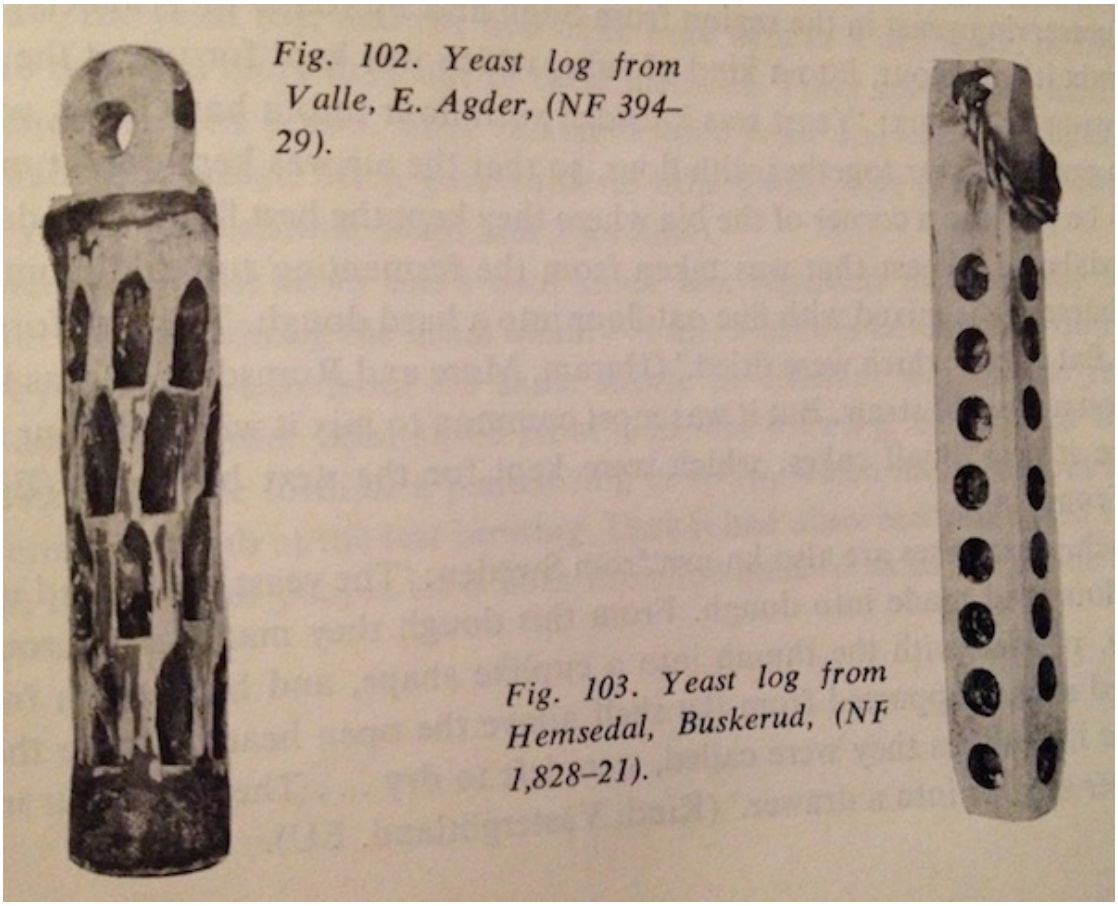


Figure 5: Two different designs of yeast logs used to preserve Kveik strains [25].

Other preservation methods for Kveik were storing it in bottles, dry it on pieces of wood or mix yeast with flour, and knead it a flat cake.

Flocculation is defined as a reversible phenomenon wherein yeast cells aggregate spontaneously and form flocs which either sediment rapidly in the culture medium or rise to the medium's surface [26]. Different yeast flocculate at different rates. High flocculation can leave high levels of diacetyl and some rest of fermentable sugars [4]. Flocculation of yeast cells happen when lectin-like proteins on the cell surface binds to mannose receptors on neighboring yeast cells. A diagram of flocculation is shown in Figure 6. Flocculation requires the presence of Calcium ions [2].

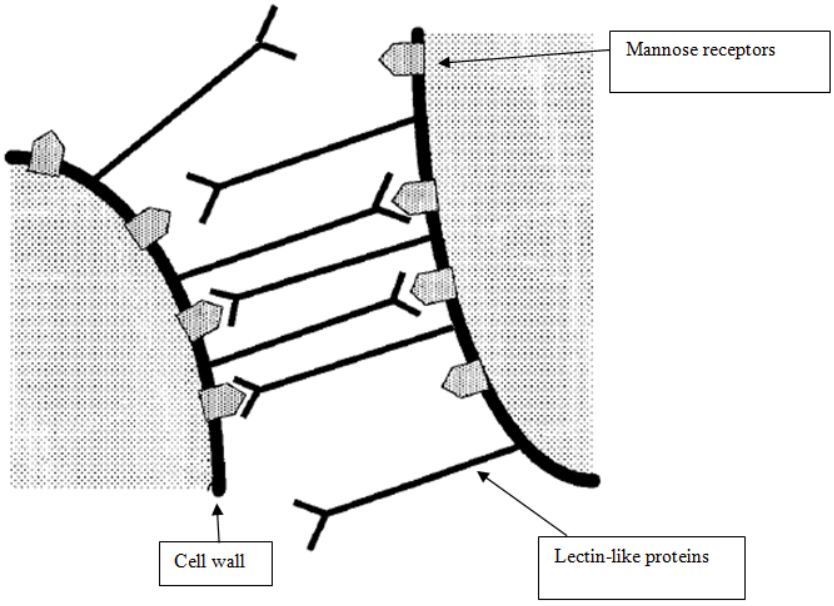


Figure 6: Diagram showing the flocculation of two yeast cells

Attenuation describes how many percent of the malt sugars that are converted to ethanol and carbon dioxide. Most yeasts have an attenuation between 70 to 80 %. Attenuation can be calculated by subtracting OG with final gravity (FG), then dividing by OG. The attenuation in the beer is dependent on type of sugars in the wort and type of yeast [4].

2.2 Analytical methodology for beer

During malting and mashing, temperature and pH control are vital to process a good tasting beer. Hydrometers are useful to track gravity changes during fermentation and FG to determine the potential strength of the beer, and spectrometer can be used to determine the color as well as concentration of some bitter compounds [27]. Sensory analysis of the final product is the deciding factor if a beer taste well or not. In order to aid a sensory analysis, it is useful to assess the production of volatile compounds the yeast produce during fermentation, the beer can be analyzed with Headspace gas chromatography and mass spectroscopy.

2.2.1 Sensory analysis

The main flavor attributes of beer are important characteristic, and a sensory panel can contribute to describe the quality of the beer. A sensory analysis of beer can be performed on a consumer and expert level, dependent on the goal of the analysis. If the test is used to detect a difference in overall flavor between two beers, a Duo-Trio test or a triangle test can be used which require the panelist to have no attribute recognition training. A flavor profile or a fresh and aged test requires a highly trained panel in order to detect small nuances dependent on flavor attributes or aging [28].

Duo-Trio test is used to determine if there is a difference in taste between two beers. Panelists are presented with three beers. One beer is identified as a reference, and of the two samples, one is equal to the reference, and the other is not equal. The duo-trio test is a forced-choice procedure; panelists are not allowed to report "no difference". Panelists who detects no difference should be instructed to randomly select one sample. When testing for a difference the number of panelists should be between 32 and 36 [29].

Flavors found in beer come from both aroma and taste, the yeast contributes to these. Esters, fusel alcohols, aldehydes, and other compounds blend together with ethanol, carbon dioxide and are properties of the fermentation. Even with the same exact ingredients the fermentation can yield different results, there are many enzymatic pathways involved, and environmental factors affect active genes and how active the yeast cells grow. The health of the cells and what sugars they consume influence the fermentation and the flavor [30]. Table 2 list esters and alcohols commonly found in beer, their flavor and threshold concentration [28] [30] [31].

Table 2: Common flavor compounds found in beer, their descriptive flavor or aroma and taste threshold

Compound	Flavor or aroma	Threshold [ppm]
Dimethylsulfid	Cooked sweet corn Cooked cabbage Processed tomatoes	$60 \cdot 10^{-3}$
Ethyl acetate	Solvent like Fuity	30
Acetaldehyde	Green apples raw apple skin bruised apple	10
Iso-amyl acetate	Banana Pear	1.2
Ethyl n-hexanoate	Apple Boiled lollies Aniseed	0.21
Iso-butyl acetate	Banana Fruity	1.6
Ethyl n-octanoate	Apple Fruity	0.9
2-phenylethyl acetate	Rose Honey Apple	3.8
Ethyl n-butyrate	Papaya Tropical fruits Mango Tinned pineapple	0.4
Ethyl decanoate	Sweet	0.5
Higher alcohols	Headace	NA
Diacetyl	Butter	0.07-0.015

Continued on next page

Table 2 – continued from previous page

Compound	Flavor or aroma	Threshold [mg/L]
Iso-butyl acetate	Fruity, floral	0.5
Isobutyl alcohol	Apple	75
Ethyl butanoate	Pineapple	0.4
Hydrogen sulfide	Rotten egg	$4 \cdot 10^{-3}$
Phenols	spicy Pepper clove Smoke Medicinal	NA
Caprylic acid	Cheesy; oily	5-10
2-phenyl ethanol	Rose	125
1-propanol	Apple Apricot Banana Beer Gin Honey Pear Sherry Tea	800
Amyl alcohol (2-Methyl-2butanol)	Fruity	50
Isoamyl alcohol (3-methyl-2-butanol)	Slight fruity flavor	70
Glycerol	faint	2500
Acetic acid	sour	60-120

2.2.2 Headspace Gas Chromatography and Mass spectroscopy

In gas chromatography, gaseous analyte is transported through the column by a gaseous mobile phase, called the carrier gas [32]. The samples are injected into the column in a split ratio to increase resolution and to not overload the column. The injector chamber is heated to a temperature where the analyte is vaporized. The carrier gas is an inert gas. Mass Spectrometer (MS) detectors are most powerful of all gas chromatography detectors.

In a GC/MS system, the mass spectrometer scans the masses continuously throughout the separation. When the sample exits the chromatography column, it passes in to the mass spectrometer. The sample is then ionized and fragmented, by an ion source. During this process, the sample is bombarded by energetic electrons which ionize the molecule by causing them to lose an electron due to electrostatic repulsion. Further bombardment causes the ions to fragment. The ions are then passed into a mass analyzer where the ions are sorted according to their mass-to-charge ratio [33]. The use of synchronous selected ion monitoring or scan acquisition on a single quadrupole mass selective detector can quickly provide full scan spectra for analyte confirmation, as well as sensitive and selective quantitation and detection of the targeted compounds [34]. The Chromatogram will point out the retention times and the mass spectrometer will use the peaks to determine what kind of molecules are exist in the mixture.

Headspace refers to the sample injected into the GC. A known mass of cell-free sample is transferred to a vial and heated for a predefined period of time to create equilibrium between gas phase and liquid phase of the sample, a needle pierces the septum of the vial and a gaseous sample is injected into the GC.

2.3 Beer yeast phylogeny

The Fungi kingdom is estimated to include more than 1.5 million species in all ecosystems. They are important part of ecosystems, acting as decomposers, mu-

tualists of plants and paratistes of various organisms [35]. The assignment of yeast species to genera and families has been based primarily on morphology of vegetative sells and sexual states. Gene sequence analysis of yeast has shown conflict between placement of specie on phylogenetic trees and their phenotypic classification. Analyses of 18s ribosomal DNA, internal transcribed spacer (ITS), s26 rDNA and cytochrome oxidase II shows a widespread disparity between phenotype and genotypes. Phenotype is a poor predictor of genetic relationship among species [36]. The ITS is located between the small subunit and large subunit rRNA genes, a map over the ITS region is given in Figure 7. The ITS sequence is divided into two sequences by the 5.8S gene [37]. With the PCR primers ITS-F_KYO2 and ITS4, the ITS can be PCR replicated as a single amplicon [35] [37].

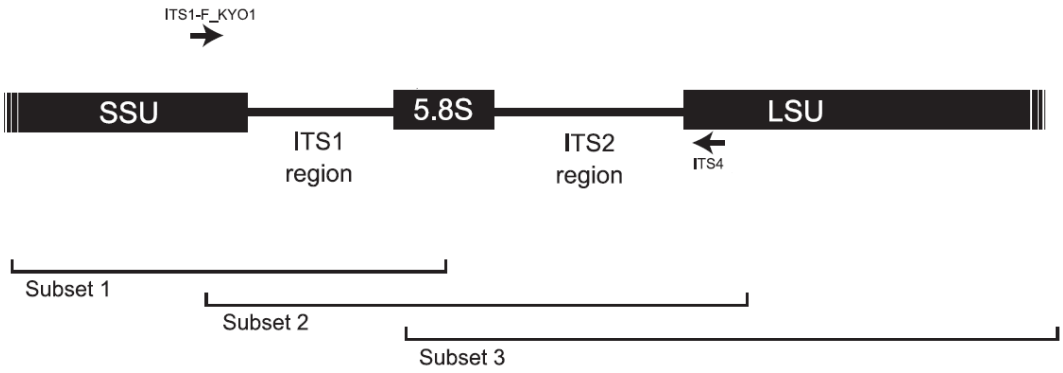


Figure 7: Map over rRNA gene and the ITS region with position of the forward ITS1-f_KYO2 and reverse ITS4 primer [35].

The subsets indicated on Figure 7 shows which parts of the rRNA gene which is amplified. Subset 2 covers the entire ITS region, and can be amplified using the primers shown. Subset 1 covers ITS region 1, Subset 3 covers ITS region 2. DNA barcoding based on nuclotide sequence of a target gene can enable rapid and accurate identification [35].

2.3.1 Real-time polymerase chain reaction

Using polymerase chain reaction (PCR), specific sequences within a DNA or cDNA template can be amplified many thousand- to a million-fold using sequence-specific

oligonucleotides, heat-stable DNA polymerase, and thermal cycling. There are three major steps in real-time PCR, denaturation, annealing and elongation. During denaturation, double stranded DNA is melted into single strands with thermostable DNA polymerase, which can withstand temperatures upto 95 °C. Complementary sequences have an opportunity to hybridize during the annealing step, the annealing temperature is dependent on the melting temperature of the primers. DNA polymerase has optimum activity at 70-72 °C and primer extension occurs at rates of up to 100 bases per second [38].

3 Method

Pale ale type of beer was brewed, as the type of hops and malt used in the brewing process will have a lesser impact on the flavor compared to other types of ale. This will in turn make the yeast have a more significant addition to taste. The mash profiles were created using BeerSmith2 software, Experiment 1 and 2 brewed a pale ale, given in Figure 8. Experiment 3 brewed a traditional Honndalsøl, given in Figure 9. All malt and hops were purchased at Bakke Brygg (`http://bakkebrygg.no/` - Fjordgata 9, 7010 Trondheim)

3.1 Beer experiment 1 and 2

The pale ale is based on 7Fjells Englafjell pale ale [39] and modified

MSc Truls ESB Sep15

Belgian Tripel (18 C)

Type: All Grain
Batch Size: 45,00 l
Boil Size: 53,88 l
Boil Time: 60 min
End of Boil Vol: 48,88 l
Final Bottling Vol: 44,00 l
Fermentation: Ale, Two Stage
Taste Notes:

Date: 28 Sep 2015
Brewer: Truls
Asst Brewer:
Equipment: Braumeister 50L
Efficiency: 74,00 %
Est Mash Efficiency: 77,3 %
Taste Rating: 30,0

Ingredients

Amt	Name	Type	#	%/IBU
55,00 l	Jonsvatnet, Trondheim Biere de garde & Belgisk pale ale	Water	1	-
13,00 g	Calcium Chloride (Mash 60,0 mins)	Water Agent	2	-
6,50 g	Gypsum (Calcium Sulfate) (Mash 60,0 mins)	Water Agent	3	-
5,50 g	Baking Soda (Mash 60,0 mins)	Water Agent	4	-
10,00 kg	Pale Malt, Maris Otter (5,9 EBC)	Grain	5	86,2 %
0,60 kg	Caramel/Crystal Malt - 60L (118,2 EBC)	Grain	6	5,2 %
0,50 kg	Corn, Flaked (2,6 EBC)	Grain	7	4,3 %
0,50 kg	Oats, Flaked (2,0 EBC)	Grain	8	4,3 %
30,00 g	Target [10,70 %] - Boil 60,0 min	Hop	9	16,9 IBUs
3,00 Items	Whirlfloc Tablet (Boil 15,0 mins)	Fining	10	-
100,00 g	Fuggles [4,10 %] - Boil 15,0 min	Hop	11	9,7 IBUs
100,00 g	Fuggles [4,10 %] - Boil 5,0 min	Hop	12	3,9 IBUs
1,0 pkg	California Ale (White Labs #WLP001) [35,49 ml]	Yeast	13	-

Gravity, Alcohol Content and Color

Est Original Gravity: 1,060 SG
Est Final Gravity: 1,016 SG
Estimated Alcohol by Vol: 5,8 %
Bitterness: 30,6 IBUs
Est Color: 16,1 EBC

Measured Original Gravity: 1,046 SG
Measured Final Gravity: 1,010 SG
Actual Alcohol by Vol: 4,7 %
Calories: 427,1 kcal/l

Mash Profile

Mash Name: Program 4 - Pale Ale
Sparge Water: 65,50 l
Sparge Temperature: 75,6 C
Adjust Temp for Equipment: TRUE

Total Grain Weight: 11,60 kg
Grain Temperature: 22,2 C
Tun Temperature: 22,2 C
Mash PH: 5,20

Mash Steps

Name	Description	Step Temperature	Step Time
Mash Step	Error: Infusion temperature above boiling. Add more water!	69,0 C	20 min
Mash Step	Add 0,00 l of water at 72,0 C	72,0 C	20 min
Mash Step	Add 0,00 l of water at 77,0 C	77,0 C	5 min

Sparge: Fly sparge with 65,50 l water at 75,6 C
Mash Notes:

Carbonation and Storage

Carbonation Type: Bottle
Pressure/Weight: 258,81 g
Keg/Bottling Temperature: 21,1 C
Fermentation: Ale, Two Stage

Volumes of CO2: 2,3
Carbonation Used: Bottle with 258,81 g Corn Sugar
Age for: 30,00 days
Storage Temperature: 18,3 C

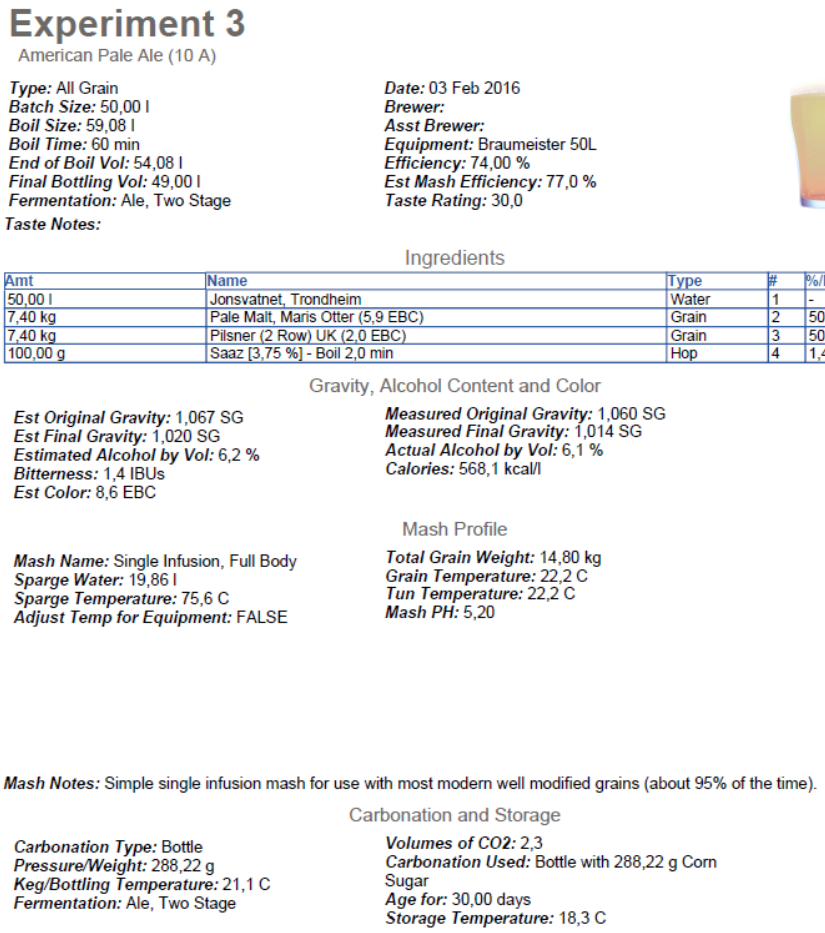
Figure 8: Mash and temperature profile of Pale Ale brewed in experiment 1 and 2, created with BeerSmith

55 L Braumaster Speidel was filled with cold tapwater. 6.5 g $CaSO_4 \cdot H_2O$ (Sigma-Aldrich 12090-1KG-R) and 13.0 g $CaCl_2$ (Sigma-Aldrich 223506-2.5KG) and 5.5 g $CaCO_3$ (Sigma-Aldrich C6763-500G) was added to 55 L. The water was heated to 69 $^{\circ}C$ before the malt given in Figure 8 was stirred in. The malt was mashed for with the temperature profile shown in "Mash Steps" in Figure 8. OG was measured before boiling by using a refractometer. The wort was boiled for 60 minutes with hops added at the applicable time indicated on Figure 8. The wort

was cooled to room temperature before addition of yeast.

3.2 Beer experiment 3

The Honndalsøl is based on a traditional recipe [40]. The mashing water used in experiment 3 simmered for 1 hour with junpier branches in order to extract resins.



Experiment 3

American Pale Ale (10 A)

Type: All Grain
Batch Size: 50,00 l
Boil Size: 59,08 l
Boil Time: 60 min
End of Boil Vol: 54,08 l
Final Bottling Vol: 49,00 l
Fermentation: Ale, Two Stage
Taste Notes:

Date: 03 Feb 2016
Brewer:
Asst Brewer:
Equipment: Braumeister 50L
Efficiency: 74,00 %
Est Mash Efficiency: 77,0 %
Taste Rating: 30,0

Ingredients

Amt	Name	Type	#	%/IBU
50,00 l	Jonsvatnet, Trondheim	Water	1	-
7,40 kg	Pale Malt, Maris Otter (5,9 EBC)	Grain	2	50,0 %
7,40 kg	Pilsner (2 Row) UK (2,0 EBC)	Grain	3	50,0 %
100,00 g	Saaz [3,75 %] - Boil 2,0 min	Hop	4	1,4 IBUs

Gravity, Alcohol Content and Color

Est Original Gravity: 1,067 SG
Est Final Gravity: 1,020 SG
Estimated Alcohol by Vol: 6,2 %
Bitterness: 1,4 IBUs
Est Color: 8,6 EBC

Measured Original Gravity: 1,060 SG
Measured Final Gravity: 1,014 SG
Actual Alcohol by Vol: 6,1 %
Calories: 568,1 kcal/l

Mash Profile

Mash Name: Single Infusion, Full Body
Sparge Water: 19,86 l
Sparge Temperature: 75,6 C
Adjust Temp for Equipment: FALSE

Total Grain Weight: 14,80 kg
Grain Temperature: 22,2 C
Tun Temperature: 22,2 C
Mash PH: 5,20

Mash Notes: Simple single infusion mash for use with most modern well modified grains (about 95% of the time).

Carbonation and Storage

Carbonation Type: Bottle
Pressure/Weight: 288,22 g
Keg/Bottling Temperature: 21,1 C
Fermentation: Ale, Two Stage

Volumes of CO2: 2,3
Carbonation Used: Bottle with 288,22 g Corn Sugar
Age for: 30,00 days
Storage Temperature: 18,3 C

Figure 9: Mash profile of Honndalsøl brewed in experiment 3, created with BeerSmith

5 % Malt Extract (ME) (Fluka 70167-500G) medium was used as growth medium for the different yeasts. 500 mL incubator flasks (Bellco) were filled with 60 mL 5 % ME medium, and each yeast type were grown for roughly 24h at 25 $°C$ and 200 rpm in order to reach max cell count. The optical density of each yeast was measured before being added to the fermentation tanks?.

3.3 Headspace GC-MS

10 g cell free samples were measured on an analytical balance (Mettler Toledo Excellence Plus) in HS-GCMS vials (Tekmar headspace vials. V 51-A 14-4440-024) and 5 PPM 1-pentanol (Vwr) was added to each sample. The vials were capped (Agilent Technologies Hdspc Al crmp cap, PTFE/si sep 20 mm 5183-4477) with a crimper (20 mm Crimper - Agilent tech 5040-4669). Samples were ran on a headspace GC-MS instrument (Agilent Technologies 7000 GC/MS Triple Quad and 7890A GC systems) with an autosampler (Teledyne Tekmar HT3 Headspace autosampler). The peaks in the chromatograms were identified by retention time using Agilent Mass hunter software, the area of each peak was automatically integrated in the software, and exported to Excel. The concentration of each compound was semi-quantitatively determined using 1-pentanol as internal standard. The head space vials were pressurized to 9 psi with Helium, and heated to 60 $^{\circ}C$ for 20 minutes to reach equilibrium
Injected sample on GC has a split ratio of 1:10. The GC column is $30m \times 250\mu m \times 1.4\mu M$ (Agilent 122-1334UT DB-624 Ultra Inert) A temperature gradient was used. Initial temperature 35 $^{\circ}C$ for 5.66 min, then 8.8 $^{\circ}C$ min^{-1} to 100 $^{\circ}C$ for 1.7 min, then 13.3 $^{\circ}C$ min^{-1} to 220 $^{\circ}C$ for 3.39 min and 22.1 $^{\circ}C$ min^{-1} to 250 $^{\circ}C$ for 3.43 min for a runtime of 31.494. Pressure in the GC was 19.871 psi and column flow was 18 $\text{mL}\,\text{min}^{-1}$. Carrier gas used was Helium.

The MS acquistion was set to scan at a low mass of 30.0 and high mass of 400.0. The MS temperatures were set to 230 $^{\circ}C$ from source and 150 $^{\circ}C$ in quadrupole.

3.4 Characterization of yeast

Malt Extract Medium Growth medium used for any strain of yeast contains sterilized (Autoclavated [matcahana S1000] 121 °C for 20 minutes) 5 % ME made with cold tap water.

Malt Extract Agar plates 2 % Agar (Agar No 1 Oxoid LP001) was suspended in 5 % ME medium for roughly 15 minutes for swelling before autoclavating at 121

°C for 20 minutes. The mixture was aliquoted in petri dishes (Sarstedt 82.1473.001 92x16 mm with cams) before left to solidify

Freeze stock Yeast were grown in 5 % ME medium for 48 hours in incubator flasks (Bellco 250 mL at 25 °C at 200 rpm (Infors AG CTA-41+ Bottminsen Minitron incubator). All content was transferred to sterile 50 mL centrifuge tubes (VWR 89039-656). Each tube were weighted and adjusted in order to create equilibrium. The tubes were centrifuged (Eppendorf Centrifuge 5804R) for 5 minutes at 3000 rpm. In a sterile bench, the supernatant was discarded and 7 mL fresh, sterile 5 % ME medium was added to the centrifuge tube with 3 mL sterile Glycerol (Sigma-Aldrich). The yeast was resuspended in the ME/glycerol mixture, and aliquoted in Cryotubes (Thermo Scientific CryoTube^{TM} Nunc 375353) and stored at -80 °C.

3.4.1 Growth rate

250 µL of freeze stock yeast sample were inoculated in 30 mL 5 % sterile ME medium. The optical density was measured in a spectrometer (VWR V-1200) at 600 nm with disposable cuvettes (Plastibrand 1.5 mL Semi-micro Disposable cuvettes 7590 15) every 60 minutes. The samples were diluted with 5 % non-sterile ME medium in order to stay within the linear region of the spectrometer.

3.4.2 Cell count

Two different methodologies were applied to get the relationship between optical density and cell concentration, Colony forming units (CFU) vs Optical density (OD), and cells using a counting chamber vs OD

CFU vs OD 30 μL yeast sample was inoculated in 30 mL 5 % ME medium in 250 mL shake flasks. The aerobic growth was followed and samples were taken during the exponential growth phase, the OD at 600 nm was measured and CFU were counted by plating out 100 μL sample from a decanic dilution series on a agar (2 %) plates containing 5 % ME medium and incubating for 2 days.

Counting chamber vs OD 30 μL yeast sample was inoculated in 30 mL 5 % ME medium in 250 mL shake flasks. The aerobic growth was followed and samples were taken during the exponential growth phase, the OD at 600 nm was measured and cell count using Buerker counting chamber was counted in a microscope.

3.4.3 Flocculation

25 mL cell culture with approximate $1 \cdot 10^8$ cells were centrifuged at 4500 G for 5 minutes at 4 °C. Supernatant was discarded and cell pellet were washed twice and resuspended in 250 mM EDTA (ACROS Organics 409930010). Supernatant was discarded and cell pellet was washed and resuspended in 250 mM NaCl (pH 2.0) (Merck K46288304 524). Supernatant was discarded and cell pellet was washed and resuspended in 250 mM NaCl (pH 4.5). Discard supernatant and suspend the pellet in 24 mL, 250 mM NaCl (pH 4.5), transfer the cells to a graduated cylinder. Add 1 mL 100 mM Ca^{2+} (Sigma-Aldrich Sodium Chloride dihydrate 223506-2.5KG). Agitate the cylinder by inverting it 18 times to promote flocculation. Measure the optical density at 600 nm at times 0s, 15s, 30s, 60s, 2min, 3min, 4min, 5min and 6min

3.4.4 Dry weight

30 μL yeast sample was inoculated in 30 mL 5 % ME medium in 250 mL shake flasks. The aerobic growth was followed and samples were taken during the exponential growth phase, the OD at 600 nm was measured, and 5 mL cell sample was dried on a pre-weighted aluminum pan for 120 °C for 24 hours and the dry cell mass was was measured on an analytical balance.

3.5 Genetic analysis

3.5.1 DNA isolation

DNA from fully grown yeast (48-72h) were extracted using DNA isolation Kit (MO BIO PowerSoil 12888-100). Nanodrop was used to check the concentration of isolated DNA before it was amplified with PCR using forward primer ITS-F_KYO2

and reverse primer ITS4 for the entire ITS region. The forward primer was used in the ITS1 region, and reverse primer was used in the ITS2 region. The technical datasheet for the primers are given in Table 3.

Table 3: The technical datasheet for the forward and reverse used to amplify the ITs region

Batch #	Oligo Name	Sequence (5'-3')	Mw	$T_M{}^\circ$	μg	nmol	GC %	μL for 100 μM
HA08385909	ITS-F_KYO2	TAGAGGAAGTAAAAGTCGTAA	6551	52.6	561.2	85.6	33.3	856
HA08385910	ITS4-R	TCCTCCGCTTATTGATATGC	6034	61.5	746.6	123.7	45	1237

The primers were dissolved in DNA free water with the volume shown in Table 3.

3.5.2 Polymerase chain reaction

Thermo Scientific Arktik Thermal Cycler PCR apparatus was used, and the PCR protocol programmed into the apparatus is given in Figure 10. BSA (BioLabs #B9001S), $MgCl_2$ (Vwr lot: 5575801) dNTP mix (10 mM Vwr ID: 510085Q) Taq DNA Polymerase (Vwr 5 units/μL ID: 510600-0100) were used.

PCR protocol

25 µL reaction volume

Navn	Volume [µL]	End concentration
10x Reaction buffer	2.5	1x
10 mM dNTP	0.5	200 µM
25 mM $MgCl_2$	1.5	3 mM
BSA	0.75	
100 µM ITS-F_KYO2 Primer	0.125	0.5 µM
100 µM ITS4-R Primer	0.125	0.5 µM
Taq DNA polymerase	0.125	
DNA template	1.0	1 ng/µL
Filtered MQ-water	18.375	
TOTAL	25.0	

Create a master mix with everything besides DNA template; divide equally in PCR tubes in a chilled container. Mark PCR tubes and add template.

PCR cycle

95 °C for 10 min (denaturing)
94 °C for 20 sec (denaturing)
47 °C for 30 sec (annealing) } 35 cycles
72 °C for 40 sec (elongation)
72 °C for 7 min (final step elong.)
10 °C ∞

Figure 10: PCR protocol

The PCR product was checked using Agarose gel electrophoresis. 1 % Agarose (SeaKem LE) was dissolved in 1x TAE buffer with GelRed nucleic acid gel stain (Biotium 41003). 5 μL PCR product with 2 μL 6x DNA loading buffer dye (Thermo scientific) were loaded on the agarose gel with 1 Kb plus ladder (Thermo scientific GeneRuler), gel ran for 45 minutes at 100 Volt (Consort E143). G:box (Syngene) was used to take a picture of the gel using the software GeneSnap. The PCR product was purified using a kit (QIAquick PCR Purification Kit 28106).

Nanodrop was used to check the DNA concentration after purification. The purified PCR product was sent to GATC-Biotech (`https://www.gatc-biotech.com/en/index.html`) for LIGHTrun Sanger sequencing.

3.6 Sensory analysis

A sensory analysis was performed with the beer from experiment 1. The sensory analysis was performed as a Duo-Trio test [29], and the objective was to determine if the beer fermented with Kveik and Idun at 19 °C could be distinguished from a beer fermented with a reference yeast. 17 assessors participated in the study, 7 female, 10 male, ages 20-60. All assessors knew which sample contained the reference, and were asked to taste two samples, where one would be equal to the reference, and the other be beer brewed with either Muri, Stranda, Hornindal, Sigmund or Idun. The assessors where asked to subjectively rate the beer which was not be equal the reference on a scale from 1-5 in comparison with the reference beer. The reference beer had a predetermined rating of 3, and the assessors were asked to score the beer not equal to the reference, either better, rating 4 and 5, with 5 having a better taste, equal, rating 3, or worse, rating 2 or 1, with 1 being the worst tasting. The testing location was evaluated to fulfill the criteria given in ISO 8589 [41].

4 Results

Kveik collected by Lars Marius Garshol (`http://www.garshol.priv.no/blog/`) have been donated to IBT for studies of genetic and beer fermentation properties. Table 4 shows an overview of the Kveik supplied.

Table 4: Overview over yeast used in this master thesis, NYNC and WLP number where available and what Norwegian county they originate from.

Yeast ID	Supplier	NCYC number	Origin	Experiment
Kveik				
Muri	Muri gård	4045	Olden	1, 2
Sigmund	Sigmund Gjernes	3995	Voss	1, 2
Hornindal			Hornindal	1, 2, 3
Hornindal + cont				1, 2, 3
Stranda	Stein Langlo	4021	Stranda	1, 2
Wendelboe	Dagfinn Wendelboe		Ljøsne, Lærdal	2
Rivenes + cont	Svein Rivenes	3545	Voss	2
Rivenes	Svein Rivenes	3548		2
Raftevold	Terje Raftevold	4051	Hornindal	2
Gausemel	Olav Sverre Gausemel		Hornindal	2
Reference yeast				
Idun Blå	Idun gjærfabrikk			1, 2
WLP001	Whitelabs			1, 2
Safale US-05	Fermentis			3
Additional yeast for genetic analysis				
Lit #1			Lithuania	
Lit #2			Lithuania	
Lit #3			Lithuania	
Lit #4			Lithuania	
Fin #1			Virrat, Finland	

The original Kveik cultures were mixed populations with presumably several Ale yeast strains and various bacterial contaminants. Thus the original cultures went through several rounds of cultivation in both liquid and solid media and with/with-

out antibacterial agent (gentamycin) addition. Some of the Kveik cultures were already deposited to The National Collection of Yeast Cultures (NCYC), hence the NCYC number ID.

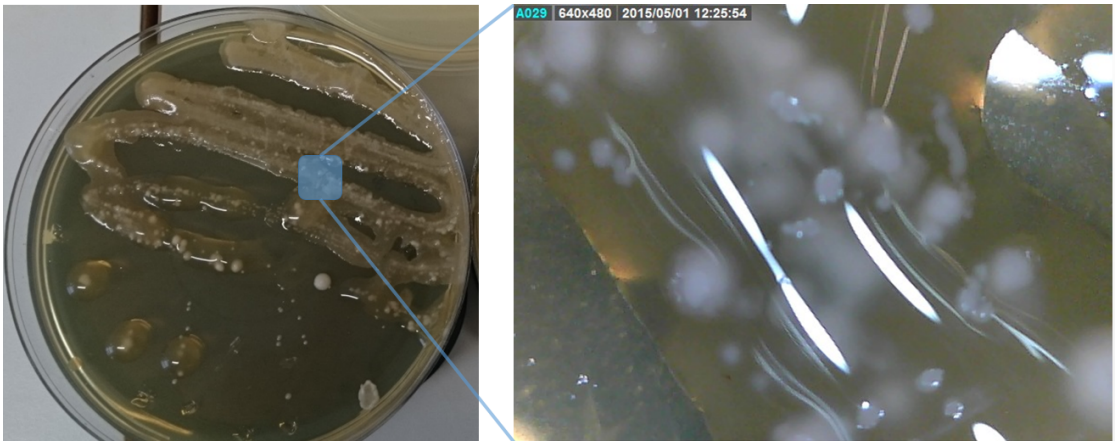


Figure 11: Hornindal Kveik before cultivation, with close up of the contaminant observed in the original culture

Figure 11 shows the original Hornindal Kveik after being plated. The Hornindal Kveik has two different yeast morphologies, Figure 11 (left) shows one larger, and one smaller morphology. The contaminant observed is producing a translucent yellow slime (right). All Kveik underwent cultivation, where one dominant colony type, were picked and transferred to liquid medium. Figure 12 shows the pure culture of the Kveik used in experiment 1. Appendix B.4 shows 40x microscopy pictures of isolated Kveik strains, all strains are between 5.0-5.5 μm in diameter using Zeiss Imager Z2.

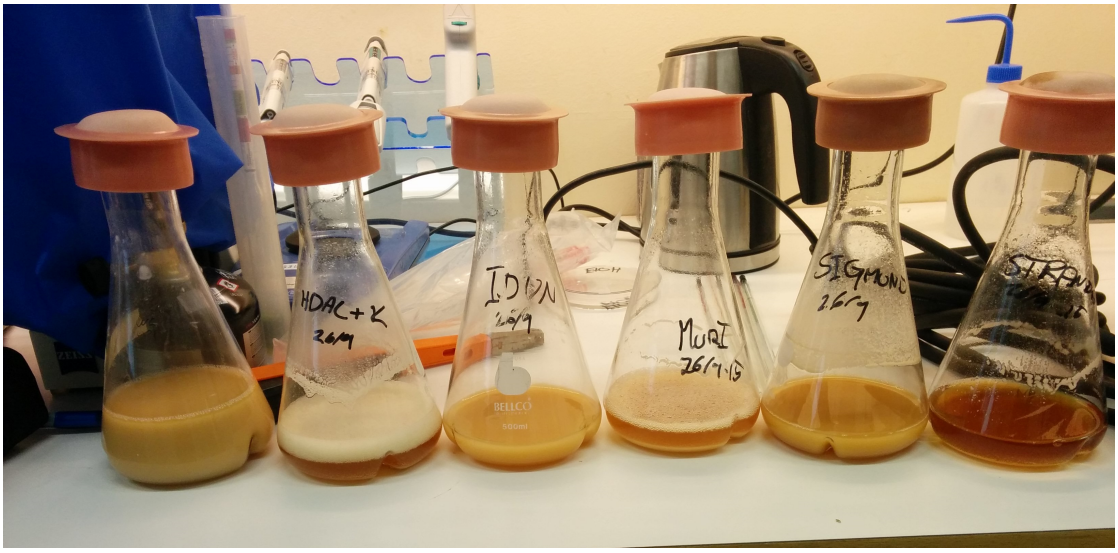


Figure 12: Kveik cultures and Idun being grown in 500 mL incubation flasks. A vial of WLP001 was suspended in 5 % ME medium before transferred to fermentation vessel. Shown from left to right are WLP001, original Hornindal, Idun, Muri, Sigmund and Stranda, 5 % ME medium for beer Experiment 1.

Figure 12 show a visual variance for the Kveik and Idun. Hornindal and contaminant have a thicker foam, Muri and Stranda Kveik settle more at the bottom at the shake flask whereas the yeast cells in both Idun and Sigmund does not flocculate.

Three brewing experiments were performed on this MSc project. A pale ale was brewed in experiment 1 and 2 (55 L) in a Braumaster Speidel The pale ale type was selected as a rather neutral beer type giving room for the Kveik flavor and aroma properties. The purpose of experiment 1 and 2 was to determine beer production properties for selected Kveik strains at 19 °C and 30 °C. Experiment 3 was desgined with an original Hornindal beer receipt. Prior to the three beer brewing experiments were the growth properties of the pure isolates characterized. These strains were also genetically analyzed at the ITS region.

4.1 Growth properties for Kveik

Kveik were grown to established a correlation between optical density and cell count. This is used to find the concentration of yeast cells in inoculum flasks. This would enable the same cell number addition to the fermentation vessel, as

the start concentration of cells will influence the fermentation.

4.1.1 Growth rate

The aerobic growth of each strain of isolate Kveik was followed for 55 hours, with sample point every hour for the first 12 hours. OD was plotted as a function of time, the plot in the exponential growth phase is shown in Figure 13.

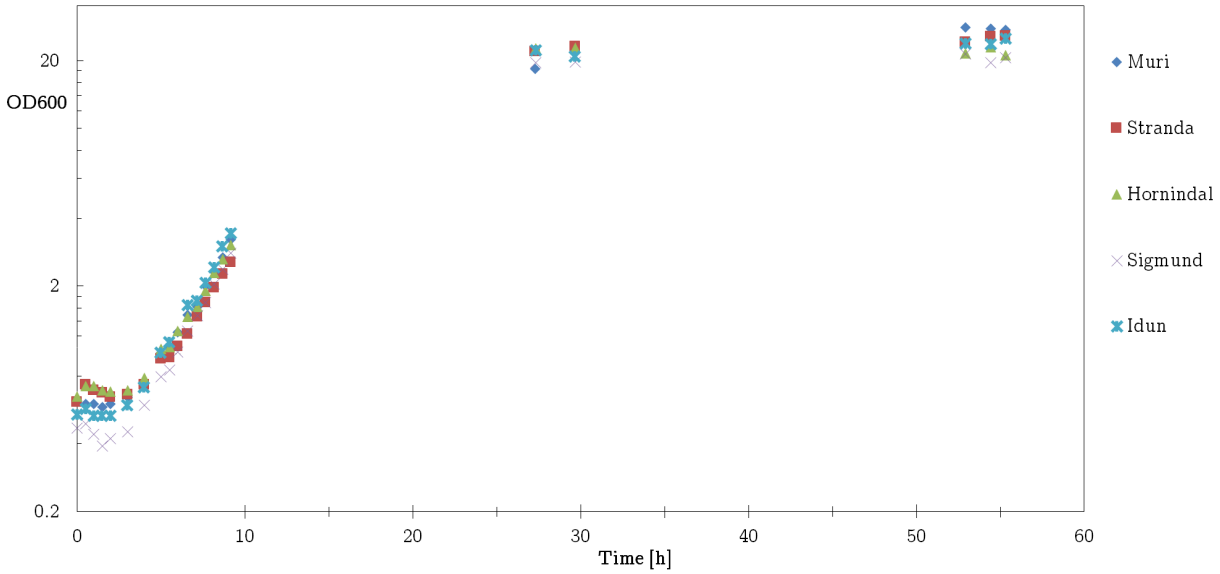


Figure 13: Optical density as a function of time for Muri, Stranda, Hornindal, Sigmund and Idun yeast.

The growth rate and generation time can be calculated from Equation 1 & 2 and the values from Appendix B.1. Maximal growth rate and generation time for the yeast are given in Table 5.

Table 5: Growth rate and generation time for Muri, Stranda, Hornindal, Sigmund and Idun

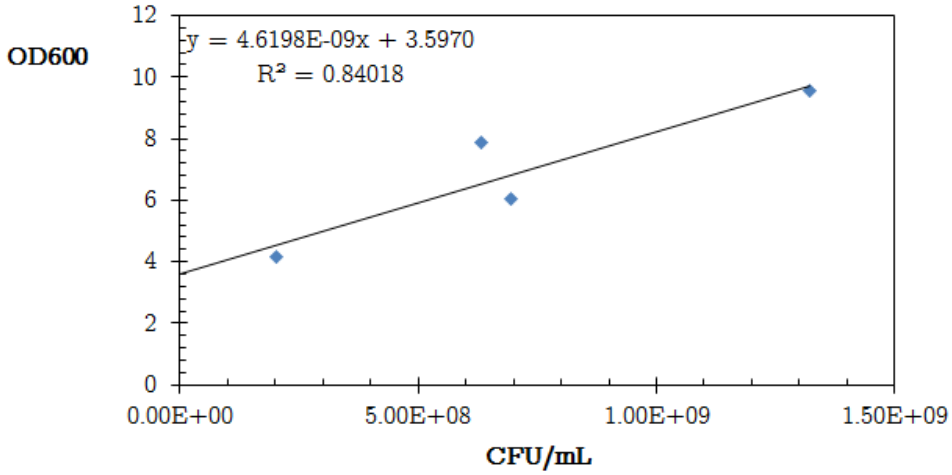
	Growth rate, μ_{max} [h^{-1}]	Gen. time, g [h]
Muri	0.28	2.45
Stranda	0.25	2.72
Hornindal	0.26	2.62
Sigmund	0.31	2.23
Idun	0.29	2.37

The max growth rate varies with over 20 % among the Kveik strains. Sigmund has the highest growth rate, 0.31 h^{-1}, and Stranda has the lowest growth rate, 0.25 h^{-1}.

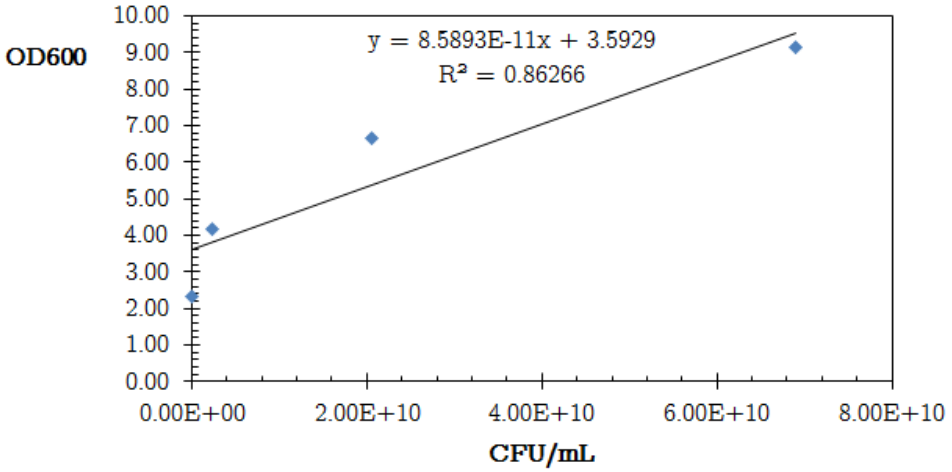
4.1.2 Cell count

The cell count was performed with two methodologies (colony-forming units and counting chamber) to determine the relationship between optical density and cell concentration. Muri and Sigmund isolates was selected as a representative Kveik strains.

CFU vs OD The optical density was plotted as a function of colony forming units. The OD_{600} and cell count data are taken from Table 52 for Sigmund and Table 51 for Muri in Appendix B.2. The plot is given in Figure 14.



(a) Muri

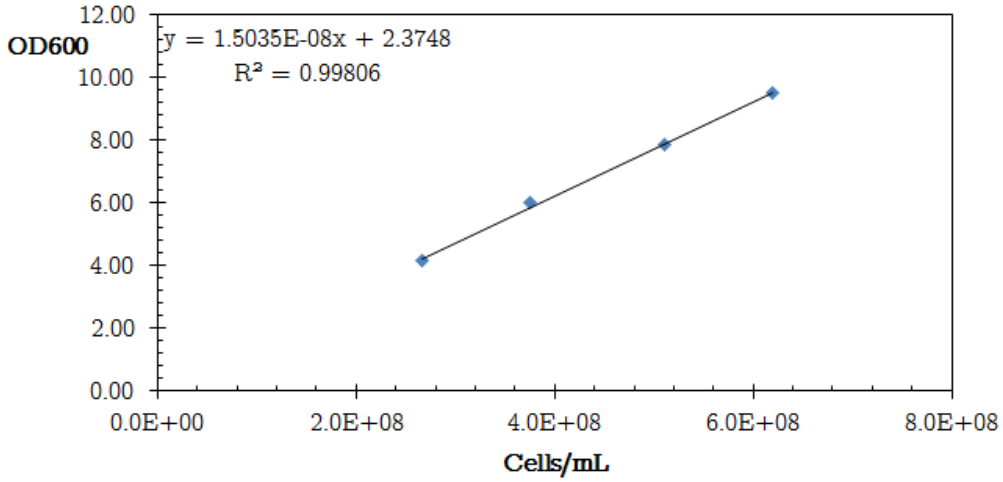


(b) Sigmund

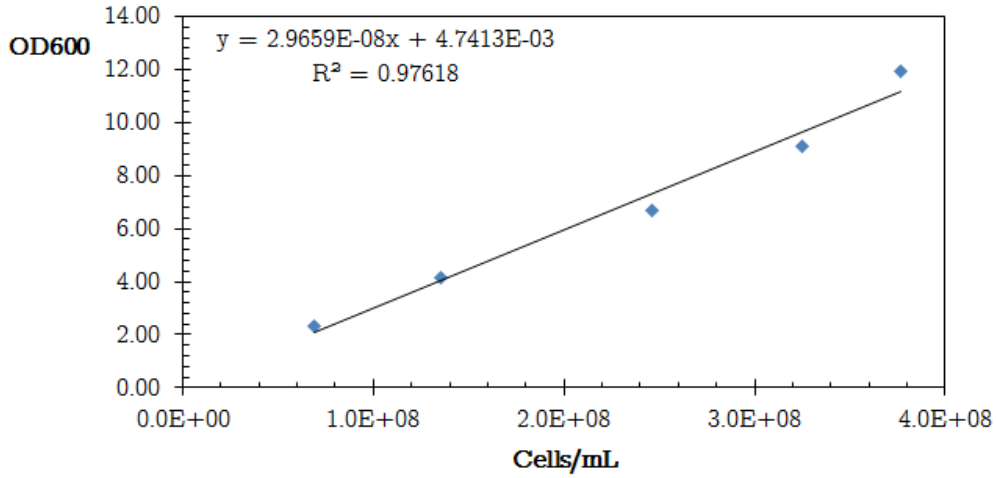
Figure 14: Measured Optical density at a given time plotted as a function of colony forming units for Muri (14a) and Sigmund (14b)

The relationship between OD and colony forming units are shown in Figure 14 for Muri and Sigmund. A linear trend line was fitted for the data. The equation shown on Figure 14a and 14b can be used to calculate the concentration of yeast cells added based on the measured optical density. The slope from Sigmund is two orders of magnitude larger than Muri. The correlation value R^2 is 0.8402 for Muri and 0.8627 for Sigmund, showing poor relationship between measure OD and CFU.

Counting chamber vs OD The optical density was plotted as a function of amount of cells. The OD_{600} and cell count data are taken from Table 57 for Sigmund in Appendix B.2 and Table 58 for Muri. The plot is given in Figure 15.



(a) Muri



(b) Sigmund

Figure 15: Measured Optical density at a given time plotted as a function of known cell concentration for Muri (15a) and Sigmund (15b)

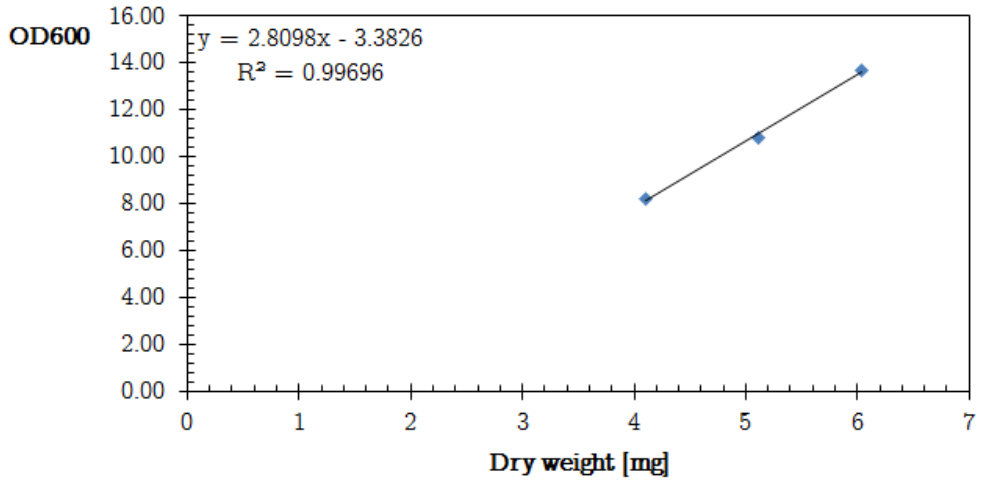
The relationship between OD and cell concentration for Muri and Sigmund is shown in Figure 15. A linear trend line was fitted for the data. The equation shown on Figure 15a and 15b can be used to calculate the concentration of yeast cells added to the fermentation vessels in the beer experiment.

Comparing the trend lines from Muri (Figure 15a) and Sigmund (Figure 15b), Sigmund has a steeper slope, leading to a higher cell concentration that is $1.5x$ the cell concentration of Muri at the same measured optical density.

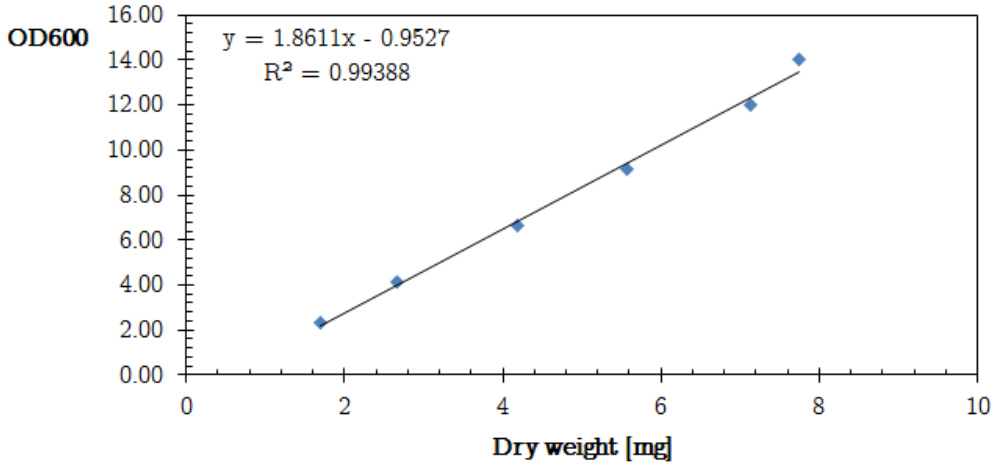
Comparing Figure 14 and 15 shows that colony forming units is one order of magnitude larger for Muri, and three orders of magnitude larger for Sigmund. The correlation factor for the hemocytometer data shows a better linear relationship between OD and counted cells compared to OD and CFU. The correlation for Muri between OD and counting chamber was used for the Kveik in experiment .

4.1.3 Dry weight analysis

OD was measured of Muri and Sigmund, and 5 mL samples was taken from each strained and dried at 120 °C for 24 hours. The optical density was plotted as a function of weight of dry samples. The plot is given in Figure 16



(a) Muri



(b) Sigmund

Figure 16: Measured Optical density at a given time plotted as a function of dry cell mass

The weight of each cell can be calculated using the trend lines on Figure 14 (CFU) and 15 (counting chamber) to calculate the amount of cells, and compare that to the weight of each individual cell. Using OD data during exponential growth, the total cells and the dry weight at that cell concentration was calculated. The weight of each cell was compared to the reported weight of $7.922 \cdot 10^{-11}$ [44]. The time and specific OD and calculated cell concentration and dry weight is given in Table 6.

Table 6: Comparison of two cell counting methodologies when comparing the weight of each individual cell to a reported value

	Muri		**Sigmund**	
Time	22		23	
OD	9.54		13.67	
	CC	CFU	CC	CFU
Cells [#]	$6.35 \cdot 10^8$	$3.22 \cdot 10^8$	$4.76 \cdot 10^8$	$1.65 \cdot 10^1 1$
Dryweight [g]	$6.78 \cdot 10^{-3}$		$8.54 \cdot 10^{-3}$	
Weight/cell [g]	$1.07 \cdot 10^{-11}$	$2.11 \cdot 10^{-11}$	$1.79 \cdot 10^{-11}$	$5.19 \cdot 10^{-14}$
Percent error [%]	-87	-73	-77	-100

There is a larger percent error observed when comparing the calculated weight per cell with the reported value, Muri is closer to the reported value when using colony forming units, however Sigmund is 100 % away from the reported value. Using counting chamber gives a value closer to the reported value. Counting chamber method is the best option to estimate cell count.

4.1.4 Flocculation

Flocculation properties is an important characteristic for beer yeast. Hence, it was of interest to determine the flocculation properties of the isolated Kveik strains. A flocculation assay based on measurement of settling time was used for this purpose. Kveik and Idun were allowed to grow until reaching stationary phase (Figure 13), roughly 24 hours. The measured values of OD are given in Appendix B.3. The percent flocculation of total cells were plotted as a function of time, and given in Figure 17.

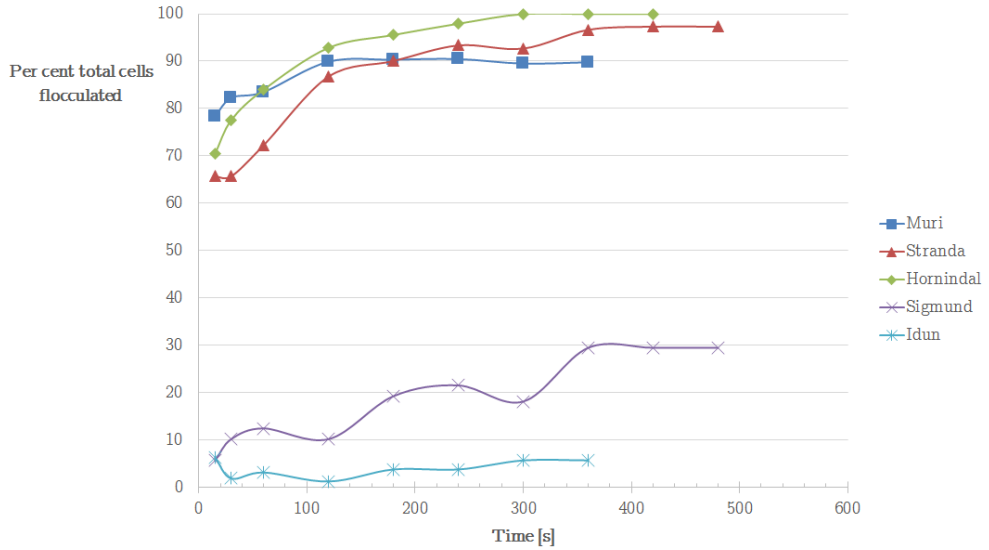


Figure 17: Percent of total settled cells as a function of time for Muri, Stranda, Hornindal, Sigmund and Idun yeast.

Hornindal and Stranda reach a flocculation close to 100 %, Muri reached a flocculation of 90 %. Idun and Sigmund has poor flocculation in comparison, 30 % Sigmund cells flocculate, and 6 % of Idun cells flocculate. Thus, it can be concluded already at this stage that Hornindal, Muri and Stranda Kveik strains have completely different flocculation properties versus Idun baker's yeast.

4.2 Genetic Analysis

DNA from fully grown yeast were extracted. DNA was amplified with PCR using primers for the entire ITS region. The forward primer was used in the ITS1 region, and reverse primer was used in the ITS2 region.

The software Chromas Lite was used to quality control the sequencing chromatograms, and the resulting FASTA sequences of the forward and reverse primer sequences were further processed in Clone Manager. A consensus region was made in Clone Manager by aligning the inverted reverse primer on the forward primer sequences. A phylogenetic tree was established for the consensus region by exhaustive pairwise alignment using Neighbor-Joining phylogeny. The resulting phylogenetic tree is given in Figure 18. (Raw data in Appendix D)

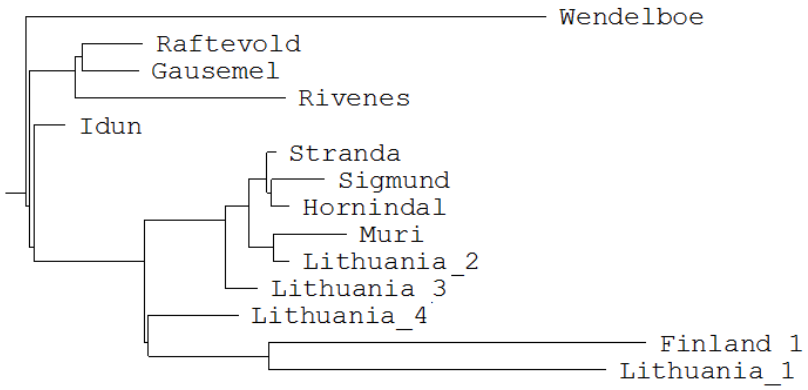


Figure 18: Neighbour-joining phylogenetic trees from analysis of entire ITS region sequences within the isolated yeast DNA

Figure 18 shows the 14 taxa evaluated in the ITS region. The tips at the end of the tree can be considered sister groups, which share a common ancestor. Wendelboe is an outgroup. Raftevold, Gausemel and Rivenes have the same ancestor. Stranda, Sigmund, and Hornindal together with Muri and Lithuania 2 have the same ancestor. The Lithuanian 3 yeast is more closely resembling the Kveik strains Stranda, Sigmund, Hornindal and Muri. Lithuania 4, 1, and Finland 1 have the same ancestor.

The consensus region of each isolated strain of Kveik were uploaded to The Ribosomal Database Project (RDP) Classifier (`http://rdp.cme.msu.edu/classifier/classifier.jsp`) [42]. 'Warcup' training set of ITS sequences was used for identification of the Kveik. in order to determine what specie the yeast belongs too, the results are given in Table 7

Table 7: Specie identification of Kveik, Lithuanian and Finnish yeast

Sample	**Specie**	**Score** [%]
Wendelboe	*S. cariocanus* [a]	24
Raftevold	*S. boulardii*	98
Gausemel	*S boulardii*	99
Rivenes	*S. boulardii* [a]	77
Idun	*S. boulardii*	84
Stranda	*S.cerevisiae*	100
Sigmund	*S. cerevisiae* [a]	78
Hornindal	*S. cerevisiae*	87
Muri	*S. bayanus / S. pastorianus*	100
Lithuania #1	*C. suzukii* [a]	6
Lithuania #2	*S. cerevisiae*	98
Lithuania #3	*S. cerevisiae*	97
Lithuania #4	*S. boulardii*	95
Finland #1	*C. humilis*	100

[a] Unclassified in different taxonomic ranks, see text below.

Wendelboe is an unclassified Fungi using the 80 % confidence threshold set in RPD classifier, the following assignment for specie *S. cariocanus* is therefore uncertain. Lithuania 1 is an unclassified Ascomycota (Phylum of the Kingdom fungi) using the same confidence threshold. For each rank assignment, the RDP Classifier automatically estimates the classification reliability using bootstrapping. Ranks where sequences could not be assigned with a bootstrap confidence estimate above the threshold are displayed under an artificial 'unclassified' taxon. The default threshold is 80 %.

Of the 14 analyzed strains, 11 was classified as the genus Saccharomyces, Rivenes and Sigmund kveik is an unclassified Saccharomyces specie. One strain was classified as the genus Candida. Raftevold, Gausemel, Idun, and Lithuania #4 was classified as *S. boulardii* . Stranda Hornindal, Lithuania #2, and #3 was classified

as *S. cerevisiae*. Muri was classified as *S. bayanus / S. pastorianus*. The Finnish yeast was identified as *C. humilis*.

Figure 18 group together *S. boulardii* (Raftevold, Gausemel, Rivenes and Idun) except Lithuania #4. *S. cerevisiae* strains are also grouped together (Stranda Sigmund Hornindal Lithuania #2 and #3. Muri (*S. bayanus / S. pastorianus*) belongs to this clade as well, since *S. bayanus / S. pastorianus* is a hybridization between *S. cerevisiae S. uvarum* and *S. eubayanus*.

4.3 Beer Experiment 1 and 2

Pale ale was brewed over two batches and fermented using 10 yeast strains. Two fermentation temperatures (19 $^{\circ}C$ and 30 $^{\circ}C$) was used. The lowest temperature was chosen since it is a common ale yeast fermentation temperature while the highest was used since it is known that the traditional Farmhouse beer brewing is using higher fermentation temperatures. 9, ten L fermentation vessels was filled with 5 L wort in experiment 1, 6 vessels being Kveik and Idun, 3 was WLP001. For experiment 2, 15 5 L fermentation vessels was filled with 3 L wort, Figure 19 shows the 19 °C incubation in experiment 2.

Figure 19: Incubation of experiment 2 with Kveik, Idun and WLP001 at 19 °C.

The Original gravity was measured measured pre-boil to be 1.060 for experiment 1 and 2 using a refractometer. The final gravity for experiment 1 and 2 is given in Table 8. The measured final gravity for experiment 1 was measured after the beer had consumed 6 g/L glucose used for carbonation. The final gravity on experiment 2 was measured before bottling. Using the gravity measurements, the alcohol by volume was calculated, which is given in Table 8.

Table 8: Measured Final gravity for experiment 1 and 2

	Final Gravity		ABV [%]	
Yeast	19 °C	30 °C	19 °C	30 °C
Muri	1.005	1.010	7.2	6.6
Stranda	1.015	1.020	5.9	5.3
Hornindal	1.012	1.020	6.3	5.3
Hornindal + cont	1.021	1.020	5.1	5.3
Sigmund	1.012	1.020	6.3	5.3
Idun	1.017	1.026	5.6	4.5
WLP001	1.010 1.020[a]	1.020	6.6 5.3[a]	5.3
Rivenes	1.025	1.030	4.6	5.3
Rivenes + cont	1.022	1.020	5.0	5.3
Raftevold	1.020	1.020	5.3	5.3

[a] FG measured in experiment 2.

The measured FG is lower at 19 °C fermentation temperature compared to 30 °C, with Idun having the largest difference between the two fermentation temperatures. Muri and Rivenes have a biggest difference when comparing the yeast at the same fermentation temperature in both experiment 1 and 2. Hornindal and Rivenes fermented with contaminant had a lower FG at 30 °C fermentation temperature.

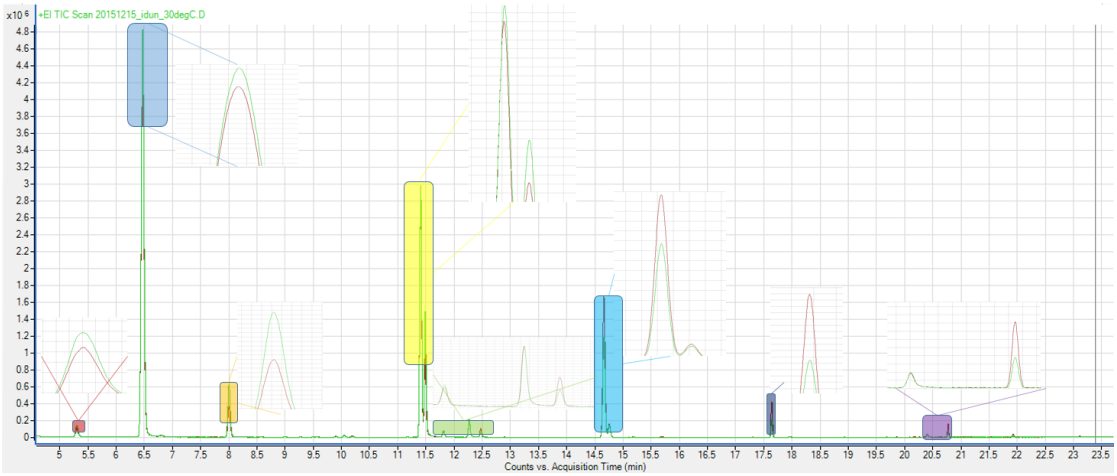
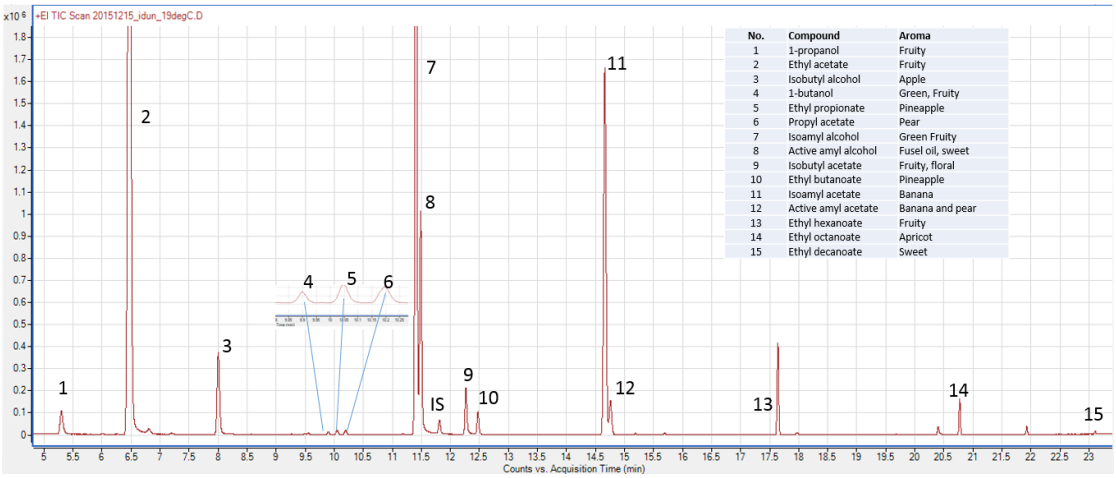


Figure 20: Total ion chromatogram of Headspace-GCMS analysis of production of volatile compounds in finished beer for Idun fermented at 19 °C and 30 °C, screen grab from Agilent mass hunter.

Green line shows the chromatogram for Idun fermented at 30 °C, red line shows Idun fermented at 19 °C. The graph shows generally higher quantities of detected compounds when fermenting at 30 °C. The compounds for each peak from Figure 20 was identified and this is shown for Idun at 19 °C in Figure 21.



No.	Compound	Aroma
1	1-propanol	Fruity
2	Ethyl acetate	Fruity
3	Isobutyl alcohol	Apple
4	1-butanol	Green, Fruity
5	Ethyl propionate	Pineapple
6	Propyl acetate	Pear
7	Isoamyl alcohol	Green Fruity
8	Active amyl alcohol	Fusel oil, sweet
9	Isobutyl acetate	Fruity, floral
10	Ethyl butanoate	Pineapple
11	Isoamyl acetate	Banana
12	Active amyl acetate	Banana and pear
13	Ethyl hexanoate	Fruity
14	Ethyl octanoate	Apricot
15	Ethyl decanoate	Sweet

Figure 21: Identification of compounds from each peak for Idun fermented at 19 °C.

Using the area of each peak, the concentration of identified compounds can be calculated using 1-pentanol as reference compound. The concentration of identified compounds from the Heads pace-GCMS analysis of Idun at 19 °C and 30 °C is given in Figure 22.

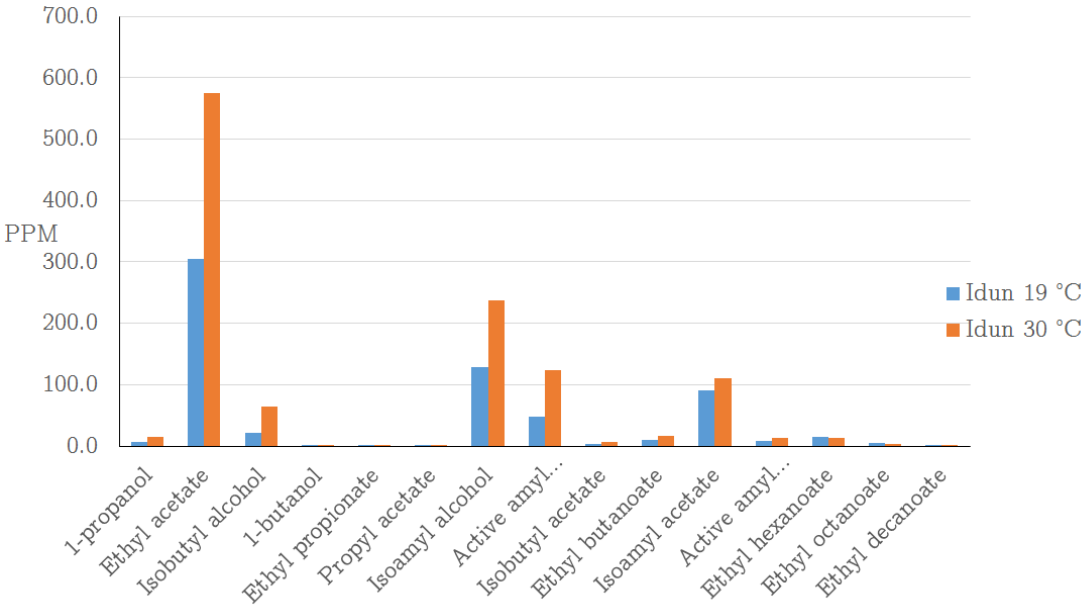


Figure 22: Concentration of identified compounds of pale ale fermented with Idun yeast at 19 °C and 30 °C.

The trend observed in Figure 22 shows that with increased temperature, the product formation is increased. Ethyl acetate is the dominant compound, which formation is almost twice as large at 30 °C. Figure 22 shows the absolute concentration of identified products. Changes in concentration for compounds like 1-butanol, ethyl propionate and propyl acetate (Peak 4, 5 and 6, Figure 22) are harder harder to observe, in order to investigate the effect of fermentation temperature, the relative concentration is used. The effect of temperature is outlined below.

4.3.1 Effect of temperature

The concentration of all identified compounds were calculated using 1-pentanol as reference compound. The concentration of each compound for the different yeast at different temperatures are given in Table 21 - 26 in Appendix A.1. In order to get an understanding of the effect of temperature for the different yeast, a heat map was generated. Concentration of compounds at 30 °C were divided by the concentration of compounds at 19 °C , and log_2 of the division was calculated. The heat map generation was made using conditional formatting. The heat map of the different yeast are given in Figure 23

Yeast	1-propanol	Ethyl acetate	Isobutyl alcohol	1-butanol	Ethyl propionate	Propyl acetate	Isoamyl alcohol	Active amyl alcohol
Muri	0.0	-0.2	0.4	-1.2	-0.3	0.3	0.1	0.1
Stranda	0.6	0.6	1.0	0.3	0.8	0.6	0.3	0.7
Hornindal	0.9	1.1	1.3	0.6	1.4	1.0	0.6	1.0
Hornindal + Cont	0.2	0.0	1.0	-0.9	1.8	-4.2	0.2	0.5
Sigmund	0.5	0.5	1.3	0.4	0.3	-0.5	0.3	0.7
Idun	1.2	0.9	1.6	1.3	1.1	0.0	0.9	1.3
Rivenes + Cont	-0.4	-0.4	-1.1	[a]	[a]	[a]	-0.1	-0.8
Rivenes	0.6	0.6	0.9	0.1	1.1	[a]	0.0	0.5
Raftevold	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
WLP001 B1	0.7	0.1	1.0	1.5	0.4	2.9	0.6	0.6
WLP001 B2	0.1	0.5	1.0	-0.8	1.1	3.7	0.2	0.5

Yeast	Isobutyl acetate	Ethyl butanoate	Isoamyl acetate	Active amyl acetate	Ethyl hexanoate	Ethyl octanoate	Ethyl decanoate
Muri	0.6	0.2	-0.6	-0.5	-0.2	-0.8	-0.7
Stranda	0.8	0.5	0.3	0.6	-0.1	0.1	-0.8
Hornindal	1.3	1.0	0.4	0.6	-0.6	-0.4	0.0
Hornindal + Cont	[a]	0.1	2.1	2.4	0.1	0.1	-0.4
Sigmund	0.7	0.5	-0.4	-0.2	-0.3	-0.7	-0.4
Idun	1.0	0.8	0.3	0.6	-0.2	-0.4	-0.6
Rivenes + Cont	-0.4	-0.5	0.4	-0.5	0.1	0.0	-0.2
Rivenes	1.1	0.2	0.3	1.0	0.2	0.1	0.1
Raftevold	0.0	0.0	0.0	0.0	0.0	0.0	0.0
WLP001 B1	0.4	0.2	0.0	-0.2	-0.7	-1.9	2.3
WLP001 B2	1.5	0.0	1.1	2.2	0.3	-0.6	0.7

[a] No peak was identified at the specific retention time, giving no detection of the compound

Figure 23: Heat map representing color coded concentration differences of yeast at different fermentation temperatures. Positive values (green color) represent concentrational differences largest at 30 °C. Negative values (red color) represent a concentrational difference largest at 19 °C. The midpoint value was defined as 0 (white color), representing no changes in concentration as a function of temperature. Values ≤ 1 has a concentration at 30 °C $\leq 2x$ the concentration at 19 °C.

The values shown in Figure 23 are relative, they do not take into consideration the real concentration. Appendix A.1, Figure 35 - 49 shows the actual concentration of the alcohols and esters used in the heat map (Figure 23). Figure 35 - 49 shows a bar graph of each detected compound, with the concentration calculated for the different yeast at 19 and 30 °C. Ethyl Acetate, isoamyl alcohol have a concentration 2-3 orders of magnitude larger than some esters like ethyl propionate, propyl acetate and ethyl decanoate. The large differences in the heat map for Propyl acetate, especially Hornindal with contaminant and WLP001 B2 might be because of low level of detection.

The general trend (Figure 23) is a higher concentration of products at a higher

fermentation temperature except the longer chained esters, ethyl hexanoate, ethyl octanoate and ethyl decanoate seems to have a larger concentration at 19 °C. The exception being Rivenes with contaminant, where the compounds has a higher concentration when fermented at 19 °C.

The concentration of alcohols 1-propanol, isobutyl alcohol, 1-butanol, isoamyl alcohol and active amyl alcohol are larger when fermenting at 30 °C, except the Kveik where the contaminant are present (Hornindal and Rivenes). The concentration of alcohol in the contaminated Kveik strains are either reduced compared to the pure culture, Hornindal for isobutyl alcohol and active amyl alcohol or the concentration is increased at 19 °C.

The opposite is observed for Rivenes with contaminant, product formation is higher when fermenting at 19 °C, however the longer chained esters isoamyl acetate, ethyl hexanoate, ethyl octanoane and ethyl decanoate are higher when fermenting at 30 °C. Hornindal with contaminant follows the same pattern as Hornindal and the rest of the Kveik, but the concentration difference is smaller for the alcohols 1-propanol and isoamyl alcohol. Isoamyl acetate and active amyl acetate have a significantly higher concentration at 30 °C.

WLP001 was brewed as a reference in experiment 1 and 2. In experiment 1 it was only fermented at 19 °C. B1 had matured for a month longer before Heads pace-GCMS analysis, and some changes in concentration can occur during maturation. B1 at 19 °C was compared to the Headspace data from experiment 2 at 19 °C. Positive values show a higher concentration of compound when B1 had matured for 1 month longer than B2, all alcohols increased in concentration during maturation, all esters increased except active amyl acetate, ethyl hexanoate and ethyl octanoate. WLP001 B2 shown in Figure 23 follows the same general trend observed for the other yeast, formation of alcohols and esters are preferred at 30 °C. With the exception of 1-Butanol, Ethyl octanoate and Ethyl decanoate.

4.3.2 Principle component analysis

The Headspace GC-MS results from Exp 1 and 2 was evaluated with Principal Component Analysis (PCA). To eliminate higher contribution from high abundant compounds was the 4th. root of the ppm values used in the PCA calculations. Figures 24 and 25 show the scores plot with two different labelings: Figure 24 with coloring yeast / Kveik strains while Figure 25 coloring on fermentation temperature. Figure 26 shows the loadings plot. The overall impression is that PC1 separates on yeast strain while PC2 partly separates on temperature. Some of the Kveik strains are close to the commercial yeast strain WLP001 while Muri and Hornindal are closer to the Idun bakers yeast strain. Sigmund and Stranda strains seem to most distant from any of the two reference strains. And not surprisingly, it is the esters (active amyl acetate, isoamyl acetate, isobutanol acetate and ethyl acetate) that are produced at higher concentration at 30 °C that contributes most to separation between the fermentation temperatures (negative values on PC2 axis in loadings plot Figure 26).

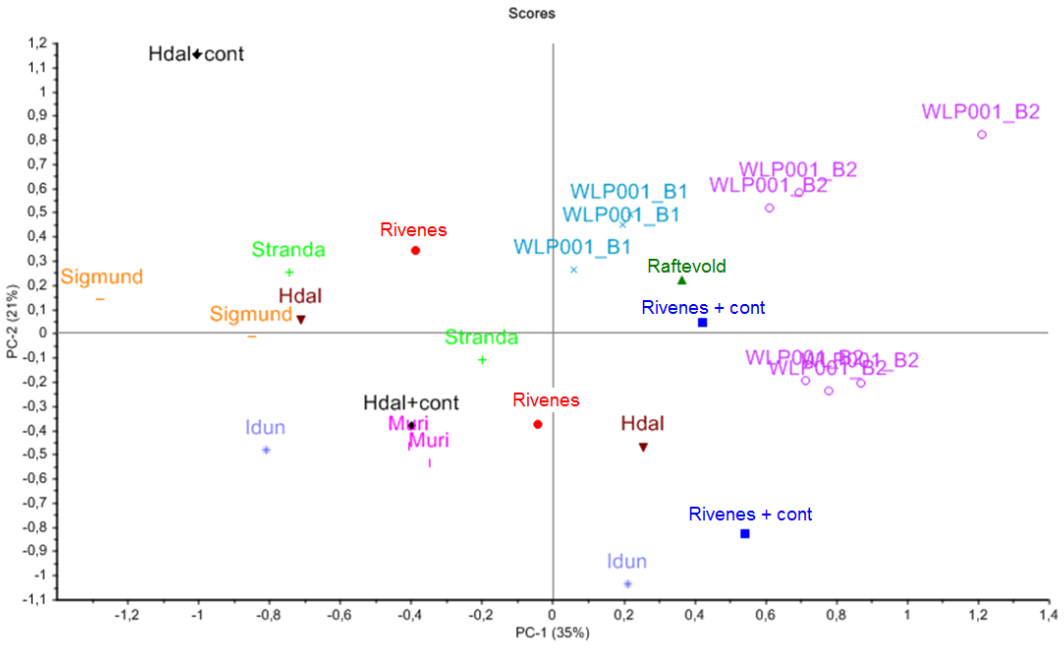


Figure 24: PCA of volatile compounds according to Kveik / yeast strains

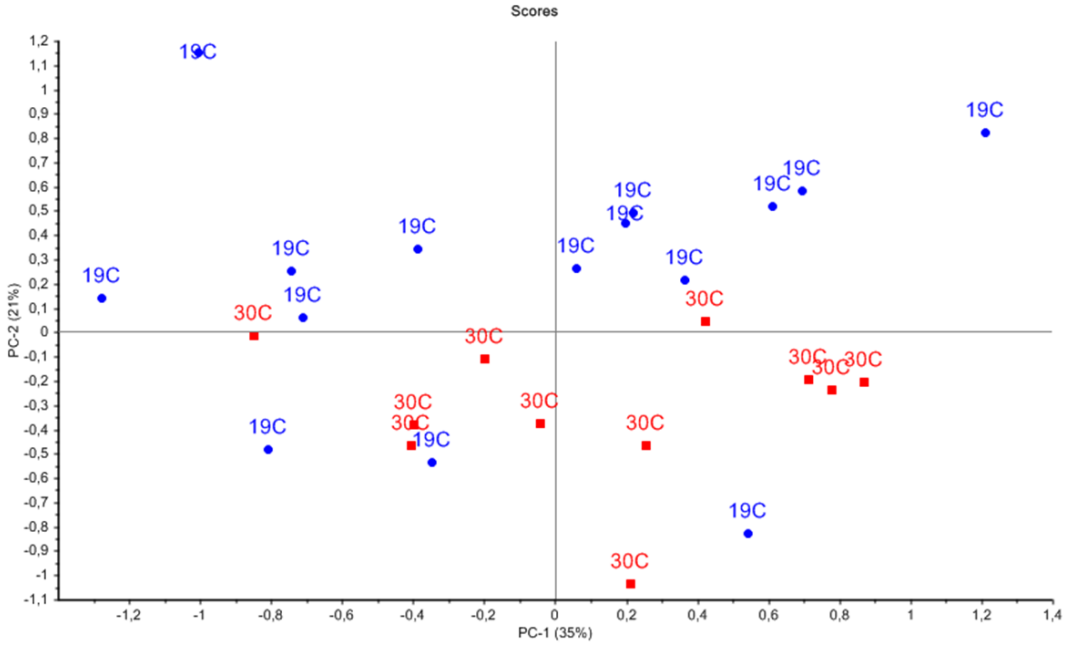


Figure 25: PCA of volatile compounds according to fermentation temperature

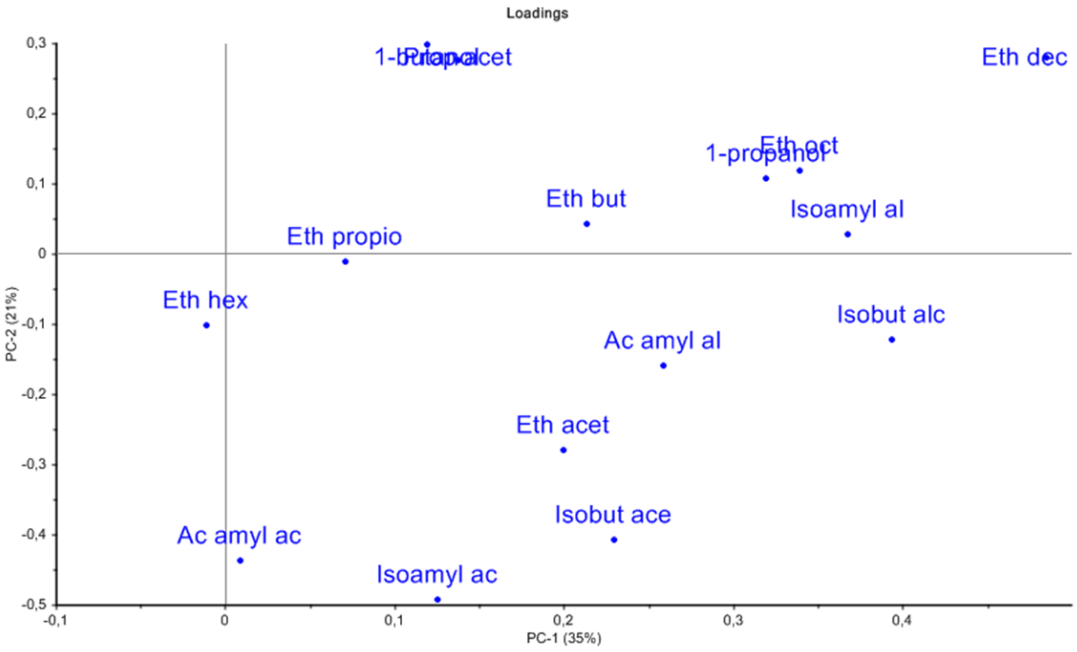


Figure 26: PCA showing the relationship among the esters and alcohols

4.3.3 Sensory analysis

A sensory analysis was performed with the beer from experiment 1. The sensory analysis was performed as a Duo-Trio test, and the objective was to determine

if the beer fermented with Kveik and Idun at 19 °C could be distinguished from WLP001 fermented beer used as a reference. 17 assessors participated in the study and were asked to taste two samples, to identify which one was equal to the reference. The assessors where asked to subjectively rate the beer which was not be equal the reference on a scale from 1-5. The number of correct responses and taste rating for each yeast is given in Table 9.

Table 9: Number of correct responses to determine if there is a significant difference in taste when comparing beer brewed with a reference yeast (WLP001) and Norwegian Kveik and Idun and a subjective rating

	Number of correct responses	Taste rating
Muri	14	3,2
Stranda	16	2,6
Hornindal	12	3,3
Sigmund	12	2,8
Idun	17	3,5

17 Assessor participated, in order to determine if there is a difference, 13 correct responses are required to conclude that a perceptible difference exists at 95 % confidence interval. From Table 9, there is a significant difference in taste between WLP001 and Muri, Stranda and Idun. Using Equation 4 (Appendix C), it can be concluded with 95 % confidence that at least 34 % of the population can recognize beer brewed with Muri yeast, 70 % can recognize Stranda, and 100 % can recognize Idun. 5 % of the population can recognize Sigmund and Hornindal.

The assessors were asked to rate the beer they determined to be different from the reference to either be equal, better or worse. Idun, Muri and Hornindal were rated better then WLP001. Sigmund and Stranda were rated to taste worse.

Too investigate the effect of yeast a heat map was generated. The concentration of

compounds for WLP001 from experiment 1 are given in Table 21 - 26 in Appendix A.1 were divided by the concentration of Muri, Stranda, Hornindal, Sigmund and Idun from Table 21 - 26 in Appendix A.1 and the log_2 of the division was used to calculate the relative difference in concentration. The heat map was generated using conditional formatting. The heat map is given in Figure 27.

Yeast	1-propanol	Ethyl acetate	Isobutyl alcohol	1-butanol	Ethyl propionate	Propyl acetate	Isoamyl alcohol	Active amyl alcohol
Muri	1.7	-0.4	1.3	1.2	0.0	2.3	0.8	0.6
Stranda	1.5	0.1	1.6	1.9	0.8	2.2	0.9	0.9
Hornindal	1.7	0.6	1.3	2.1	1.1	1.9	0.8	0.6
Sigmund	2.6	0.6	2.3	3.6	1.4	2.2	1.5	1.4
Idun	2.1	0.2	1.9	3.4	-0.1	1.1	1.2	1.0
Rivenes	0.6	-0.8	-1.2	[a]	[a]	[a]	-0.4	-1.1
Raftevold	0.7	-0.3	-0.2	-0.3	0.4	-3.6	-0.6	-0.5

Yeast	Isobutyl acetate	Ethyl butanoate	Isoamyl acetate	Active amyl acetate	Ethyl hexanoate	Ethyl octanoate	Ethyl decanoate
Muri	0.2	1.3	-1.7	-2.6	-0.8	0.8	3.9
Stranda	0.9	1.0	0.1	-0.6	-0.8	1.1	4.3
Hornindal	0.4	1.2	-0.2	-1.5	-0.8	1.0	4.9
Sigmund	0.8	1.4	0.0	-1.2	-0.8	0.9	5.4
Idun	-0.3	1.2	-1.4	-2.7	-0.9	0.8	5.2
Rivenes	-1.1	-0.3	-0.5	-1.8	-0.5	0.5	-0.6
Raftevold	-0.1	0.8	-0.1	-0.7	-0.2	1.0	0.1

[a] No peak was identified at the specific retention time, giving no detection of the compound

Figure 27: Heat map representing color coded concentration differences of yeast compared to WLP001. Three-point color scale, negative values (red color) represent a higher concentration of the given compound for the Kveik and Idun, positive values (green color) represent a higher concentration of the given compound for WLP001. The midpoint value was defined as 0 (white color), representing no changes in concentration.

Idun was the highest rated beer (3.5), followed by Hornindal and Muri. Figure 27 shows a higher concentration of active amyl acetate and isoamyl acetate for mentioned Kveik. These compounds have an aroma that is considered to be banana- and banana/pear-like. Two assessors noted that Idun tasted like banana or was fruity which gave the Idun beer a sweeter taste. Hornindal was not recognized to be significantly different from WLP001, but was rated higher than WLP001, the untrained panel generally might rate sweeter tasting beer higher than non-sweet beer, Stranda has concentrations of isoamyl acetate and active amyl acetate closer to the value of WLP001, but had the worst taste rating at 2.6.

The sensory analysis was only performed with the the boxed in values in Figure 27, Rivenes and Raftevold yeast was brewed in experiment 2, and did not take part in the sensory analysis. WLP001 has a higher concentration of all observed alcohols. The esters are reduced, but for some still larger for the fermentation of WLP001, isoamyl acetate (Sigmund and Stranda equal WLP001), active amyl acetate and ethyl hexanoate are larger for the Kveik. It does not seem to be a correlation between the sensory analysis (Table 9), and the difference in concentration when compared to WLP001 (Figure 27).

The threshold for flavor common flavor compounds found in beer is given in Table 2, these thresholds can be compared to the concentrations in the samples (Table 21 - 26 Appendix A.1). The data are compared, and given in Figure 28

	1-propanol	Ethyl acetate	Isobutyl alcohol	Isoamyl alcohol	Active amyl alcohol	Isobutyl acetate	Ethyl butanoate	Isoamyl acetate	Ethyl hexanoate	Ethyl octanoate	Ethyl decanoate
Muri 19 °C	0	1	0	1	1	1	1	1	1	1	1
Muri 30 °C	0	1	0	1	1	1	1	1	1	1	1
Stranda 19 °C	0	1	0	1	1	1	1	1	1	1	1
Stranda 30 °C	0	1	0	1	1	1	1	1	1	1	0
Hornindal 19 °C	0	1	0	1	1	1	1	1	1	1	0
Hornindal 30 °C	0	1	1	1	1	1	1	1	1	1	0
Hornindal + K 19 °C	0	1	0	1	1	0	1	1	1	1	0
Hornindal + K 30 °C	0	1	0	1	1	1	1	1	1	1	0
Sigmund 19 °C	0	1	0	1	0	1	1	1	1	1	0
Sigmund 30 °C	0	1	0	1	1	1	1	1	1	1	0
Idun 19 °C	0	1	0	1	0	1	1	1	1	1	0
Idun 30 °C	0	1	0	1	1	1	1	1	1	1	0
Rivenes + K 19 °C	0	1	1	1	1	1	1	1	1	1	1
Rivenes + K 30 °C	0	1	0	1	1	1	1	1	1	1	1
Rivenes 19 °C	0	1	0	1	1	1	1	1	1	1	1
Rivenes 30 °C	0	1	0	1	1	1	1	1	1	1	1
Raftevold 19 °C	0	1	0	1	1	1	1	1	1	1	1
WLP001 19 B1 °C	0	1	1	1	1	1	1	1	1	1	1
WLP001 30 B2 °C	0	1	1	1	1	1	1	1	1	1	1
WLP001 19 B2 °C	0	1	0	1	1	1	1	1	1	1	1

Figure 28: Comparing detected concentration of flavors with reference values, red values are below the taste threshold, green values are above the threshold for beer in experiment 1 and 2. Highlighted values (Orange) are Kveik used in the Duo-trio test.

1-propanol is below the reported threshold value of 800 ppm for all Kveik strains, isobutyl alcohol is above the taste threshold in pure Hornindal at °C, Rivenes with contaminant at 19 °C and WLP 001 at 19 (batch 1) and 30 °C. Sigmund and Idun are below the threshold of active amyl alcohol. Isobutyl acetate is below the taste threshold for Hornindal + contaminant at 19 °C. Many strains are below the threshold of ethyl decanoate.

4.4 Beer Experiment 3

The Hornindal Kveik was investigated further, and experiment 3 brewed a traditional Honndalsøl, which is brewed with juniper branches. The batch was divided into 12, 5 L fermentation vessels each filled with 3 L wort. 4 with Hornindal yeast, 4 with Hornindal + contaminant yeast, and 4 with Safale US-05 dry yeast. Each culture of yeast were fermented at different temperatures;

1. 19 °C
2. 26 °C
3. 33 °C
4. 33 °C for 2 days, then 19 °C for the rest of the fermentation

All vessels were fermented for 14 days. Sample points were taken after two days, and at the end of fermentation before bottling. The samples were analyzed on Headspace GC-MS. The total ion chromatogram comparing Hornindal + contaminant fermented at 33 °C after 2 days and 33 °C at the end of fermentation is given in Figure 29

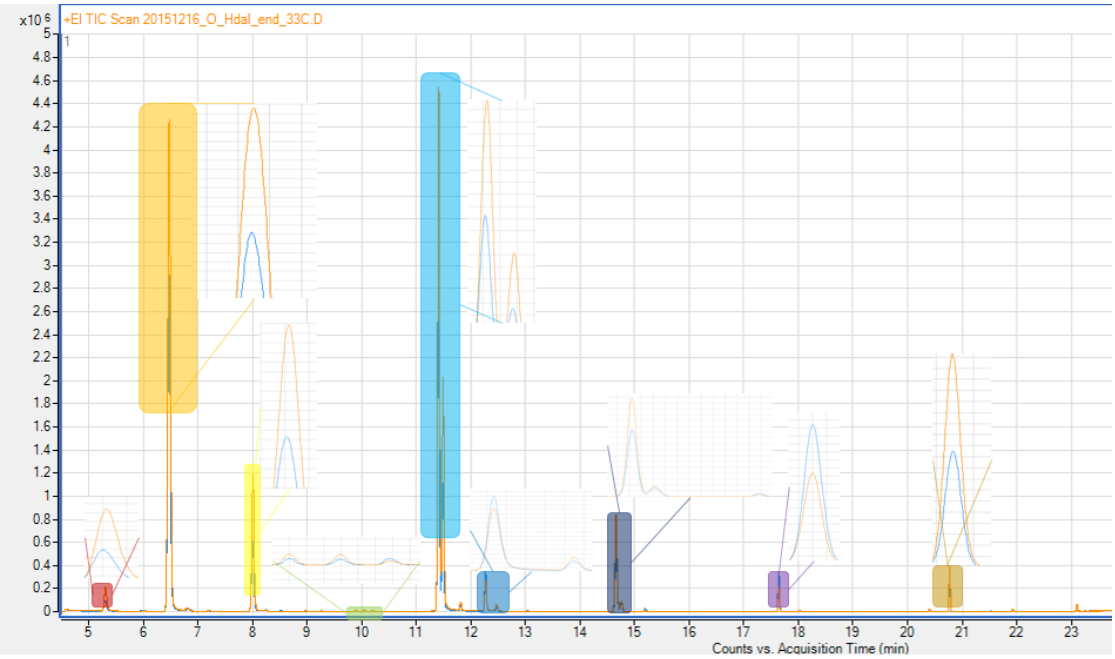


Figure 29: Total ion chromatogram of Headspace GC-MS analysis comparing Hornindal with contaminant fermented at 33 °C after 2 days and 33 °C at the end of fermentation, screen grab from Agilent mass hunter.

Orange line on Figure 29 is Hornindal at the end of fermentation, blue line is after 2 days. The count is related to quantity, and is generally higher at the end of fermentation. The concentration of many compounds is higher at the end of the fermentation.

OG was measured to be 1.060 for experiment 3. FG was measured before bottling, using OG and FG data. The alcohol by volume was calculated, it is shown in Table 10.

Table 10: Measured FG from experiment 3 and calculated ABV

	Final Gravity	ABV [%]
Hornindal + cont 19 °C	1.022	5.0
Hornindal + cont 26 °C	1.020	5.3
Hornindal + cont 33 °C	1.016	5.8
Hornindal + cont 33-19 °C	1.014	6.0
Hornindal 19 °C	1.017	5.6
Hornindal 26 °C	1.017	5.6
Hornindal 33 °C	1.017	5.6
Hornindal 33-19 °C	1.017	5.6
Safale US-05 19 °C	1.016	5.8
Safale US-05 26 °C	1.012	6.3
Safale US-05 33 °C	1.017	5.6
Safale US-05 33-19 °C	1.017	5.6

Hornindal with contaminant decrease in gravity as fermentation temperature increase. Alcohol by volume differs by 18 % between the highest and lowest temperature. Hornindal reached the same FG on all fermentation temperatures.

Using 1-pentanol as internal standard, the concentration of compound detected on Headspace GC-MS was calculated. A bar graph was created, comparing the fermentation after 2 days 14 days, the graph is given in Figure 30.

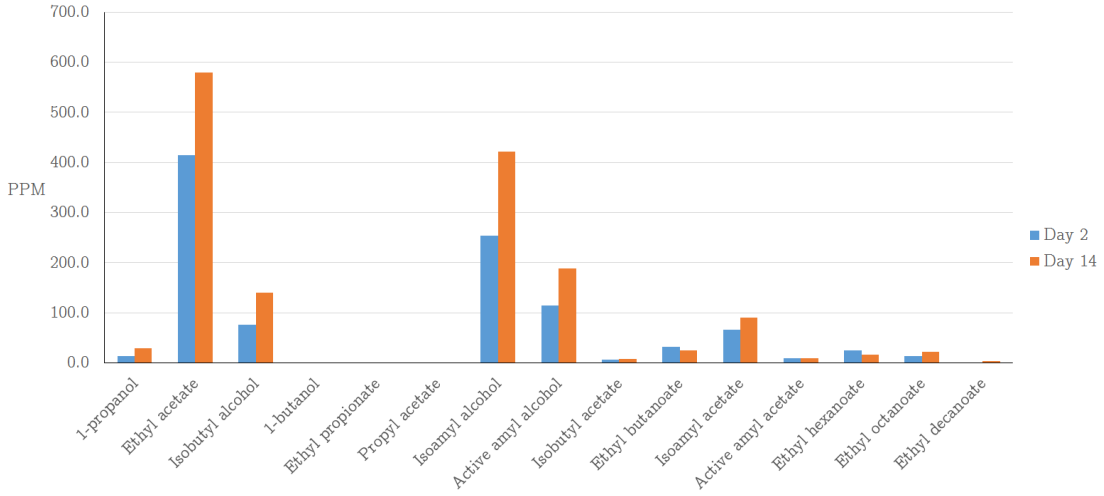


Figure 30: Concentration of identified compounds from the fermentation of Hondalsøl with Hornindal with contaminant fermented at 33 °C for 2 days (blue bar), followed by 19 °C for 12 days (orange bar).

As the fermentation progresses, the concentration of alcohols increase, and esters increase except propyl acetate, ethyl butanoate and ethyl hexanoate. To compare the timeline of fermentation for the other fermentation temperatures for Hornindal with and without contaminant, accompanied with Safale US-05 as reference, a heat map was generated. The heat map is given in Figure 31

Yeast	1-propanol	Ethyl acetate	Isobutyl alcohol	1-butanol	Ethyl propionate	Propyl acetate	Isoamyl alcohol	Active amyl alcohol
Hornindal 19 °C	0,94	0,12	0,44	0,49	[a]	[a]	0,71	0,60
Hornindal 26 °C	0,01	-0,19	-0,07	0,47	[a]	[a]	0,33	0,05
Hornindal 33-19 °C	0,17	-0,19	0,20	-0,41	[a]	-0,36	0,37	0,24
Hornindal 33 °C	0,21	-0,04	0,15	a	[a]	[a]	0,18	0,15
Hornindal + Cont 19 °C	0,70	-0,18	0,08	0,37	[a]	[a]	0,76	0,48
Hornindal + Cont 26 °C	0,35	-0,08	-0,15	[a]	[a]	[a]	0,30	0,09
Hornindal + Cont 33-19 °C	0,20	-0,11	0,40	[a]	[a]	[a]	0,49	0,31
Hornindal + Cont 33 °C	-1,15	-0,48	-0,89	-0,72	-0,78	0,83	-0,73	-0,73
Safale US-05 19 °C	0,69	-0,03	0,83	0,86	-1,55	-3,06	0,87	0,77
Safale US-05 26 °C	-0,03	-0,31	0,06	0,27	[a]	[a]	0,22	0,08
Safale US-05 33-19 °C	0,21	-0,28	0,15	-0,07	-0,05	[a]	0,19	0,11
Safale US-05 33 °C	-0,21	-0,21	-0,21	[a]	[a]	[a]	-0,18	-0,24

Yeast	Isobutyl acetate	Ethyl butanoate	Isoamyl acetate	Active amyl acetate	Ethyl hexanoate	Ethyl octanoate	Ethyl decanoate
Hornindal 19 °C	1,01	1,29	0,67	1,19	0,82	-1,06	-0,96
Hornindal 26 °C	0,24	1,06	0,35	0,83	0,93	-0,92	-1,22
Hornindal 33-19 °C	0,70	0,66	0,00	0,38	0,29	-0,87	-0,64
Hornindal 33 °C	0,15	0,22	0,54	0,68	1,40	0,52	-0,24
Hornindal + Cont 19 °C	1,24	1,62	0,71	0,96	0,59	-1,25	-0,82
Hornindal + Cont 26 °C	0,74	1,07	1,03	1,41	0,63	-0,58	-1,15
Hornindal + Cont 33-19 °C	1,07	-0,04	1,78	1,81	1,13	0,69	0,64
Hornindal + Cont 33 °C	-0,32	0,38	-0,46	-0,15	0,68	-0,76	-1,01
Safale US-05 19 °C	1,03	1,23	0,84	1,59	1,48	-0,38	0,34
Safale US-05 26 °C	0,59	0,52	0,57	1,19	1,05	-1,07	-1,02
Safale US-05 33-19 °C	0,27	0,33	0,05	0,40	0,46	-1,11	-1,76
Safale US-05 33 °C	0,18	-0,17	0,35	0,61	0,79	-0,55	-0,93

[a] No peak was identified at the specific retention time, giving no detection of the compound

Figure 31: Heat map representing color coded concentration differences of Hornindal, Hornindal + contaminant and Safale US-05 after 2 and 14 days fermentation. Three-point color scale, positive values (green color) represent a higher concentration of the given compound after 2 days fermentation. Negative values (red color) represent a higher concentration of the given compound after 14 days fermentation, The midpoint value was defined as 0 (white color), representing no changes in concentration.

The general trend of the fermentation time line shown in Figure 31 is that as the fermentation progress, the concentration of alcohols decrease. Most esters decrease over time except ethyl acetate, ethyl octanoate and ethyl decanoate. Hornindal with contaminant (Figure 30) is exempt from this trend.

Ethyl propionate and propyl acetate was not detected after 2 days fermentation for Hornindal 19 °C, 26 °C, 33-19 °C (Hornindal 33 °C was not detected at 14 days). Hornindal + cont at 19 °C, 26 °C, 33-19 °C was not detected at 2 days fermentation. Safale US-05 was not detected at 26 °C, 33 °C. Ethyl propionate and propyl acetate was detected after 14 days fermentation.

There is a difference in concentration when comparing beer fermented at 33 °C for 2 days, then 19 °C for an additional 12 days as to beer fermented at 33 °C for 14 days. For the fermentation of Hornindal, alcohols isobutyl alcohol, 1-butanol, isoamyl alcohol, active amyl alcohol are highest after 2 days at 19 °C, then 33-19 °C followed by 33 °C. This suggest that the production of mentioned alcohols happen in the first couple of days of the fermentation at a lower temperature, and is consumed over time at a lower temperature. The ester isobutyl acetate has the highest difference of concentration at 19 °C, then 33-19 °C, compared to 26 and 33 °C, this suggest that the consumption of isobutyl acetate happens primarily after two days fermentation, at a lower temperature. Ethyl octanoate increase over time for 19, 26 and 33-19 °C whereas decrease for 33 °C, more is consumed at higher temperatures, and increased production at lower temperature. Ethyl decanoate increase over time, it increase less at higher temperatures and most is produced early in the fermentation. The same observations happens for the alcohols when fermenting Hornindal with contaminant, excluding 33 °C. Esters isoamyl acetate, active amyl acetate and ehtyl hexanoate is absorbed at a higher rate when fermentation start at 33 °C, and then decreased to 19 °C, which is the opposite of the fermentation pattern for Hornindal.

4.4.1 Effect of temperature on end product

The concentration of all identified compounds were calculated. The concentration of each compound for Hornindal, Hornindal + contaminant and Safale US-05 at the different temperatures are given in Table 38 - 43 in Appendix A.2. A heat map was generated comparing the concentration of compounds at 33 °C with either 19 °C, 26 °C, or 33-19 19 °C and is given in in Figure 32.

Yeast	1-propanol	Ethyl acetate	Isobutyl alcohol	1-butanol	Ethyl propionate	Propyl acetate	Isoamyl alcohol	Active amyl alcohol
Hornindal 19 °C	1,27	0,67	1,46	[a]	[a]	1,18	1,22	1,10
Hornindal 26 °C	0,58	0,24	0,94	[a]	[a]	1,36	0,83	0,82
Hornindal 33-19 °C	0,52	-0,16	0,66	[a]	[a]	1,13	0,56	0,55
Hornindal + Cont 19 °C	2,23	0,79	2,17	0,17	[a]	-0,98	1,68	1,68
Hornindal + Cont 26 °C	1,57	0,68	1,37	0,61	1,62	-0,57	1,25	1,14
Hornindal + Cont 33-19 °C	0,81	0,37	0,73	0,91	1,01	-0,72	0,77	0,61
Safale US-05 19 °C	0,13	0,33	1,07	-1,47	0,60	-0,77	0,56	0,51
Safale US-05 26 °C	0,19	-0,29	1,00	-0,17	0,02	1,11	0,52	0,50
Safale US-05 33-19 °C	0,61	-0,17	0,56	0,16	-0,05	2,51	0,30	0,31

Yeast	Isobutyl acetate	Ethyl butanoate	Isoamyl acetate	Active amyl acetate	Ethyl hexanoate	Ethyl octanoate	Ethyl decanoate
Hornindal 19 °C	1,65	0,81	1,05	1,15	-0,91	-1,33	-0,13
Hornindal 26 °C	1,14	0,41	0,96	1,25	-0,72	-0,74	0,43
Hornindal 33-19 °C	0,89	0,28	0,07	0,34	-1,00	-0,88	0,46
Hornindal + Cont 19 °C	2,59	0,56	1,74	1,83	-0,02	0,03	1,16
Hornindal + Cont 26 °C	1,66	0,44	1,78	1,84	-0,18	0,29	0,10
Hornindal + Cont 33-19 °C	0,89	0,13	1,60	1,37	0,57	1,05	0,99
Safale US-05 19 °C	1,13	0,28	0,90	1,13	-0,39	-0,84	0,30
Safale US-05 26 °C	0,63	0,18	-0,12	0,32	-0,30	-0,89	-0,78
Safale US-05 33-19 °C	0,09	0,32	-0,57	-0,45	-0,42	-0,36	-0,07

[a] No peak was identified at the specific retention time, giving no detection of the compound

Figure 32: Heat map representing color coded concentration differences of Hornindal, Hornindal + contaminant and Safale US-05 at different fermentation temperatures. Three-point scale, green positive values have a higher concentration at 33 °C, red negative values have a higher concentration at the given temperature. The midpoint value was defined as 0 (white color), representing no changes in concentration as a function of temperature.

The general trend observed in Figure 32 is for the most part that the concentration of alcohols and esters are larger when fermentation at a higher temperature. The same applies for samples fermented for two days at 33 °C before being lowered to 19 °C. However the esters ethyl hexanoate and ethyl octanoate favors fermentation at a lower temperature besides Hornindal with contaminant where ethyl octanoate levels are increased at a higher temperature. The concentration of esters are either larger when fermenting at a lower temperature, and the levels remain relatively the same, or as the temperature increase, the concentration also increase.

As the fermentation temperature increase, the concentration of compound increase. For Hornindal with contaminant, propyl acetate levels are similar, but generally larger when fermenting at a lower temperature compared to Hornindal.

Safale US-05 was used as a control yeast in the experiment, fermentation of many

alcohols are larger at 33 °C, besides 1-butanol. There is a very slight change in concentration of alcohols when comparing 19 and 26 °C, the batch fermented at 33 °C for two days have a lower value of alcohols compared to 33 °C, and the concentration of alcohol is very different than the 19 and 26 °C samples.

Effect of Kveik compared to reference Safale US-05 was used as a reference beer for the sensory evaluation of Honndalsøl from experiment 3. A heat map was generated when comparing the concentration of compounds for Hornindal with and without contaminant with Safale US-05, the heat map is given in Figure 33.

Yeast	1-propanol	Ethyl acetate	Isobutyl alcohol	1-butanol	Ethyl propionate	Propyl acetate	Isoamyl alcohol	Active amyl alcohol
Hornindal 19 °C	-1,74	0,79	-1,47	-1,30	0,02	-0,93	-0,57	-0,42
Hornindal 26 °C	-0,99	0,60	-1,03	-0,17	-0,34	0,77	-0,23	-0,15
Hornindal 33-19 °C	-0,51	1,12	-1,19	0,72	0,35	2,39	-0,18	-0,07
Hornindal 33 °C	-0,60	1,13	-1,09	[a]	[a]	1,02	0,08	0,17
Hornindal + Cont 19 °C	-1,94	0,81	-1,82	-1,28	[a]	-1,50	-0,75	-0,67
Hornindal + Cont 26 °C	-1,22	0,31	-1,09	-0,42	-0,60	-0,03	-0,37	-0,15
Hornindal + Cont 33-19 °C	-0,04	0,73	-0,90	-0,39	-0,06	1,52	-0,10	0,19
Hornindal + Cont 33 °C	0,16	1,28	-0,73	0,36	1,00	-1,71	0,37	0,50

Yeast	Isobutyl acetate	Ethyl butanoate	Isoamyl acetate	Active amyl acetate	Ethyl hexanoate	Ethyl octanoate	Ethyl decanoate
Hornindal 19 °C	-0,21	-0,24	1,23	1,64	0,98	0,16	1,20
Hornindal 26 °C	-0,21	0,06	0,31	0,72	0,89	-0,49	-0,43
Hornindal 33-19 °C	-0,51	0,33	0,75	0,87	1,04	0,18	0,25
Hornindal 33 °C	0,30	0,29	1,38	1,65	0,47	-0,34	0,78
Hornindal + Cont 19 °C	-1,19	0,00	0,62	1,04	1,06	-0,02	-0,01
Hornindal + Cont 26 °C	-0,76	0,02	-0,44	0,21	1,31	-0,34	-0,03
Hornindal + Cont 33-19 °C	-0,54	0,47	-0,71	-0,08	0,44	-0,57	-0,21
Hornindal + Cont 33 °C	0,27	0,28	1,46	1,74	1,43	0,84	0,85

[a] No peak was identified at the specific retention time, giving no detection of the compound

Figure 33: Heat map representing color coded concentration differences when comparing Hornindal with and without contaminant at different fermentation temperatures with Safale US-05 as reference yeast. Red color indicates larger concentration for Safale US-05, green color indicates a larger concentration for either Hornindal with and without contaminant at the given temperature.

As the temperature increase, products formation are increased for both Hornindal variants. Alcohols concentration are mostly larger for Safale US-05. From the description of Safale US-05 brewed beer, after 26 °C, the yeast starts to get stressed, this can explain the jump in concentration difference for 1-propanol, ethyl acetate, isobutyl alcohol and propyl acetate. There is a large relative difference in concen-

tration for many compounds, from the sensory evaluation it was very clear which one was Safale US-05, and which was Hornindal.

Effect of contaminant Hornindal with contaminant is very different from Hornindal without the contaminant. Throughout all experiments, it was observed that the beer brewed with the contaminant had a a perceptible larger viscosity which can be the result of the yellow colonies morphology observed on the agar plate (Figure 11). A comparison of the original culture compared pure culture is given in Figure 34.

Yeast	1-propanol	Ethyl acetate	Isobutyl alcohol	1-butanol	Ethyl propionate	Propyl acetate	Isoamyl alcohol	Active amyl alcohol
Hornindal 19 °C B3	0,19	-0,02	0,35	-0,02	[a]	0,57	0,17	0,25
Hornindal 26 °C B3	0,23	0,29	0,07	0,25	0,26	0,80	0,14	0,00
Hornindal 33-19 °C B3	-0,47	0,38	-0,29	1,10	0,41	0,87	-0,08	-0,26
Hornindal 33 °C B3	-0,76	-0,15	-0,36	[a]	[a]	2,73	-0,28	-0,33
Rivenes 19 °C	-1,29	-1,13	-1,48	[a]	[a]	[a]	-0,78	-1,37
Rivenes 30 °C	-0,27	-0,16	0,44	-0,90	0,10	[a]	-0,68	-0,10
Hornindal 19 B1	0,23	-0,65	0,22	-0,14	0,91	-3,19	0,10	0,04
Hornindal 30 B2	0,89	0,46	0,59	1,36	0,56	1,93	0,52	0,52

Yeast	Isobutyl acetate	Ethyl butanoate	Isoamyl acetate	Active amyl acetate	Ethyl hexanoate	Ethyl octanoate	Ethyl decanoate
Hornindal 19 °C B3	0,98	-0,24	0,61	0,60	-0,08	0,18	1,21
Hornindal 26 °C B3	0,55	0,05	0,75	0,51	-0,42	-0,15	-0,41
Hornindal 33-19 °C B3	0,03	-0,14	1,46	0,94	0,61	0,75	0,46
Hornindal 33 °C B3	0,04	0,01	-0,08	-0,08	-0,96	-1,18	-0,07
Rivenes 19 °C	-1,01	-1,05	-0,42	-1,10	-0,26	-0,53	-0,61
Rivenes 30 °C	0,49	-0,30	-0,57	0,36	-0,20	-0,43	-0,30
Hornindal 19 °C B1	[a]	-0,31	1,93	2,03	0,43	0,15	0,27
Hornindal 30 °C B2	0,35	0,58	0,25	0,31	-0,24	-0,28	0,65

[a] No peak was identified at the specific retention time, giving no detection of the compound

Figure 34: Heat map representing color coded concentration differences of Hornindal (Experiment 1, 2 and 3) and Rivenes when compared to the same temperature with contaminant. Positive green values has a higher concentration of end product for pure culture, negative red colors have a higher concentration of end product in original culture.

Hornindal B3 at different temperatures was tried in the sensory evaluation. The Hornindal B1 did not participate in the Duo-Trio test as it was deemed to be too easy to differentiate both taste and physical characteristics. Hornindal B1 with contaminant had no visible foam and was very viscous, and from the internal test deemed too low quality to test. Figure 34 shows the difference between beer brewed with and without contaminant in experiment 1, 2 and 3. At lower temperature,

the concentration is higher in the pure culture, the yeast is dominating, at higher temperature, the concentration is higher in the original culture, the contaminant can help the yeast create some products.

4.4.2 Sensory evaluation

Anders Christensen (`http://anders.geekhouse.no/blog`) is an employee at NTNU and an avid beer enthusiast, he was asked to evaluate the sensory properties of the beer brewed with Hornindal with and without contaminant and Safale US-05 at different temperatures, his opinions on the beer were recorded.

Hornindal with contaminant at 19 °C tasted different from Hornindal at 19 °C, with contaminant the beer tasted a lot more citrus, whereas Hornindal without contaminant had a touch of sulfur, as well as having a drying effect on the mouth. Safale US-05 had resin flavor from the juniper, but pure and clear. There was a clear difference in taste between the three types.

At 26 °C, Hornindal with contaminant had an astringent flavor, still tasting like citrus and very different from Hornindal. Hornindal had lost some sulfur, but its still traceable. The reference had become more fruity at 26 °C, however noticeable that the yeast is under stress but still remained relatively clean although more yeast flavor.

The astringency noticed at 26 °C remained at 33 °C, without being a direct result of low pH, it might be a hint of tannin. Anders Christensen noted a slight bitterness resembling a Orval Trappist in the Hornindal with contaminant. The Hornindal with contaminant felt lighter on the malt flavor. Hornindal without contaminant at 33 °C was darker, with a smooth malt rich basis for the beer. 33-19 °C was not judged by Anders Christensen

As the temperature increase, both Hornindal with contaminant and without tasted better, whereas Safale US-05 did not perform well at higher fermentation temper-

atures. This was the same general consensus from an internal sensory evaluation (7 panelist) performed on the same beer, with the panelist being untrained in recognizing flavor.

5 Discussion

5.1 Growth parameters

The two methodologies applied to estimate cell count were CFU and counting chamber. CFU should be lower than counting chamber, because the counting chamber does not differentiate between dead or alive cells, and only alive, colony forming cells are allowed to grow on agar plate. It is possible to stain the yeast cells using methylene blue in order count only alive cells [43]. This is critical in the case of Muri. Since counting chamber had nearly twice as many counted cells as CFU. The large difference between the two methods are the result of dilution range. The counting chamber method needs the samples to be in a 10^{-2} dilution in order to observe an appropriate amount of cells in the microscope, while the CFU method requires the samples to be in the 10^{-6} - 10^{-9} range. It was observed that strains with very high flocculations (Muri, Stranda and Hornindal) had cell concentration values from the CFU method in the same order of magnitude as the counting chamber method, oppositely Idun and Sigmund with poor flocculation had a cell concentration from the CFU method 2-3 orders of magnitude larger than counting chamber.

The reported value of $7.922 \cdot 10^{-11} g/_{yeast_{cell}}$ [44] refers yeast cells and does not specify which specie, however the weight is highly dependent on the diameter of the cell, a 3.5 μm cell has a weight of $2.4 \cdot 10^{-11}$, and a 7.0 μm cell has a weight of $19.2 \cdot 10^{-11}$ with the average being the reported value, which represent the weight of a cell with diameter of roughly 5.0 - 5.5 μm. 5.0 - 5.5 μm cell diameter is what was observed for the Kveik strains (Appendix B.4).

The flocculation patters shows that there are phenotypic differences between Muri,

Hornindal, Stranda compared to Sigmund and Idun, however phenotypic differences are not a clear indicator of specie [36], the different flocculation patterns can be the result of the farmers who used those Kveik had a specific preference when it comes to collecting yeast after fermentation.

5.2 Genetic analysis

The chromatograms from the Sanger sequencing have a larger variance in quality, especially Rivenes, Raftevold, Sigmund and Wendelboe which makes the alignment of forward and inverse reverse sequencing data to create a consensus region difficult, this is reflected from the result of the RDP classification, Wendelboe is an unclassified Fungi, Rivenes and Sigmund is of an unclassified Saccharomyces specie. Lithuania 1 was an unclassified Ascomycetes, the ITS primers used are based on the identification of Ascomycetes and Basidiomycetes [35].

The Idun and Lithuania #4 and the Kveik strains Raftevold, Gausemel, and Rivenes was classified as *S. boulardii* , which is more commonly used as a biotherapeutic agent for the prevention and treatment of several gastrointestinal diseases and has been prescribed in the past 30 years for prophylaxis and treatment of diarrheal diseases caused by bacteria [45]. A study showed that *S. boulardii* is genetically very close or nearly identical to *S. cerevisiae*. Metabolically and physiologically, however, it shows a very different behavior, particularly in relation to growth yield and resistance to temperature and acidic stresses [46]. Another study concluded that PCR-amplified intergenic transcribed spacer regions (including the 5.8S ribosomal DNA) showed that the three isolates of S. boulardii were not separable from authentic isolates of Saccharomyces cerevisiae [47]. In order to test if Raftevold, Gausemel, Rivenes, Idun and Lithuania 4 are *S. boulardii* , they can be grown on medium containing galactose as carbon source, *S. boulardii* cannot use galactose while *S. cerevisiae* can [48]. Galactose is neither present in malt nor the produced wort. *S. boulardii* has an higher optimum growth temperature 37 °C vs 30 °C for *S. cerevisiae* [49]. It is possible that Idun, Lithuania #4, Raftevold,

Gausemel, and Rivenes are *S. cerevisiae*. The Phylogenetic tree showed that the Kveik grouped together in clades containing either *S. boulardii* or *S. cerevisiae*. The Muri strain was closer to *S. cerevisiae*. This shows that there is a difference between the different classifications, however it is still possible that they belong to the same specie.

Idun baker's yeast production started in 1883 under the name "Christiania pressgjær fabrikk" [50]. Through fusion, Idun baker's yeast was incorporated into Orkla in 1991. The original Idun baker's yeast lost its tariff protection in 2005, and Orklas Swedish subsidiary Jästbolaget in Stockholm, started to supply the Norwegian marked with their yeast under the Idun baker's yeast name [51]. The Idun yeast used in this thesis will most likely belong to the strain introduced in 2005, however there is no certain way to prove this. When Idun yeast was introduced to the Norwegian market in 1883, it quickly drove out the private strains that people used to keep at home [24]. The age of Muri, Stranda, Hornindal, Sigmund, Raftevold, Wendelboe and Rivenes is not know, so it is uncertain if it dates back before Idun baker's yeast was introduced in 1883 or after. It is fairly certain that the Kveik strains are not the Idun strain introduced in 2005.

5.3 Beer experiment 1 and 2

The beer from experiment 1 matured or a couple of weeks longer than experiment 2 (Figure 23). All alcohols increased in concentration, all esters except active amyl acetate, ethyl hexanoate and ethyl octanoate during maturation. Various carbonyl compounds, some ethyl esters, Maillard compounds, dioxolanes, and furanic ethers showed a marked increase over a 6 month maturation, due to oxidative and nonoxidative reactions compared to fresh beer, the esters amyl acetate, ethyl hexanoate and ethyl octanoate all decreased in concentration [52]. Maturation influence the chemical evolution of beer.

The OG and FG data from Table 8 for experiment 1 show Muri having a lower FG compared to the other strains, this is most likely due to Muri being classified

as *S. bayanus / S. pastorianus* and can ferment more sugars, the strain can transport fructose via a proton symport mechanism [22], *S. cerevisiae* can only ferment some mono-, di-, and trihexoses [53]. Idun has a higher FG compared to the other strains, which oppositely means that there are some wort sugars that Idun cannot ferment. The FG from experiment 1 (19 °C fermentation) was measured on beer that had been carbonated for 2 weeks on 6 g/L glucose, all glucose should have been consumed over this time, and should not influence FG. Hornindal with contaminant has a higher FG, the contaminant can have consumed some carbohydrates the yeast would ferment, however at 30 °C, this does not happen, the contaminant might not be as dominant, or it is possible that the contaminant can digest other carbohydrates the yeast cannot. Isolated Rivenes strain has higher FG, and with the contaminant, FG is lowered at both fermentation temperatures, it is possible that the contaminant digest some di or trisaccharides, and leaving the monomer for the yeast strain to ferment. When the contaminant is present for Rivenes with contaminant at 30 °C, the FG for the Kveik ends at the same FG as the other Kveik

Figure 11 show the contaminant for Hornindal, a yellow translucent is being produced by it, no analyses have been performed to identify the contaminant, it is uncertain if it is the same contaminant present in Rivenes. It is suspected that the Hornindal contaminant is a form of lactic acid bacteria, this is based on the morphology.

Rivenes and Raftevold are closest to WLP001 of the Kveik in the PCA plots (Figure 24 - 26), these taxa are also grouped together in the same clade in the phylogenetic tree (Figure 18). The same trend is observed for Stranda, Hornindal and Sigmund. It is possible that similar phylogeny result in similar fermentation patters.

5.4 Sensory analysis and threshold values

The Duo-trio test from experiment 1 had too few assessors participating, according to the ISO standard, the typical number of assessors for a Duo-trio test when

testing for a difference is 32 - 36 participants, and it is suggested to replicate evaluations to produce a sufficient number of total evaluations [29].
Figure 28 show the thresholds of flavor components in beer, where the theoretical threshold value is compared to the determined concentration. The highlighted values (orange) represent those who participated in the sensory analysis. Active amyl alcohol is below the taste threshold in Idun and Sigmund, they were on the opposite side of the taste rating, 3.5 and 2.8 respectively, and all assessors recognized Idun to be different from WLP001, but only 12/17 recognized Sigmund from WLP001. Active amyl alcohol does not seem to have a large influence on the overall flavor and rating of the beer. Ethyl decanoate (sweet ester) were below the flavor threshold for Hornindal, Sigmund and Idun. Hornindal together with Idun were the highest rated beer, ethyl decanoate might not have a large contribution on the flavor. Active amyl acetate (banana or pear flavor) had a higher concentration in the beer that was rated higher than WLP001, the flavor thresholds in beer for this compound was not found.

5.5 Beer experiment 3

The FG data from experiment 3 (Table 10) show that Hornindal with contaminant decrease in FG as temperature increase, the contaminant looks to either consume sugars or potentially have enzymes that cut larger sugars still in the wort into smaller carbohydrates that the yeast can consume FG is lower at 33-19 °C for Hornindal + contaminant compared to Hornindal. The contaminant have an effect on the final product. As the fermentation temperature increase, the concentration of volatile compounds are higher in the beer fermented with Kveik and contaminant. The contaminant might grow better at 19 °C, and as a result outperform the yeast and consume more wort nutrients. Following at 33 °C, the yeast has a higher growth rate and outperform the contaminant, however not enough about the contaminant is know.

The sensory evaluation of experiment 3 performed with Anders Christensen this is a description of flavors when each beer sample is known before hand, and always

compared to Safale US-05. There was no doubt that there was a very different flavor in Hornindal with and without contaminant when comparing it to Safale US-05. At higher fermentation temperatures, Safale US-05 is a poor reference, as it is clearly stressed.

5.6 Further Work

A continuation of the work presented here can focus on a single strain of Kveik, and isolate more than one dominant yeast strain observed in the original Kveik culture and perform a similar set of experiment that can examine the genotypic characterization as well as fermentation properties. It is of interest to identify the contaminants present in the current selection of Kveik, to further assess their responsibilities in the fermentation process. Expanding the genotypic characterization should also involve representative yeast strains from England, America, Belgium and Germany and also reference strains from *S. cerevisiae* and *S. pastorianus*, this can broaden the understanding of the origin of the Norwegian Kveik strains

A continued collaboration with Lars Marius Garshol to identify and gather more Kveik before they potentially vanish from Norwegian farms, these traditional Kveik strains should be preserved as they are a part of Norwegian cultural history. Following the preservation of Kveik strains, it would be interesting to contact Idun Industri AS and inquire if they have baker's yeast strain dating back before the introduction of the Swedish version, and compare that one to the fermentation properties and genetic phylogeny of the current Kveik strains.

Analyzing the carbohydrate content on final beer in order to determine if there are any difference between the Kveik strains, and what the contaminant do with the carbohydrates in the wort.

6 Conclusion

Fourteen isolated strains of yeast were genotypically characterized using the ITS region, eight Norwegian Kveik, Idun Baker's yeast, four Lithuanian yeast and one Finnish yeast. Two Norwegian strains were classified as *S. boulardii* (Raftevold and Gausemel), two as *S. cerevisiae* (Stranda and Hornindal), one as *S. bayanus / S. pastorianus* (Muri), two were unclassified specie of the Saccharomyces genus (Rivenes and Sigmund), one was an unclassified Fungi (Wendelboe). Two Lithuanian yeast was classified as *S. cerevisiae* (#2 and #3) and one was *S. boulardii* (#4), one Lithuanian yeast was an unclassified Ascomycota (#1). The Finnish yeast was classified as *C. humulis*. A phylogenetic neighbor-joining tree showed that The Norwegian Kveik grouped together in clades containing either *S. boulardii* or *S. cerevisiae*. The Muri strain was closer to *S. cerevisiae*. The Lithuanian yeast grouped together. The Finnish yeast was closed to the Lithuanian yeast.

Two representative isolated Kveik strains underwent a phenotypic characterization, and the relationship between optical density and cell concentration using two different methodologies was established. The counting chamber method was quicker and more reliable method in estimating the cell concentration. A flocculation assay was performed on five of the eight Kveik strains. Muri, Hornindal and Stranda strains had great flocculation, while Sigmund and Idun had poor flocculation.

A pale ale was brewed with 10 yeast strains fermented at 19 °C and 30 °C. Two strains had a contamination present, one strain was WLP001 used as reference yeast. As fermentation temperature increased, the concentration of volatile compounds increased except longer chained esters. The beer fermented with contaminant was still higher for the Kveik strain Hornindal, but the difference in concentration of volatile compounds was closer between the two fermentation temperatures. For Rivenes with contaminant, the concentration of volatile compounds was higher when fermenting at 19 °C. A PCA plot show PC1 separates on yeast strain while

PC2 partly separates on temperatures. The esters are produced at a higher concentration at 30 °C. A sensory analysis was performed on the pale ale brewed with Muri, Stranda, Hornindal and Idun yeast. Muri, Stranda and Idun was recognized to be different from the reference yeast. Beer with higher concentration of active amyl acetate, a sweet tasting ester, got a higher taste rating when comparing it to the reference yeast.

A traditional Honndalsøl was brewed using Hornindal Kveik with and without contaminant and Safale US-05 as reference at 19 °C, 26 °C, 33 °C, and 33 °C for 2 days followed by 19 °C for 12 days. The evolution of volatile compounds was assessed by taking samples after two days fermentation for all fermentation temperatures and comparing them to the end of the fermentation. As the fermentation progress, the concentration of alcohols decrease. When comparing the beer end point, the concentration of alcohols and esters are larger at higher fermentation temperatures, independent if the contaminant is present or not. A sensory evaluation of Hornindal with and without contaminant showed large variance between the samples. Hornindal with contaminant fermented at 33 °C had the best subjective flavor. As temperature increased, both Hornindal variants tasted better. At 33 °C, Hornindal with contaminant had an astringent note, which was not a direct result of low pH, the astringent note was favorable for the overall impression of the beer.

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Appendix A Rawdata: Headspace

A.1 Beer experiment 1 and 2

1-pentanol was used as an internal standard, 18.50 mg 1-pentanol was measured in a 10.00 mL volumetric flask. 1.25 mL of the 1850 ppm solution was transferred to a 5.00 mL volumetric flask and diluted to 462.5 ppm. 100 μL of standard was transferred to each sample to be run on Headspace GC-MS. Each sample was weighted in Headspace GS-MS vials on an analytical balance, the measured weight from experiment one and two with the concentration of 1-pentanol is given in Table 11

Table 11: Sample weight and concentration of 1-pentanol ran on headspace GC-MS

Sample	**Weight [g]**	**1-pentanol [ppm]**
wlp001_19degC_b2_1	10.0310	4.611
wlp001_19degC_b2_2	10.0414	4.606
wlp001_19degC_b2_3	10.0269	4.613
wlp001_19degC_b1_1	10.0018	4.624
wlp001_19degC_b1_2	10.0384	4.607
wlp001_19degC_b1_3	10.0431	4.605
wlp001_30degC_1	10.0204	4.616
wlp001_30degC_2	10.0081	4.621
wlp001_30degC_3	10.0175	4.617
3545_19degC	10.0247	4.614
3545_30degC	10.0217	4.615
3548_19degC	10.0258	4.613
3548_30degC	10.0368	4.608
4051_19degC	10.0140	4.619
Hdal+kont_19degC	10.0039	4.623
Hdal+kont_30degC	10.0140	4.619
hornindal_19degC	10.0383	4.607
hornindal_30degC	10.0140	4.619
idun_19degC	10.0107	4.620
idun_30degC	10.0350	4.609
muri_19degC	10.0369	4.608
muri_30degC	10.0295	4.611
sigmund_19degC	10.0031	4.624
sigmund_30degC	10.0184	4.617
stranda_19degC	10.0178	4.617
stranda_30degC	10.0329	4.610

Each peak was identified by retention time, the retention time of all identified

peaks are given in Table 12 -14

Table 12: Identification of 1-propanol, ethyl acetate, isobutyl alcohol, 1-butanol and ethyl propionate from the chromatograms based on retention time

Gjær og temp	**1-propanol**	**Ethyl acetate**	**Isobutyl alcohol**	**1-butanol**	**Ethyl propionate**
wlp001_19degC_brygg2_1	5.317	6.479	8.013	9.904	10.053
wlp001_19degC_brygg2_2	5.315	6.479	8.014	9.904	10.055
wlp001_19degC_brygg2_3	5.315	6.478	8.013	9.902	10.054
wlp001_19degC_brygg1_1	5.31	6.476	8.009	9.900	10.049
wlp001_19degC_brygg1_2	5.305	6.474	8.008	9.900	10.049
wlp001_19degC_brygg1_3	5.31	6.474	8.008	9.900	10.052
wlp001_30degC_1	5.315	6.477	8.012	9.904	10.048
wlp001_30degC_2	5.316	6.479	8.014	9.902	10.05
wlp001_30degC_3	5.313	6.477	8.012	9.908	10.053
3545_19degC	5.318	6.479	8.013		
3545_30degC	5.314	6.478	8.013	9.902	10.049
3548_19degC	5.311	6.478	8.011	9.905	10.051
3548_30degC	5.311	6.477	8.011	9.899	10.051
4051_19degC	5.316	6.48	8.014	9.902	10.05
Hdal+kont_19degC	5.305	6.473	8.005	9.900	10.054
Hdal+kont_30degC	5.304	6.474	8.007	9.902	10.053
Hornindal_19degC	5.307	6.474	8.008	9.900	10.048
Hornindal_30degC	5.303	6.472	8.006	9.898	10.048
idun_19degC	5.302	6.471	8.004	9.899	10.047
idun_30degC	5.302	6.472	8.005	9.901	10.048
muri_19degC	5.315	6.478	8.011	9.900	10.048
muri_30degC	5.311	6.476	8.009	9.903	10.052
sigmund_19degC	5.303	6.471	8.005	9.901	10.048
sigmund_30degC	5.301	6.472	8.005	9.905	10.049
stranda_19degC	5.305	6.473	8.006	9.902	10.052
stranda_30degC	5.304	6.473	8.006	9.902	10.047

Table 13: Identification of propyl acetate, isoamyl alcohol, active amyl alcohol, isobutyl acetate and ethyl butanoate from the chromatograms based on retention time

Yeast and temp	Propyl acetate	Isoamyl alcohol	Active amyl alcohol	Isobutyl acetate	Ethyl butanoate
wlp001_19degC_b2_1	10.196	11.413	11.493	11.817	12.275
wlp001_19degC_b2_2	10.195	11.412	11.492	11.816	12.273
wlp001_19degC_b2_3		11.412	11.492	11.813	12.273
wlp001_19degC_b1_1	10.2	11.411	11.491	11.818	12.271
wlp001_19degC_b1_2	10.202	11.41	11.49	11.815	12.272
wlp001_19degC_b1_3	10.199	11.412	11.491	11.814	12.272
wlp001_30degC_1	10.194	11.412	11.491	11.812	12.272
wlp001_30degC_2	10.196	11.413	11.492	11.817	12.274
wlp001_30degC_3	10.196	11.414	11.492	11.815	12.274
3545_19degC		11.415	11.495	11.817	12.273
3545_30degC	10.205	11.414	11.494	11.817	12.275
3548_19degC	10.207	11.414	11.494	11.816	12.273
3548_30degC		11.413	11.492	11.816	12.272
4051_19degC	10.211	11.418	11.495	11.817	12.274
Hdal+kont_19degC	10.195	11.409	11.49		12.27
Hdal+kont_30degC	10.2	11.41	11.49	11.814	12.272
hornindal_19degC	10.206	11.411	11.491	11.814	12.272
hornindal_30degC	10.197	11.409	11.489	11.813	12.271
idun_19degC	10.195	11.407	11.489	11.813	12.269
idun_30degC	10.198	11.409	11.488	11.814	12.271
muri_19degC	10.202	11.412	11.492	11.817	12.271
muri_30degC	10.204	11.411	11.491	11.814	12.271
sigmund_19degC	10.201	11.408	11.488	11.814	12.269
sigmund_30degC	10.203	11.407	11.488	11.814	12.269
stranda_19degC	10.208	11.409	11.49	11.816	12.271
stranda_30degC	10.197	11.408	11.489	11.815	12.272

Table 14: Identification of isoamyl acetate, active amyl acetate, ethyl hexanoate, ehyl ocanoate, ethyl decanoate and 1-pentanol from the chromatograms based on retention time

Gjær og temp	Isoamyl acetate	Active amyl acetate	Ethyl hexanoate	Ethyl octanoate	Ethyl decanoate	1-pentanol
wlp001_19degC_b2_1	14.666	14.76	17.644	20.774	23.106	12.478
wlp001_19degC_b2_2	14.665	14.758	17.643	20.774	23.106	12.476
wlp001_19degC_b2_3	14.664	14.762	17.642	20.774	23.106	12.476
wlp001_19degC_b1_1	14.664	14.754	17.643	20.773	23.104	12.477
wlp001_19degC_b1_2	14.662	14.754	17.642	20.774	23.105	12.476
wlp001_19degC_b1_3	14.664	14.755	17.644	20.774	23.105	12.476
wlp001_30degC_1	14.662	14.757	17.642	20.773	23.107	12.475
wlp001_30degC_2	14.665	14.761	17.642	20.774	23.107	12.479
wlp001_30degC_3	14.664	14.664	17.642	20.775	23.108	12.476
3545_19degC	14.665	14.763	17.642	20.774	23.108	12.479
3545_30degC	14.665	14.761	17.641	20.774	23.108	12.478
3548_19degC	14.665	14.761	17.643	20.774	23.107	12.478
3548_30degC	14.663	14.76	17.642	20.773	23.107	12.479
4051_19degC	14.664	14.762	17.643	20.775	23.108	12.477
Hdal+kont_19degC	14.662	14.758	17.642	20.774	23.103	12.476
Hdal+kont_30degC	14.663	14.761	17.641	20.774	23.107	12.474
hornindal_19degC	14.663	14.757	17.641	20.774	23.105	12.474
hornindal_30degC	14.662	14.759	17.641	20.773	23.107	12.474
idun_19degC	14.662	14.756	17.641	20.774	23.108	12.475
idun_30degC	14.663	14.757	17.641	20.774	23.107	12.476
muri_19degC	14.663	14.759	17.641	20.773	23.105	12.476
muri_30degC	14.663	14.757	17.641	20.774	23.108	12.476
sigmund_19degC	14.662	14.756	17.641	20.774	23.107	12.474
sigmund_30degC	14.664	14.759	17.641	20.774	23.106	12.475
stranda_19degC	14.661	14.758	17.642	20.774	23.105	12.475
stranda_30degC	14.661	14.756	17.641	20.774	23.103	12.475

The area of each peak from the retention times (Table 12 - 14) was extracted. The areas of each peak are given in Table 15 - 20

Table 15: Area of each identified compounds for WLP001 brewed at 19 °C for experiment 2 and 1

Compound	wlp001_19degC_b2_1	wlp001_19degC_b2_2	wlp001_19degC_b2_3	wlp001_19degC_br1_1
1-propanol	591 914	512 108	513 138	727 270
Ethyl acetate	9 795 964	9 042 864	9 202 168	8 388 915
Isobutyl alcohol	1 202 324	1 142 054	1 161 681	1 914 647
1-butanol	53 424	51 408	51 455	118 174
Ethyl propionate	20 536	19 603	19 680	21 943
Propyl acetate	12 021	11 973		48 932
Isoamyl alcohol	5 942 585	5 724 931	5 850 668	7 137 711
Active amyl alcohol	1 872 113	1 828 453	1 857 086	2 323 262
Isobutyl acetate	68 281	62 488	62 079	66 179
Ethyl butanoate	587 975	571 860	587 868	556 738
Isoamyl acetate	997 172	936 382	949 551	816 650
Active amyl acetate	46 006	46 642	41 213	32 379
Ethyl hexanoate	375 528	361 065	359 523	200 599
Ethyl octanoate	916 783	932 861	964 706	274 665
Ethyl decanoate	82 058	100 316	76 314	792 028
1-pentanol	137 297	131 702	134 387	109 942

Table 16: Area of each identified compounds for WLP001 brewed at 19 °C for experiment 1 and 30 °C

Compound	wlp001_19degC_b1_2	wlp001_19degC_b1_3	wlp001_30degC_1	wlp001_30degC_2
1-propanol	615 966	704 428	707 289	612 142
Ethyl acetate	8 160 498	7 805 177	14 867 853	14 623 110
Isobutyl alcohol	1 797 839	1 834 868	2 587 354	2 465 259
1-butanol	118 808	110 737	32 314	32 189
Ethyl propionate	20 379	20 021	49 007	47 627
Propyl acetate	48 793	48 428	115 667	115 032
Isoamyl alcohol	6 695 603	6 838 476	7 476 221	7 130 895
Active amyl alcohol	2 201 386	2 224 467	2 875 663	2 787 814
Isobutyl acetate	73 352	67 745	195 315	205 962
Ethyl butanoate	523 498	532 141	642 631	623 267
Isoamyl acetate	790 458	762 008	2 261 582	2 253 200
Active amyl acetate	31 355	30 771	221 824	220 952
Ethyl hexanoate	175 275	169 850	511 955	513 154
Ethyl octanoate	172 732	163 946	637 258	669 159
Ethyl decanoate	163 471	73 345	125 996	158 379
1-pentanol	108 118	105 634	149 271	153 255

Table 17: Area of each identified compounds for WLP001 brewed at 30 °C for and NYCY 3545 at 19 & 30 °C and NYCY 3548 at 19 °C

Compound	**wlp001_30degC_3**	**3545_19degC**	**3545_30degC**	**3548_19degC**
1-propanol	575 781	354 226	347 490	291 484
Ethyl acetate	13 631 153	15 216 470	15 216 062	13 950 066
Isobutyl alcohol	2 414 076	2 517 347	1 622 284	1 817 459
1-butanol	30 372		50 995	37 440
Ethyl propionate	45 263		23 929	18 169
Propyl acetate	111 502		126 148	68 686
Isoamyl alcohol	7 031 868	7 644 307	9 768 820	8 931 780
Active amyl alcohol	2 810 087	3 914 786	3 093 765	3 052 494
Isobutyl acetate	190 540	136 120	135 021	136 066
Ethyl butanoate	599 319	680 741	630 608	658 305
Isoamyl acetate	2 130 468	1 354 532	2 409 417	2 034 744
Active amyl acetate	205 950	150 925	143 742	141 413
Ethyl hexanoate	478 766	489 476	695 396	819 661
Ethyl octanoate	673 183	641 708	882 147	892 364
Ethyl decanoate	174 261	122 406	143 269	160 909
1-pentanol	138 732	129 756	173 367	260 680

Table 18: Area of each identified compounds for NYCY 3548 at 30 °C, NYCY 4051 at 19 °C Hdal+kont at 19 & 30 °C

Compound	3548_30degC	4051_19degC	Hdal+kont_19degC	Hdal+kont_30degC
1-propanol	370 075	470 093	347 375	363 504
Ethyl acetate	17 411 679	16 213 269	17 378 838	15 321 974
Isobutyl alcohol	2 826 435	1 861 873	1 309 141	2 290 172
1-butanol	34 949	91 494	59 890	29 426
Ethyl propionate	32 849	20 725	10 335	31 771
Propyl acetate		139 467	230 219	11 434
Isoamyl alcohol	7 784 304	12 650 201	7 187 881	7 124 601
Active amyl alcohol	3 700 663	3 582 741	2 842 351	3 558 239
Isobutyl acetate	243 150	104 038		176 295
Ethyl butanoate	653 957	668 743	571 696	560 599
Isoamyl acetate	2 077 192	2 178 901	489 752	1 829 426
Active amyl acetate	236 911	114 118	44 770	203 235
Ethyl hexanoate	775 882	564 904	475 644	452 272
Ethyl octanoate	835 852	562 519	180 156	168 957
Ethyl decanoate	148 631	121 575	18 786	13 074
1-pentanol	221 563	189 805	215 476	191 614

Table 19: Area of each identified compounds for Hornindal at 19 & 30 °C, Idun at 19 & 30 °C and muri at 19 °C

Compound	hornindal_19degC	hornindal_30degC	idun_19degC	idun_30degC	muri_19degC
1-propanol	444 186	434 391	364 636	479 951	408 755
Ethyl acetate	12 018 280	13 605 497	16 363 683	17 721 879	21 037 142
Isobutyl alcohol	1 665 297	2 221 453	1 151 502	1 970 617	1 501 641
1-butanol	59 076	48 801	26 051	36 422	102 343
Ethyl propionate	21 159	30 265	51 754	62 721	42 533
Propyl acetate	27 392	28 159	52 272	29 985	19 387
Isoamyl alcohol	8 385 877	6 617 089	6 876 273	7 330 606	8 004 255
Active amyl alcohol	3 190 633	3 290 565	2 609 390	3 793 490	3 081 043
Isobutyl acetate	115 289	145 727	192 393	217 874	122 709
Ethyl butanoate	502 713	540 140	522 300	507 329	447 986
Isoamyl acetate	2 028 087	1 404 892	4 857 414	3 382 489	5 008 090
Active amyl acetate	198 489	163 162	465 642	404 231	387 462
Ethyl hexanoate	696 016	248 009	787 241	387 575	645 316
Ethyl octanoate	216 936	89 730	260 744	115 375	241 373
Ethyl decanoate	24 659	13 231	21 360	7 973	44 773
1-pentanol	233 777	123 843	248 460	141 838	216 700

Table 20: Area of each identified compounds for muri at 30 °C, sigmund at 19 & 30 °C and stranda at 19 °C & 30 °C

Compound	muri_30degC	sigmund_19degC	sigmund_30degC	stranda_19degC	stranda_30degC
1-propanol	386 202	278 509	301 340	479 302	514 083
Ethyl acetate	17 058 463	13 489 557	14 895 969	14 322 157	14 888 459
Isobutyl alcohol	1 908 639	926 072	1 841 609	1 146 714	1 627 056
1-butanol	41 501	23 623	24 199	61 382	51 728
Ethyl propionate	32 074	19 321	18 694	23 160	28 525
Propyl acetate	22 206	25 368	14 681	19 915	20 581
Isoamyl alcohol	8 354 148	5 896 784	5 900 032	6 947 412	5 823 959
Active amyl alcohol	3 043 387	2 135 507	2 751 757	2 283 209	2 512 855
Isobutyl acetate	180 007	96 828	121 124	71 355	83 175
Ethyl butanoate	495 728	485 767	537 327	522 704	510 723
Isoamyl acetate	3 205 821	1 953 473	1 140 658	1 470 323	1 218 057
Active amyl acetate	263 796	180 152	122 909	91 863	98 687
Ethyl hexanoate	550 264	749 369	481 647	632 958	402 404
Ethyl octanoate	133 690	260 882	130 391	178 636	133 271
Ethyl decanoate	26 080	20 195	12 428	32 786	13 280
1-pentanol	205 366	264 085	208 634	209 460	144 847

Using 1-pentanol as internal standard, the concentration of the compounds can be calculated by dividing the area of a specific peak with the product of the area of 1-pentanol with the concentration. The concentration of 1-pentanol is given in Table 11, and the area of 1-pentanol and the given compound is given in Table 15 - 20.

Example calculation for the concentration of 1-Propanol in WLP001_19degC_brygg2_1

$$C_{(1-Propanol)}[ppm] = \frac{A_{1-propanol}}{C_{1-pentanol} \cdot A_{1-pentanol}}$$

$$C_{(1-Propanol)} = \frac{591914}{4.611 \cdot 137297}$$

$$C_{(1-Propanol)} = 19.9ppm$$

The concentration of each compound for the different yeast fermented at 19 and 30 °C is given in Table 21 - 25

Table 21: Concentration of identified compounds in parts per million for WLP001 at 19 °C from experiment 1 and 2

Compound	wlp001_19degC_b2_1	wlp001_19degC_b2_2	wlp001_19degC_b2_3	wlp001_19degC_b1_1
1-propanol	19.9	17.9	17.6	30.6
Ethyl acetate	329.0	316.3	315.8	352.8
Isobutyl alcohol	40.4	39.9	39.9	80.5
1-butanol	1.8	1.8	1.8	5.0
Ethyl propionate	0.7	0.7	0.7	0.9
Propyl acetate	0.4	0.4	0.0	2.1
Isoamyl alcohol	199.6	200.2	200.8	300.2
Active amyl alcohol	62.9	63.9	63.7	97.7
Isobutyl acetate	2.3	2.2	2.1	2.8
Ethyl butanoate	19.7	20.0	20.2	23.4
Isoamyl acetate	33.5	32.7	32.6	34.3
Active amyl acetate	1.5	1.6	1.4	1.4
Ethyl hexanoate	12.6	12.6	12.3	8.4
Ethyl octanoate	30.8	32.6	33.1	11.6
Ethyl decanoate	2.8	3.5	2.6	33.3

Table 22: Concentration of identified compounds in parts per million for WLP001 at 19 °C from experiment 1 and 2 and 30 °C

Compound	wlp001_19degC_b1_2	wlp001_19degC_b1_3	wlp001_30degC_1	wlp001_30degC_2
1-propanol	26.2	30.7	21.9	18.5
Ethyl acetate	347.7	340.3	459.7	440.9
Isobutyl alcohol	76.6	80.0	80.0	74.3
1-butanol	5.1	4.8	1.0	1.0
Ethyl propionate	0.9	0.9	1.5	1.4
Propyl acetate	2.1	2.1	3.6	3.5
Isoamyl alcohol	285.3	298.1	231.2	215.0
Active amyl alcohol	93.8	97.0	88.9	84.1
Isobutyl acetate	3.1	3.0	6.0	6.2
Ethyl butanoate	22.3	23.2	19.9	18.8
Isoamyl acetate	33.7	33.2	69.9	67.9
Active amyl acetate	1.3	1.3	6.9	6.7
Ethyl hexanoate	7.5	7.4	15.8	15.5
Ethyl octanoate	7.4	7.1	19.7	20.2
Ethyl decanoate	7.0	3.2	3.9	4.8

Table 23: Concentration of identified compounds in parts per million for WLP001 at 30 °C, NYCY 3545 at 19 and 30 °C and 3548 at 19 °C

Compound	wlp001_30degC_3	3545_19degC	3545_30degC	3548_19degC
1-propanol	19.2	12.6	9.3	5.2
Ethyl acetate	453.6	541.0	405.0	246.9
Isobutyl alcohol	80.3	89.5	43.2	32.2
1-butanol	1.0	0.0	1.4	0.7
Ethyl propionate	1.5	0.0	0.6	0.3
Propyl acetate	3.7	0.0	3.4	1.2
Isoamyl alcohol	234.0	271.8	260.0	158.1
Active amyl alcohol	93.5	139.2	82.4	54.0
Isobutyl acetate	6.3	4.8	3.6	2.4
Ethyl butanoate	19.9	24.2	16.8	11.6
Isoamyl acetate	70.9	48.2	64.1	36.0
Active amyl acetate	6.9	5.4	3.8	2.5
Ethyl hexanoate	15.9	17.4	18.5	14.5
Ethyl octanoate	22.4	22.8	23.5	15.8
Ethyl decanoate	5.8	4.4	3.8	2.8

Table 24: Concentration of identified compounds in parts per million for NYCY 3548 at 30 °C, 4051 at 19 °C, Hdal+cont at 19 and 30 °C

Compound	3548_30degC	4051_19degC	Hdal+kont_19degC	Hdal+kont_30degC
1-propanol	7.7	11.4	7.5	8.8
Ethyl acetate	362.1	394.5	372.9	369.3
Isobutyl alcohol	58.8	45.3	28.1	55.2
1-butanol	0.7	2.2	1.3	0.7
Ethyl propionate	0.7	0.5	0.2	0.8
Propyl acetate	0.0	3.4	4.9	0.3
Isoamyl alcohol	161.9	307.8	154.2	171.7
Active amyl alcohol	77.0	87.2	61.0	85.8
Isobutyl acetate	5.1	2.5	0.0	4.2
Ethyl butanoate	13.6	16.3	12.3	13.5
Isoamyl acetate	43.2	53.0	10.5	44.1
Active amyl acetate	4.9	2.8	1.0	4.9
Ethyl hexanoate	16.1	13.7	10.2	10.9
Ethyl octanoate	17.4	13.7	3.9	4.1
Ethyl decanoate	3.1	3.0	0.4	0.3

Table 25: Concentration of identified compounds in parts per million for hornindal at 19 and 30 °C, idun at 19 and 30 °C, muri at 19 °C

Compound	hornindal_19degC	hornindal_30degC	idun_19degC	idun_30degC	muri_19degC
1-propanol	8.8	16.2	6.8	15.6	8.7
Ethyl acetate	236.9	507.4	304.3	575.9	447.3
Isobutyl alcohol	32.8	82.8	21.4	64.0	31.9
1-butanol	1.2	1.8	0.5	1.2	2.2
Ethyl propionate	0.4	1.1	1.0	2.0	0.9
Propyl acetate	0.5	1.1	1.0	1.0	0.4
Isoamyl alcohol	165.3	246.8	127.9	238.2	170.2
Active amyl alcohol	62.9	122.7	48.5	123.3	65.5
Isobutyl acetate	2.3	5.4	3.6	7.1	2.6
Ethyl butanoate	9.9	20.1	9.7	16.5	9.5
Isoamyl acetate	40.0	52.4	90.3	109.9	106.5
Active amyl acetate	3.9	6.1	8.7	13.1	8.2
Ethyl hexanoate	13.7	9.2	14.6	12.6	13.7
Ethyl octanoate	4.3	3.3	4.8	3.7	5.1
Ethyl decanoate	0.5	0.5	0.4	0.3	1.0

Table 26: Concentration of identified compounds in parts per million for muri at 30 °C, sigmund at 19 and 30 °C, stranda at 19 and 30 °C

Compound	muri_30degC	sigmund_19degC	sigmund_30degC	stranda_19degC	stranda_30degC
1-propanol	8.7	4.9	6.7	10.6	16.4
Ethyl acetate	383.0	236.2	329.6	315.7	473.8
Isobutyl alcohol	42.9	16.2	40.7	25.3	51.8
1-butanol	0.9	0.4	0.5	1.4	1.6
Ethyl propionate	0.7	0.3	0.4	0.5	0.9
Propyl acetate	0.5	0.4	0.3	0.4	0.7
Isoamyl alcohol	187.6	103.2	130.6	153.1	185.4
Active amyl alcohol	68.3	37.4	60.9	50.3	80.0
Isobutyl acetate	4.0	1.7	2.7	1.6	2.6
Ethyl butanoate	11.1	8.5	11.9	11.5	16.3
Isoamyl acetate	72.0	34.2	25.2	32.4	38.8
Active amyl acetate	5.9	3.2	2.7	2.0	3.1
Ethyl hexanoate	12.4	13.1	10.7	14.0	12.8
Ethyl octanoate	3.0	4.6	2.9	3.9	4.2
Ethyl decanoate	0.6	0.4	0.3	0.7	0.4

A.1.1 Plots of each compound at different temperature

A bar graph was made comparing 19 and 30 °C from the concentration of identified compound form Table 21 - 26. The concentration of each compound is given in

Figure 35 - Figure 49

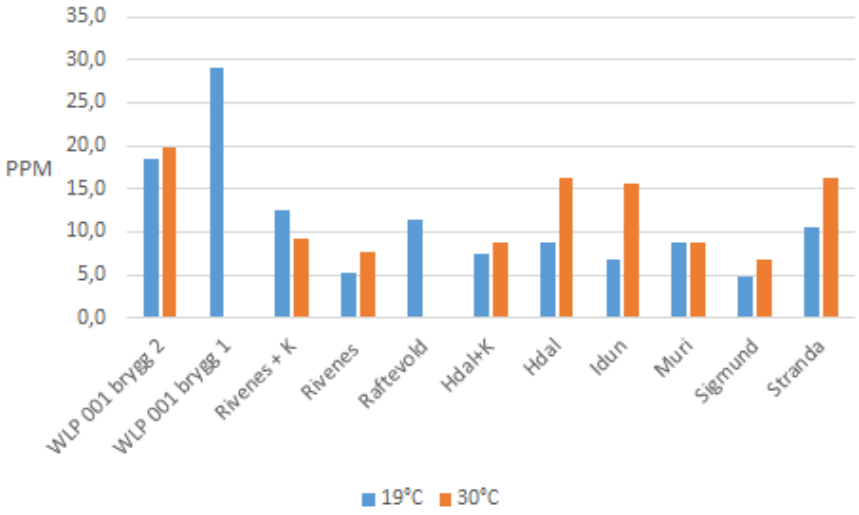

Figure 35: Parts per million of 1-propanol from Dynamic Headspace-GCMS analysis for WLP001 batch #1 and #2, Rivenes + contaminant, Rivenes, Raftevold, Hornidal, Hornindal + contaminant, Idun, Muri, Sigmund and Stranda at 19 °C and 30 °C

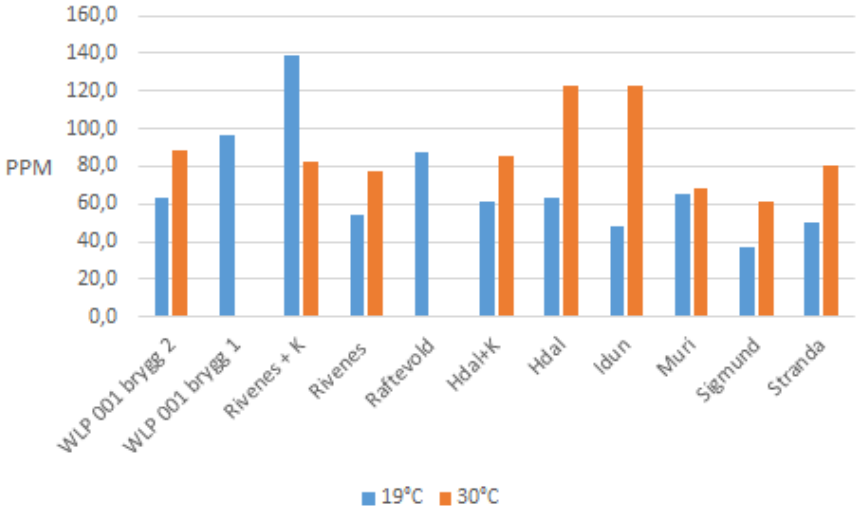

Figure 36: Parts per million of activeamyl from Dynamic Headspace-GCMS analysis for WLP001 batch #1 and #2, Rivenes + contaminant, Rivenes, Raftevold, Hornidal, Hornindal + contaminant, Idun, Muri, Sigmund and Stranda at 19 °C and 30 °C

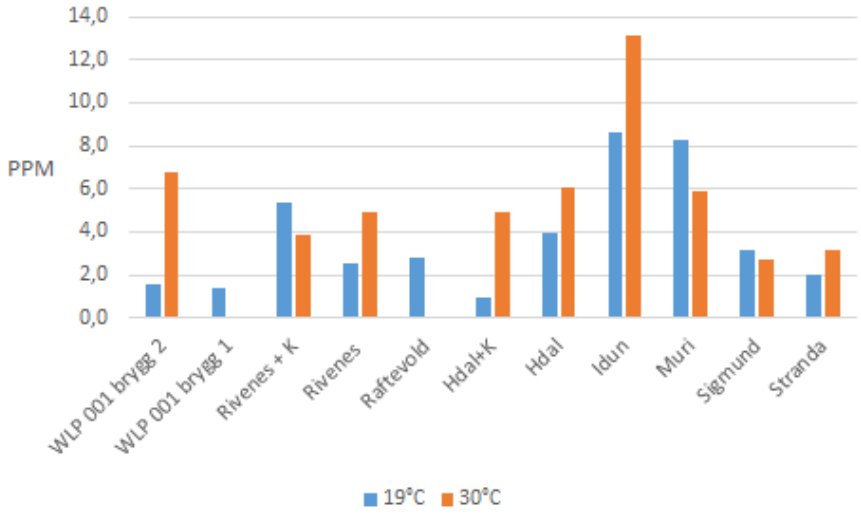

Figure 37: Parts per million of activeamylacetate from Dynamic Headspace-GCMS analysis for WLP001 batch #1 and #2, Rivenes + contaminant, Rivenes, Raftevold, Hornidal, Hornindal + contaminant, Idun, Muri, Sigmund and Stranda at 19 °C and 30 °C

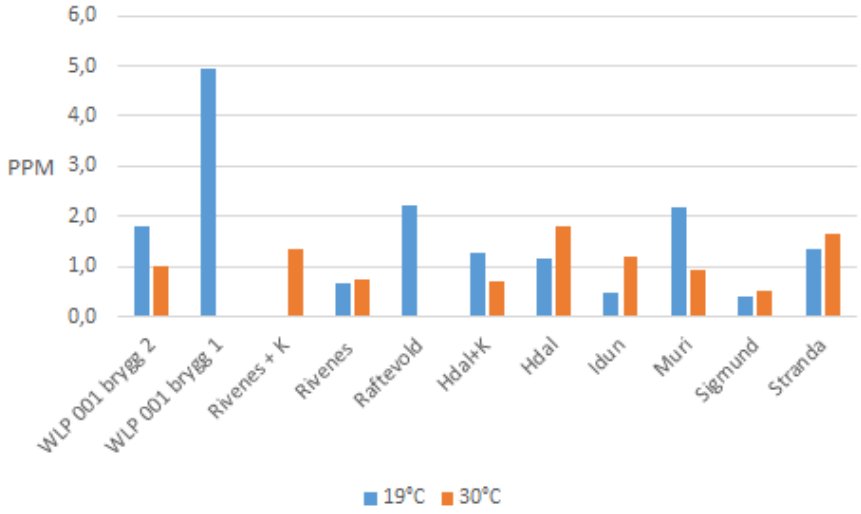

Figure 38: Parts per million of butanol from Dynamic Headspace-GCMS analysis for WLP001 batch #1 and #2, Rivenes + contaminant, Rivenes, Raftevold, Hornidal, Hornindal + contaminant, Idun, Muri, Sigmund and Stranda at 19 °C and 30 °C

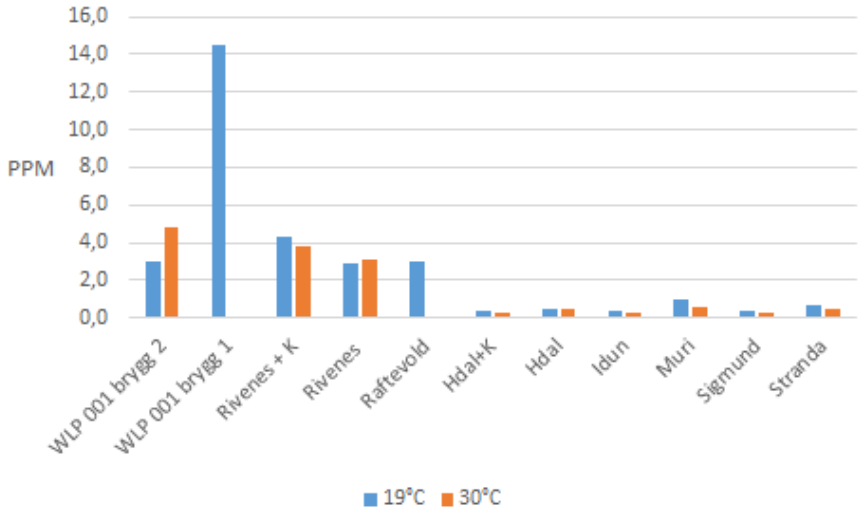

Figure 39: Parts per million of dedononate from Dynamic Headspace-GCMS analysis for WLP001 batch #1 and #2, Rivenes + contaminant, Rivenes, Raftevold, Hornidal, Hornindal + contaminant, Idun, Muri, Sigmund and Stranda at 19 °C and 30 °C

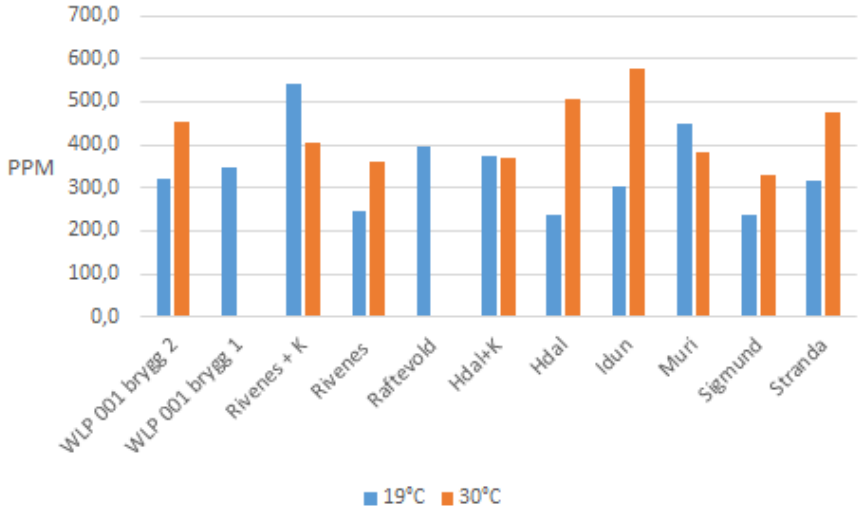

Figure 40: Parts per million of ethylacetate from Dynamic Headspace-GCMS analysis for WLP001 batch #1 and #2, Rivenes + contaminant, Rivenes, Raftevold, Hornidal, Hornindal + contaminant, Idun, Muri, Sigmund and Stranda at 19 °C and 30 °C

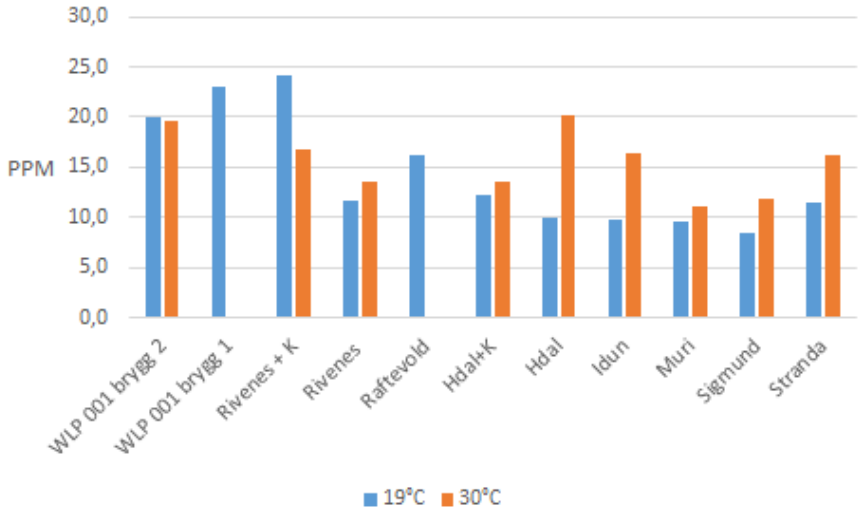

Figure 41: Parts per million of ethylbutanoate from Dynamic Headspace-GCMS analysis for WLP001 batch #1 and #2, Rivenes + contaminant, Rivenes, Raftevold, Hornidal, Hornindal + contaminant, Idun, Muri, Sigmund and Stranda at 19 °C and 30 °C

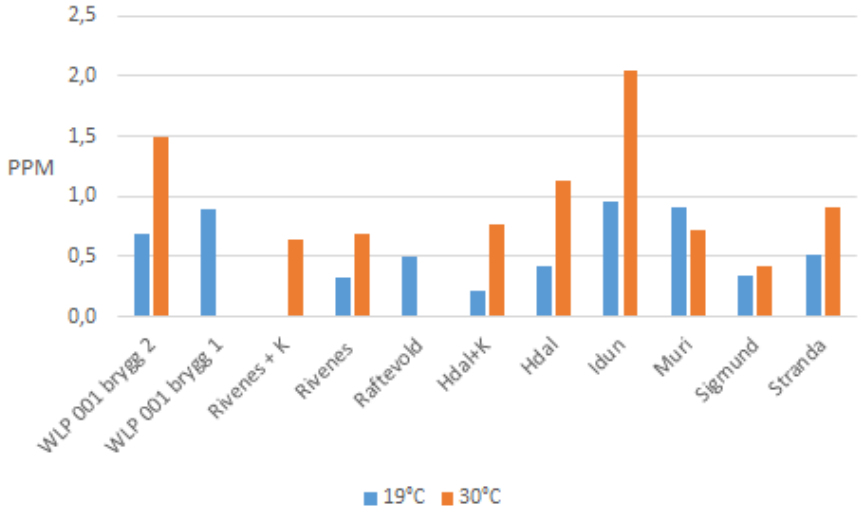

Figure 42: Parts per million of ethylpropionate from Dynamic Headspace-GCMS analysis for WLP001 batch #1 and #2, Rivenes + contaminant, Rivenes, Raftevold, Hornidal, Hornindal + contaminant, Idun, Muri, Sigmund and Stranda at 19 °C and 30 °C

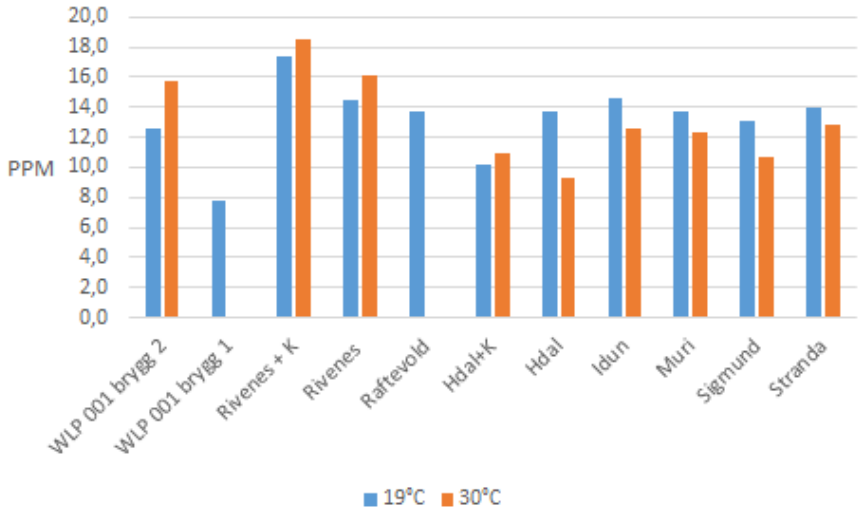

Figure 43: Parts per million of hexonate from Dynamic Headspace-GCMS analysis for WLP001 batch #1 and #2, Rivenes + contaminant, Rivenes, Raftevold, Hornidal, Hornindal + contaminant, Idun, Muri, Sigmund and Stranda at 19 °C and 30 °C

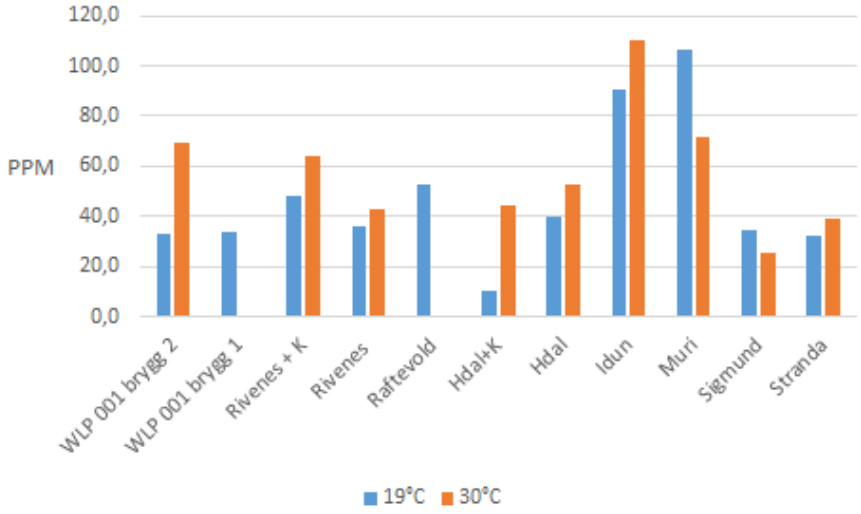

Figure 44: Parts per million of isoamylacetate from Dynamic Headspace-GCMS analysis for WLP001 batch #1 and #2, Rivenes + contaminant, Rivenes, Raftevold, Hornidal, Hornindal + contaminant, Idun, Muri, Sigmund and Stranda at 19 °C and 30 °C

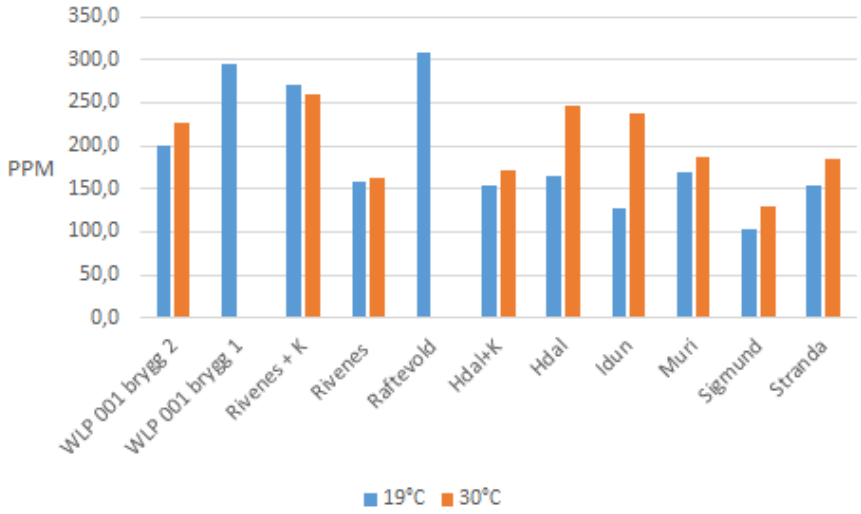

Figure 45: Parts per million of isoamylalcohol from Dynamic Headspace-GCMS analysis for WLP001 batch #1 and #2, Rivenes + contaminant, Rivenes, Raftevold, Hornidal, Hornindal + contaminant, Idun, Muri, Sigmund and Stranda at 19 °C and 30 °C

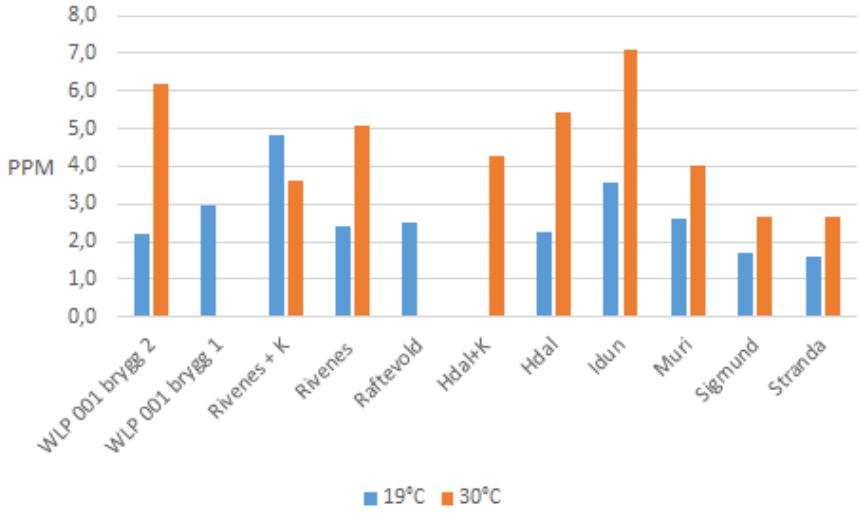

Figure 46: Parts per million of isobutamyl from Dynamic Headspace-GCMS analysis for WLP001 batch #1 and #2, Rivenes + contaminant, Rivenes, Raftevold, Hornidal, Hornindal + contaminant, Idun, Muri, Sigmund and Stranda at 19 °C and 30 °C

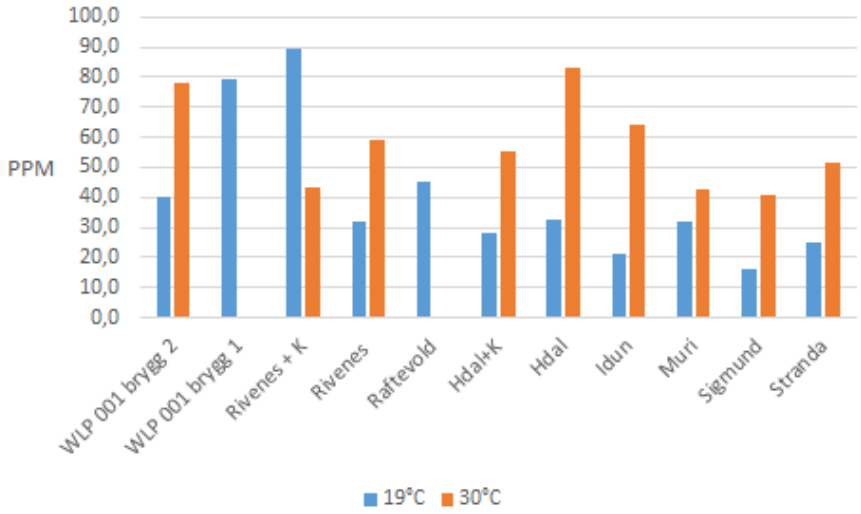

Figure 47: Parts per million of isobutyl from Dynamic Headspace-GCMS analysis for WLP001 batch #1 and #2, Rivenes + contaminant, Rivenes, Raftevold, Hornidal, Hornindal + contaminant, Idun, Muri, Sigmund and Stranda at 19 °C and 30 °C

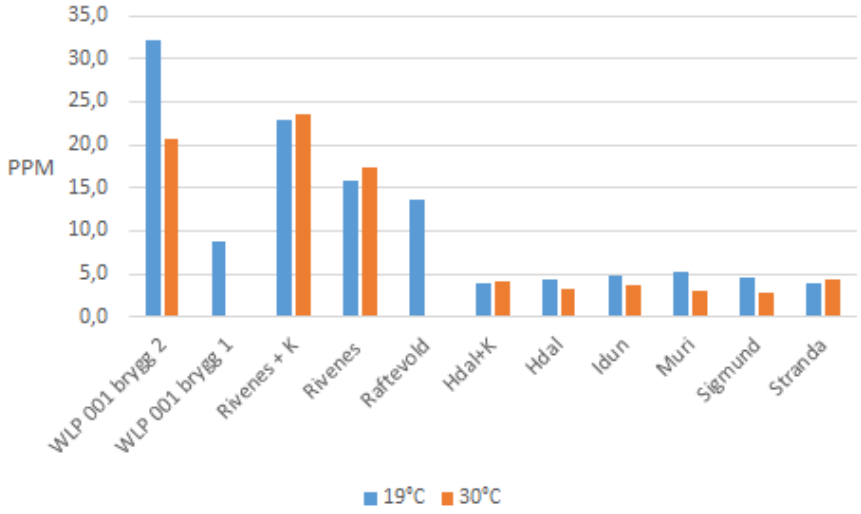

Figure 48: Parts per million of octanonate from Dynamic Headspace-GCMS analysis for WLP001 batch #1 and #2, Rivenes + contaminant, Rivenes, Raftevold, Hornidal, Hornindal + contaminant, Idun, Muri, Sigmund and Stranda at 19 °C and 30 °C

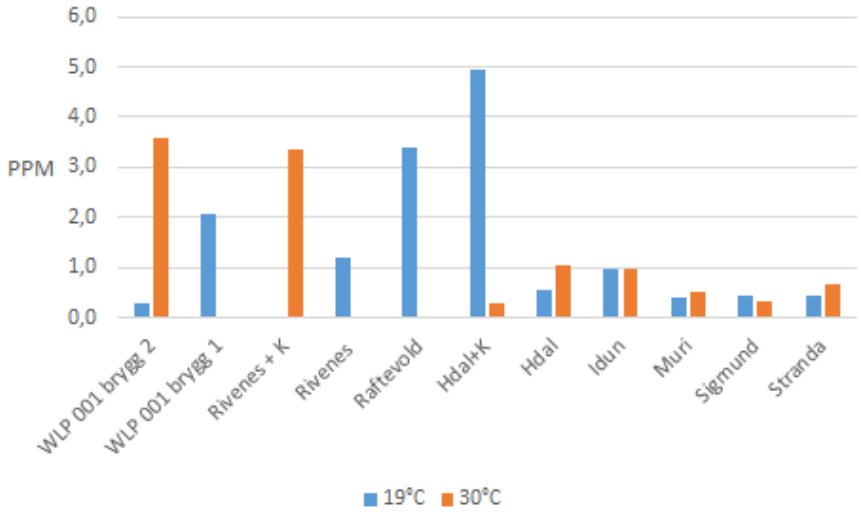

Figure 49: Parts per million of propylacetate from Dynamic Headspace-GCMS analysis for WLP001 batch #1 and #2, Rivenes + contaminant, Rivenes, Raftevold, Hornidal, Hornindal + contaminant, Idun, Muri, Sigmund and Stranda at 19 °C and 30 °C

A.2 Beer experiment 3

1-pentanol was used as an internal standard, 23.30 mg 1-pentanol was measured in a 10.00 mL volumetric flask. 1.25 mL of the 2030 ppm solution was transferred to a 5.00 mL volumetric flask and diluted to 5.00 mL, concentration of 1-pentanol 507.5 ppm. 100 μL of standard was transferred to each sample to be run on Headspace GC-MS. Each sample was weighted in Headspace GS-MS vials on an analytical balance, the measured weight from experiment one and two with the concentration of 1-pentanol is given in Table 27

Table 27: Sample weight and concentration of 1-pentanol

Yeast	**Weight [g]**	**1-pentanol [ppm]**
05_2dag_19C_1	10.0244	5.063
05_2dag_19C_2	10.0060	5.072
05_2dag_19C_3	8.0119	6.334
Hdal_2dag_19C	10.0022	5.074

Continued on next page

Table 27 – continued from previous page

Yeast	Weight [g]	1-pentanol [ppm]
Hdal_2dag_26C	10.0007	5.075
Hdal_2dag_33-19C	10.0128	5.069
hdal_2dag_33C_1	10.0293	5.060
hdal_2dag_33C_2	10.0051	5.072
hdal_2dag_33C_3	8.2749	6.133
Hdal_end_19C	10.0003	5.075
Hdal_end_26C	9.9996	5.075
Hdal_end_33-19C	10.0058	5.072
Hdal_end_33C	10.0033	5.073
O_Hdal_2dag_19C	10.0005	5.075
O_Hdal_2dag_26C	10.0099	5.070
O_Hdal_2dag_33-19C	10.0057	5.072
O_Hdal_2dag_33C	10.0052	5.072
O_Hdal_end_19C	10.0003	5.075
O_Hdal_end_26C	10.0053	5.072
O_Hdal_end_33-19C	10.0173	5.066
O_Hdal_end_33C	10.0019	5.074
S05_2dag_26C	10.0012	5.074
S05_2dag_33-19C	10.0128	5.069
S05_2dag_33C	10.0247	5.062
S05_end_19C	10.0089	5.070
S05_end_26C	9.9977	5.076
S05_end_33-19C	10.0131	5.068
S05_end_33C	10.0116	5.069

Each peak was identified by retention time, the retention time of all identified peaks are given in Table 28 - 30

Table 28: Retentiontime of 1-propanol (A), ethyl acetate (B), isobutyl alcohol (C) and 1-butanol (D)

Yeast	**(A)**	**(B)**	**(C)**	**(D)**
05_2dag_19C_1	5.309	6.474	8.01	9.899
05_2dag_19C_2	5.309	6.474	8.008	9.899
05_2dag_19C_3	5.302	6.472	8.006	9.898
Hdal_2dag_19C	5.297	6.47	8.003	9.897
Hdal_2dag_26C	5.297	6.471	8.002	9.9
Hdal_2dag_33-19C	5.306	6.472	8.005	9.902
hdal_2dag_33C_1	5.312	6.475	8.007	9.899
hdal_2dag_33C_2	5.304	6.474	8.006	9.903
hdal_2dag_33C_3	5.31	6.476	8.009	
Hdal_end_19C	5.313	6.477	8.008	9.899
Hdal_end_26C	5.309	6.477	8.007	9.897
Hdal_end_33-19C	5.314	6.476	8.007	9.899
Hdal_end_33C	5.314	6.476	8.011	
O_Hdal_2dag_19C	5.292	6.468	7.998	9.898
O_Hdal_2dag_26C	5.294	6.469	7.999	
O_Hdal_2dag_33-19C	5.31	6.476	8.009	
O_Hdal_2dag_33C	5.303	6.471	8.005	9.901
O_Hdal_end_19C	5.307	6.473	8.005	9.896
O_Hdal_end_26C	5.316	6.477	8.008	9.901
O_Hdal_end_33-19C	5.314	6.477	8.011	9.901
O_Hdal_end_33C	5.31	6.474	8.008	9.899
S05_2dag_26C	5.306	6.476	8.008	9.899
S05_2dag_33-19C	5.316	6.479	8.016	9.9
S05_2dag_33C	5.311	6.476	8.017	
S05_end_19C	5.31	6.476	8.012	9.899
S05_end_26C	5.31	6.474	8.01	9.901

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Table 28 – continued from previous page

Yeast	(A)	(B)	(C)	(D)
S05_end_33-19C	5.31	6.474	8.013	9.9
S05_end_33C	5.315	6.477	8.017	9.899

Table 29: Retentiontime of ethyl propionate (E), Propyl acetate (F), Isoamyl alcohol (G) and Active amyl alcohol (H)

Yeast	(E)	(F)	(G)	(H)
05_2dag_19C_1		10.194	11.41	11.49
05_2dag_19C_2	10.052	10.19	11.409	11.49
05_2dag_19C_3			11.408	11.488
Hdal_2dag_19C			11.405	11.487
Hdal_2dag_26C			11.405	11.487
Hdal_2dag_33-19C		10.195	11.407	11.488
hdal_2dag_33C_1	10.047		11.411	11.489
hdal_2dag_33C_2			11.409	11.49
hdal_2dag_33C_3			11.411	11.491
Hdal_end_19C	10.049	10.19	11.409	11.489
Hdal_end_26C	10.048	10.193	11.408	11.488
Hdal_end_33-19C	10.047	10.188	11.408	11.489
Hdal_end_33C		10.194	11.411	11.491
O_Hdal_2dag_19C			11.403	11.486
O_Hdal_2dag_26C			11.405	11.484
O_Hdal_2dag_33-19C			11.413	11.491
O_Hdal_2dag_33C	10.048	10.191	11.407	11.488
O_Hdal_end_19C		10.188	11.406	11.485
O_Hdal_end_26C	10.049	10.196	11.409	11.489
O_Hdal_end_33-19C	10.054	10.195	11.41	11.49

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Table 29 – continued from previous page

Yeast	(E)	(F)	(G)	(H)
O_Hdal_end_33C	10.051	10.192	11.411	11.491
S05_2dag_26C			11.409	11.49
S05_2dag_33-19C	10.051		11.414	11.494
S05_2dag_33C			11.414	11.492
S05_end_19C	10.05	10.186	11.409	11.49
S05_end_26C	10.047	10.196	11.409	11.489
S05_end_33-19C	10.048	10.201	11.411	11.491
S05_end_33C	10.05	10.198	11.412	11.492

Table 30: Retentiontime of Isobutyl acetate (I), Ethyl butanoate (J), Isoamyl acetate (K) and Active amyl acetate (L)

Yeast	(I)	(J)	(K)	(L)
05_2dag_19C_1	11.813	12.269	14.662	14.759
05_2dag_19C_2	11.813	12.27	14.661	14.755
05_2dag_19C_3	11.812	12.268	14.661	14.754
Hdal_2dag_19C	11.814	12.268	14.661	14.756
Hdal_2dag_26C	11.814	12.268	14.66	14.757
Hdal_2dag_33-19C	11.813	12.268	14.66	14.755
hdal_2dag_33C_1	11.811	12.268	14.658	14.754
hdal_2dag_33C_2	11.812	12.271	14.661	14.756
hdal_2dag_33C_3	11.814	12.27	14.661	14.756
Hdal_end_19C	11.814	12.269	14.66	14.756
Hdal_end_26C	11.812	12.267	14.66	14.754
Hdal_end_33-19C	11.812	12.268	14.659	14.754
Hdal_end_33C	11.813	12.269	14.661	14.755
O_Hdal_2dag_19C	11.814	12.267	14.661	14.756

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Table 30 – continued from previous page

Yeast	(I)	(J)	(K)	(L)
O_Hdal_2dag_26C	11.813	12.269	14.662	14.758
O_Hdal_2dag_33-19C	11.813	12.27	14.66	14.758
O_Hdal_2dag_33C	11.814	12.269	14.661	14.757
O_Hdal_end_19C	11.811	12.268	14.661	14.756
O_Hdal_end_26C	11.816	12.269	14.66	14.757
O_Hdal_end_33-19C	11.816	12.27	14.662	14.759
O_Hdal_end_33C	11.814	12.269	14.661	14.756
S05_2dag_26C	11.815	12.27	14.661	14.756
S05_2dag_33-19C	11.813	12.27	14.662	14.757
S05_2dag_33C	11.813	12.27	14.661	14.756
S05_end_19C	11.812	12.268	14.66	14.752
S05_end_26C	11.813	12.269	14.66	14.754
S05_end_33-19C	11.812	12.269	14.66	14.757
S05_end_33C	11.814	12.268	14.66	14.754

Table 31: Retentiontime of Ethyl hexanoate (M), Ethyl octanoate (N), Ethyl decanoate (O) and 1-pentanol (P)

Yeast	(M)	(N)	(O)	(P)
05_2dag_19C_1	17.64	20.773	23.108	12.473
05_2dag_19C_2	17.64	20.773	23.107	12.474
05_2dag_19C_3	17.639	20.772	23.105	12.471
Hdal_2dag_19C	17.64	20.773	23.104	12.476
Hdal_2dag_26C	17.642	20.773	23.107	12.474
Hdal_2dag_33-19C	17.641	20.773	23.106	12.473
hdal_2dag_33C_1	17.638	20.772	23.106	12.474
hdal_2dag_33C_2	17.64	20.774	23.106	12.474

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Table 31 – continued from previous page

Yeast	(M)	(N)	(O)	(P)
hdal_2dag_33C_3	17.64	20.772	23.107	12.476
Hdal_end_19C	17.639	20.771	23.105	12.474
Hdal_end_26C	17.64	20.772	23.104	12.473
Hdal_end_33-19C	17.639	20.772	23.107	12.473
Hdal_end_33C	17.64	20.772	23.106	12.475
O_Hdal_2dag_19C	17.641	20.773	23.106	12.473
O_Hdal_2dag_26C	17.641	20.774	23.108	12.474
O_Hdal_2dag_33-19C	17.641	20.774	23.107	12.475
O_Hdal_2dag_33C	17.64	20.773	23.104	12.476
O_Hdal_end_19C	17.639	20.773	23.107	12.473
O_Hdal_end_26C	17.639	20.772	23.105	12.474
O_Hdal_end_33-19C	17.64	20.773	23.106	12.475
O_Hdal_end_33C	17.641	20.773	23.106	12.475
S05_2dag_26C	17.641	20.773	23.107	12.476
S05_2dag_33-19C	17.641	20.773	23.105	12.475
S05_2dag_33C	17.641	20.774	23.104	12.475
S05_end_19C	17.64	20.771	23.105	12.474
S05_end_26C	17.64	20.772	23.108	12.473
S05_end_33-19C	17.64	20.771	23.106	12.475
S05_end_33C	17.64	20.773	23.105	12.475

The area of each peak from the retention times (Table 28 - 31) was extracted. The areas of each peak are given in Table 32 - 37

Table 32: Area of each peak for Safale US 05 after 2 days fermentation at 19 °C and Hornindal 2 days fermentation at 19 °C

Compound	05_2dag_19C_1	05_2dag_19C_2	05_2dag_19C_3	Hdal_2dag_19C	Hdal_2dag_26C
1-propanol	784 695	817 588	773 519.37	285 547.43	233 109.00
Ethyl acetate	3 806 406	3 879 501	3 876 745.41	7 486 215.21	7 793 213.28
Isobutyl alcohol	4 033 549	4 070 468	3 989 630.65	1 126 753.82	1 079 986.72
1-butanol	107 423	110 992	109 664.65	34 856.96	29 412.34
Ethyl propionate		10 780			
Propyl acetate	10 893	12 312			
Isoamyl alcohol	8 345 610	8 573 719	8 182 333.60	5 101 235.27	4 928 386.63
Active amyl alcohol	3 254 607	3 397 164	3 278 781.34	2 234 990.54	1 775 565.06
Isobutyl acetate	129 130	123 458	130 657.47	110 632.18	87 947.87
Ethyl butanoate	744 431	768 775	925 686.57	731 214.59	791 333.11
Isoamyl acetate	663 170	676 224	630 325.13	1 382 818.71	1 134 745.29
Active amyl acetate	82 362	86 062	81 682.95	198 884.78	138 245.72
Ethyl hexanoate	453 218	461 818	419 434.66	561 627.75	512 243.46
Ethyl octanoate	324 632	359 743	371 142.38	247 725.31	174 176.58
Ethyl decanoate	18 988	30 145	58 505.77	35 461.57	19 257.81
1-pentanol	109 906	117 035	113 584	106186	101952.91

Table 33: Area of each peak for Hornindal 2 days fermentation at 33 °C

Compound	Hdal_2dag_33-19C	hdal_2dag_33C_1	hdal_2dag_33C_2	hdal_2dag_33C_3	Hdal_end_19C
1-propanol	307 829.64	582 752.45	456 727.57	503 994.99	354 883.20
Ethyl acetate	11 711 126.73	13 425 384.22	12 965 877.29	12 978 904.62	16 464 195.15
Isobutyl alcohol	1 812 530.03	3 285 031.91	2 939 995.03	3 184 431.68	1 979 292.68
1-butanol	26 769.76	33 731.68	33 269.69		59 441.10
Ethyl propionate		30 935.99			23 139.90
Propyl acetate	27 747.39				75 608.06
Isoamyl alcohol	6 974 394.52	10 807 779.37	9 508 102.67	10 322 674.11	7 449 160.53
Active amyl alcohol	2 788 655.41	4 487 372.56	4 091 932.01	4 412 011.22	3 519 298.59
Isobutyl acetate	164 886.96	236 252.35	227 671.74	243 047.69	130 889.68
Ethyl butanoate	742 806.31	750 184.36	641 468.13	868 739.40	713 959.97
Isoamyl acetate	1 880 689.34	3 383 198.20	3 244 330.84	3 087 478.84	2 071 916.41
Active amyl acetate	217 800.52	399 584.70	385 814.93	364 819.11	208 340.29
Ethyl hexanoate	454 019.89	586 980.80	556 066.54	520 319.77	761 071.64
Ethyl octanoate	225 693.74	388 369.72	355 709.64	351 294.47	1 236 997.01
Ethyl decanoate	32 149.37	82 757.51	39 895.62	77 010.97	164 699.23
1-pentanol	116038.89	138435.29	127800.85	154926.1	253751.56

Table 34: Area of each peak for Hornindal after 14 days fermentation at 26 and 33 °C and 33 for two days followed by 12 days at 19 °C and original Hornindal after 2 days fermentation at 19 °C

Compound	Hdal_end_26C	Hdal_end_33-19C	Hdal_end_33C	O_Hdal_2dag_19C	O_Hdal_2dag_26C
1-propanol	454 876.85	395 112.04	522 453.79	146 960	272 734.33
Ethyl acetate	17 490 618.99	19 245 247.74	15 915 749.12	4 305 086	7 474 409.67
Isobutyl alcohol	2 240 782.72	2 270 530.70	3 312 197.23	480 943	1 070 981.01
1-butanol	41 926.12	51 268.31		22 740	
Ethyl propionate	21 338.39	30 011.88			
Propyl acetate	52 780.35	51 369.69	104 216.07		
Isoamyl alcohol	7 737 231.41	7 748 297.91	10 573 139.32	3 261 950	4 772 733.53
Active amyl alcohol	3 390 281.76	3 394 343.81	4 593 625.95	1 196 107	1 992 244.20
Isobutyl acetate	146 986.68	145 671.29	249 945.16	45 549	92 401.69
Ethyl butanoate	746 533.29	676 954.07	761 399.15	754 095	841 721.02
Isoamyl acetate	1 754 919.65	2 705 287.23	2 623 106.39	649 283	1 186 192.28
Active amyl acetate	153 383.11	240 478.31	280 962.40	77 776	158 581.30
Ethyl hexanoate	528 354.97	534 186.21	247 656.88	351 910	609 020.83
Ethyl octanoate	651 631.57	595 436.20	299 477.92	134 265	268 431.57
Ethyl decanoate	88 538.11	72 349.11	92 043.43	11 689	29 294.05
1-pentanol	200937.13	167197.38	154612.69	73 957	111126.1

Table 35: Area of each peak for original Hornindal after 2 days fermentation at 33 °C, original Hornindal at the end of fermentation at 19 and 26 °C and original Hornindal at the end of fermentation after 2 days at 33 °C followed by 12 days at 19 °C

Compound	O_Hdal_2dag_33-19C	O_Hdal_2dag_33C	O_Hdal_end_19C	O_Hdal_end_26C	O_Hdal_end_33-19C
1-propanol	605 745.92	331 565.54	255 744.33	396 704.51	521 575.79
Ethyl acetate	13 200 604.41	10 478 962.53	13 802 821.32	14 649 230.94	14 077 388.78
Isobutyl alcohol	3 553 775.02	1 910 450.07	1 282 844.74	2 193 432.78	2 655 762.27
1-butanol		20862.85	49 632.91	36 083.66	22 763.30
Ethyl propionate		20 378.68		18 256.02	21 627.13
Propyl acetate		23 184.42	41 912.74	31 054.37	26 821.85
Isoamyl alcohol	11 037 677.44	6 426 898.72	5 445 161.23	7 190 202.86	7 798 509.64
Active amyl alcohol	4 869 389.30	2 885 347.63	2 431 894.52	3 476 388.85	3 892 016.18
Isobutyl acetate	288 321.85	161 617.00	54 679.18	102 542.44	135 851.59
Ethyl butanoate	701 477.78	816 235.79	695 457.59	740 591.60	713 444.61
Isoamyl acetate	3 258 477.57	1 669 581.95	1 120 771.04	1 071 909.91	939 590.98
Active amyl acetate	421 996.88	222 339.89	113 094.36	110 440.88	119 244.23
Ethyl hexanoate	741 800.12	642 336.00	661 772.94	725 975.01	335 009.47
Ethyl octanoate	552 034.93	332 385.10	901 709.17	740 155.34	337 865.86
Ethyl decanoate	79 352.58	39 756.52	58 525.20	120 273.64	50 331.30
1-pentanol	161273.03	128306.62	209163.76	205783.15	159386.21

Table 36: Area of each peak for original Hornindal at the end of fermentation at 33 °C, Safale US05 after 2 days at 26 and 33 °C

Compound	**O_Hdal_end_33C**	**S05_2dag_26C**	**S05_2dag_33-19C**	**S05_2dag_33C**
1-propanol	780 725.49	643 867.75	777 717.65	911 479.16
Ethyl acetate	15 569 726.49	6 796 875.07	8 698 155.00	8 363 619.34
Isobutyl alcohol	3 757 568.46	3 469 197.53	6 832 394.30	8 119 128.03
1-butanol	36 465.17	41 275.91	35 608.38	
Ethyl propionate	37 212.22		27 181.88	
Propyl acetate	13 880.37			
Isoamyl alcohol	11 350 805.76	7 684 848.98	11 882 969.34	11 726 553.27
Active amyl alcohol	5 084 821.29	2 892 896.67	4 588 517.63	4 591 083.97
Isobutyl acetate	214 999.62	186 049.50	298 200.92	305 951.32
Ethyl butanoate	667 098.45	748 545.46	806 962.37	738 673.91
Isoamyl acetate	2 440 980.81	1 541 048.35	1 985 419.76	1 704 564.65
Active amyl acetate	262 444.69	154 270.82	207 525.10	181 675.33
Ethyl hexanoate	425 251.46	430 973.10	424 184.27	412 260.38
Ethyl octanoate	599 576.04	318 084.39	292 116.91	344 575.72
Ethyl decanoate	85 215.62	42 885.51	21 488.94	37 440.52
1-pentanol	136356.48	146578.83	199367.73	205029.85

Table 37: Area of each peak for Safale US-05 at the end of fermentation at 19, 26, 33 °C and 33 for 2 days followed by 12 days at 19 °C

Compound	**S05_end_19C**	**S05_end_26C**	**S05_end_33-19C**	**S05_end_33C**
1-propanol	1 022 169.36	890 333.94	755 471.45	954 404.22
Ethyl acetate	8 207 463.46	11 424 139.04	11 907 240.65	8 794 743.42
Isobutyl alcohol	4 734 041.86	4 510 806.78	6 948 452.51	8 492 722.97
1-butanol	125 683.94	46 435.70	41 894.71	38 815.41
Ethyl propionate	19 684.01	26 691.33	31 592.15	25 389.92
Propyl acetate	124 132.97	30 552.14	13 136.37	61 954.46
Isoamyl alcohol	9 529 694.64	8 946 515.50	11 750 753.34	12 029 657.84
Active amyl alcohol	4 043 070.80	3 707 057.74	4 791 943.40	4 929 737.36
Isobutyl acetate	130 431.46	167 730.15	277 491.93	244 507.80
Ethyl butanoate	727 538.90	705 786.80	723 980.24	752 638.87
Isoamyl acetate	761 631.39	1 402 658.69	2 163 747.26	1 214 042.92
Active amyl acetate	57 583.77	91 851.55	176 737.99	107 788.24
Ethyl hexanoate	331 171.17	282 420.99	347 677.58	216 184.26
Ethyl octanoate	955 704.15	901 595.97	706 809.97	457 313.67
Ethyl decanoate	61 580.05	118 044.14	81 660.20	64 608.90
1-pentanol	218190.9	198518.37	224209.39	186244.03

Using 1-pentanol as internal standard, the concentration of the compounds can be calculated by dividing the area of a specific peak with the product of the area of 1-pentanol with the concentration. The concentration of 1-pentanol is given in Table 27, and the area of 1-pentanol and the given compound is given in Table 32 - 37.

Example calculation for the concentration of 1-Propanol in Safale US 05 2 day at 19 °C 1

$$C_{(1-Propanol)}[ppm] = \frac{A_{1-propanol}}{C_{1-pentanol} \cdot A_{1-pentanol}}$$

$$C_{(1-Propanol)} = \frac{784695}{5.063 \cdot 109906}$$

$$C_{(1-Propanol)} = 36.1ppm$$

The concentration of each compound for the different yeast fermented at 19 and 30 °C is given in Table 38 - 43

Table 38: Concentration [ppm] of identified compounds for Safale US 05 after 2 days fermentation at 19 °C and Hornindal 2 days fermentation at 19 °C

Compound	**05_2dag_19C_1**	**05_2dag_19C_2**	**05_2dag_19C_3**	**Hdal_2dag_19C**	**Hdal_2dag_26C**
1-propanol	36.1	35.4	43.1	13.6	11.6
Ethyl acetate	175.3	168.1	216.2	357.7	387.9
Isobutyl alcohol	185.8	176.4	222.5	53.8	53.8
1-butanol	4.9	4.8	6.1	1.7	1.5
Ethyl propionate	0.0	0.5	0.0	0.0	0.0
Propyl acetate	0.5	0.5	0.0	0.0	0.0
Isoamyl alcohol	384.4	371.6	456.3	243.8	245.3
Active amyl alcohol	149.9	147.2	182.9	106.8	88.4
Isobutyl acetate	5.9	5.4	7.3	5.3	4.4
Ethyl butanoate	34.3	33.3	51.6	34.9	39.4
Isoamyl acetate	30.5	29.3	35.2	66.1	56.5
Active amyl acetate	3.8	3.7	4.6	9.5	6.9
Ethyl hexanoate	20.9	20.0	23.4	26.8	25.5
Ethyl octanoate	15.0	15.6	20.7	11.8	8.7
Ethyl decanoate	0.9	1.3	3.3	1.7	1.0

Table 39: Concentration [ppm] of identified compounds for Hornindal 2 days fermentation at 33 °C

Compound	**Hdal_2dag_33-19C**	**hdal_2dag_33C_1**	**hdal_2dag_33C_2**	**hdal_2dag_33C_3**	**Hdal_end_19C**
1-propanol	13.4	21.3	18.1	20.0	7.1
Ethyl acetate	511.5	490.7	514.6	513.8	329.3
Isobutyl alcohol	79.2	120.1	116.7	126.1	39.6
1-butanol	1.2	1.2	1.3	0.0	1.2
Ethyl propionate	0.0	1.1	0.0	0.0	0.5
Propyl acetate	1.2	0.0	0.0	0.0	1.5
Isoamyl alcohol	304.6	395.1	377.4	408.6	149.0
Active amyl alcohol	121.8	164.0	162.4	174.7	70.4
Isobutyl acetate	7.2	8.6	9.0	9.6	2.6
Ethyl butanoate	32.4	27.4	25.5	34.4	14.3
Isoamyl acetate	82.1	123.7	128.8	122.2	41.4
Active amyl acetate	9.5	14.6	15.3	14.4	4.2
Ethyl hexanoate	19.8	21.5	22.1	20.6	15.2
Ethyl octanoate	9.9	14.2	14.1	13.9	24.7
Ethyl decanoate	1.4	3.0	1.6	3.0	3.3

Table 40: Concentration [ppm] of identified compounds for Hornindal after 14 days fermentation at 26 and 33 °C and 33 for two days followed by 12 days at 19 °C and original Hornindal after 2 days fermentation at 19 °C

Compound	Hdal_end_26C	Hdal_end_33-19C	Hdal_end_33C	O_Hdal_2dag_19C	O_Hdal_2dag_26C
1-propanol	11.5	12.0	17.1	10.1	12.4
Ethyl acetate	441.8	583.8	522.2	295.4	341.0
Isobutyl alcohol	56.6	68.9	108.7	33.0	48.9
1-butanol	1.1	1.6	0.0	1.6	0.0
Ethyl propionate	0.5	0.9	0.0	0.0	0.0
Propyl acetate	1.3	1.6	3.4	0.0	0.0
Isoamyl alcohol	195.4	235.1	346.9	223.8	217.7
Active amyl alcohol	85.6	103.0	150.7	82.1	90.9
Isobutyl acetate	3.7	4.4	8.2	3.1	4.2
Ethyl butanoate	18.9	20.5	25.0	51.7	38.4
Isoamyl acetate	44.3	82.1	86.1	44.6	54.1
Active amyl acetate	3.9	7.3	9.2	5.3	7.2
Ethyl hexanoate	13.3	16.2	8.1	24.1	27.8
Ethyl octanoate	16.5	18.1	9.8	9.2	12.2
Ethyl decanoate	2.2	2.2	3.0	0.8	1.3

Table 41: Concentration [ppm] of identified compounds for original Hornindal after 2 days fermentation at 33 °C, original Hornindal at the end of fermentation at 19 and 26 °C and original Hornindal at the end of fermentation after 2 days at 33 °C followed by 12 days at 19 °C

Compound	O_Hdal_2dag_33-19C	O_Hdal_2dag_33C	O_Hdal_end_19C	O_Hdal_end_26C	O_Hdal_end_33-19C
1-propanol	19.1	13.1	6.2	9.8	16.6
Ethyl acetate	415.2	414.3	334.9	361.1	447.5
Isobutyl alcohol	111.8	75.5	31.1	54.1	84.4
1-butanol	0.0	0.8	1.2	0.9	0.7
Ethyl propionate	0.0	0.8	0.0	0.4	0.7
Propyl acetate	0.0	0.9	1.0	0.8	0.9
Isoamyl alcohol	347.1	254.1	132.1	177.2	247.9
Active amyl alcohol	153.1	114.1	59.0	85.7	123.7
Isobutyl acetate	9.1	6.4	1.3	2.5	4.3
Ethyl butanoate	22.1	32.3	16.9	18.3	22.7
Isoamyl acetate	102.5	66.0	27.2	26.4	29.9
Active amyl acetate	13.3	8.8	2.7	2.7	3.8
Ethyl hexanoate	23.3	25.4	16.1	17.9	10.6
Ethyl octanoate	17.4	13.1	21.9	18.2	10.7
Ethyl decanoate	2.5	1.6	1.4	3.0	1.6

Table 42: Concentration [ppm] of identified compounds for original Hornindal at the end of fermentation at 33 °C, Safale US05 after 2 days at 26 and 33 °C

Compound	**O_Hdal_end_33C**	**S05_2dag_26C**	**S05_2dag_33-19C**	**S05_2dag_33C**
1-propanol	29.1	22.3	19.8	22.5
Ethyl acetate	579.4	235.3	221.1	206.5
Isobutyl alcohol	139.8	120.1	173.7	200.5
1-butanol	1.4	1.4	0.9	0.0
Ethyl propionate	1.4	0.0	0.7	0.0
Propyl acetate	0.5	0.0	0.0	0.0
Isoamyl alcohol	422.4	266.0	302.1	289.5
Active amyl alcohol	189.2	100.1	116.7	113.4
Isobutyl acetate	8.0	6.4	7.6	7.6
Ethyl butanoate	24.8	25.9	20.5	18.2
Isoamyl acetate	90.8	53.3	50.5	42.1
Active amyl acetate	9.8	5.3	5.3	4.5
Ethyl hexanoate	15.8	14.9	10.8	10.2
Ethyl octanoate	22.3	11.0	7.4	8.5
Ethyl decanoate	3.2	1.5	0.5	0.9

Table 43: Concentration [ppm] of identified compounds for Safale US-05 at the end of fermentation at 19, 26, 33 °C and 33 for 2 days followed by 12 days at 19 °C

Compound	**S05_end_19C**	**S05_end_26C**	**S05_end_33-19C**	**S05_end_33C**
1-propanol	23.8	22.8	17.1	26.0
Ethyl acetate	190.7	292.1	269.2	239.4
Isobutyl alcohol	110.0	115.3	157.1	231.2
1-butanol	2.9	1.2	0.9	1.1
Ethyl propionate	0.5	0.7	0.7	0.7
Propyl acetate	2.9	0.8	0.3	1.7
Isoamyl alcohol	221.5	228.8	265.6	327.4
Active amyl alcohol	94.0	94.8	108.3	134.2
Isobutyl acetate	3.0	4.3	6.3	6.7
Ethyl butanoate	16.9	18.0	16.4	20.5
Isoamyl acetate	17.7	35.9	48.9	33.0
Active amyl acetate	1.3	2.3	4.0	2.9
Ethyl hexanoate	7.7	7.2	7.9	5.9
Ethyl octanoate	22.2	23.1	16.0	12.4
Ethyl decanoate	1.4	3.0	1.8	1.8

Appendix B Rawdata: Characterization of yeast

Growth of microorganisms Under laboratory condition, the growth of microorganisms follows a typical bacterial growth curve, which is illustrated in Figure 50

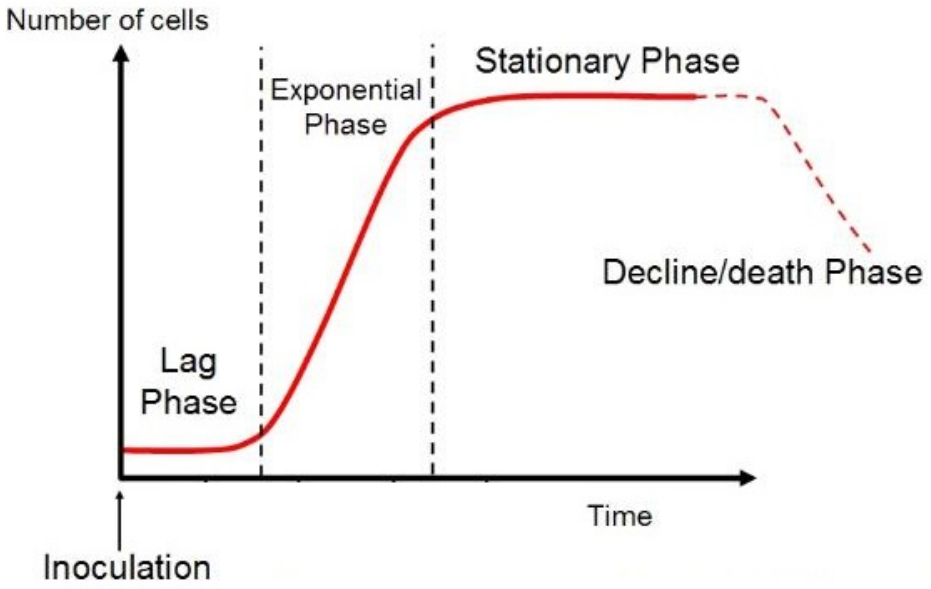

Figure 50: Illustration of typical microbial growth in fresh liquid medium [54]

Immediately after inoculation of the cells into fresh medium, the population remains temporarily unchanged, this is called the Lag Phase. Although there is no apparent cell division occurring, the cells may be growing in volume or mass, synthesizing enzymes, proteins, RNA, etc., and increasing in metabolic activity.

The length of the lag phase is apparently dependent on a wide variety of factors including the size of the inoculum; time necessary to recover from physical damage or shock in the transfer; time required for synthesis of essential coenzymes or division factors; and time required for synthesis of new (inducible) enzymes that are necessary to metabolize the substrates present in the medium.

The exponential phase of growth is a pattern of balanced growth wherein all the cells are dividing regularly by binary fission, and are growing by geometric progression. The cells divide at a constant rate depending upon the composition of the growth medium and the conditions of incubation. The rate of exponential growth of a bacterial culture is expressed as generation time, also the doubling time of the bacterial population. The rate of exponential growth can be calculated using

Equation 1

$$\mu = \frac{lnOD_2 - lnOD_1}{t_2 - t_1} \tag{1}$$

Where μ is the growth rate expressed in h^{-1}. $lnOD_{1or2}$ is the optical density at two time intervals. T is the time as which the OD is measured in h.

The generation time can be calculated using Equation 2

$$g = \frac{ln2}{\mu} \tag{2}$$

Where μ is the growth rate calculated in Equation 1.
Stationary Phase. Exponential growth cannot be continued forever in a batch culture (e.g. a closed system such as a test tube or flask). Population growth is limited by one of three factors: 1. exhaustion of available nutrients; 2. accumulation of inhibitory metabolites or end products; 3. exhaustion of space, in this case called a lack of "biological space".

During the stationary phase, if viable cells are being counted, it cannot be determined whether some cells are dying and an equal number of cells are dividing, or the population of cells has simply stopped growing and dividing. The stationary phase, like the lag phase, is not necessarily a period of quiescence. Bacteria that produce secondary metabolites, such as antibiotics, do so during the stationary phase of the growth cycle (Secondary metabolites are defined as metabolites produced after the active stage of growth). It is during the stationary phase that spore-forming bacteria have to induce or unmask the activity of dozens of genes that may be involved in sporulation process.

4. Death Phase. If incubation continues after the population reaches stationary phase, a death phase follows, in which the viable cell population declines. (Note, if counting by turbidimetric measurements or microscopic counts, the death phase cannot be observed.). During the death phase, the number of viable cells de-

creases geometrically (exponentially), essentially the reverse of growth during the log phase. [55]

Hemocytometer The types of counting chambers differ in counting grids and the depths of the chambers. Orthogonal lines form grids which become visible by magnifying them with a microscope. The grid of a counting chamber is engraved into the surface of its base. The depth of the Buerker (Figure 51) counting chamber is 0.1 mm. Its counting grid covers 9 mm^2 and is subdivided by triple lines into 9 large squares. The distances between the middle lines are 1 mm. Each of these large squares is subdivided into 16 small squares by double lines with a space of 0.05 mm between them. The inner lines of these small squares form areas of 0.2 x 0.2 mm^2. The crossings of the double lines form small squares of 0.05 x 0.05 mm^2. These squares are suitable for counting thrombocytes and erythrocytes. [56]

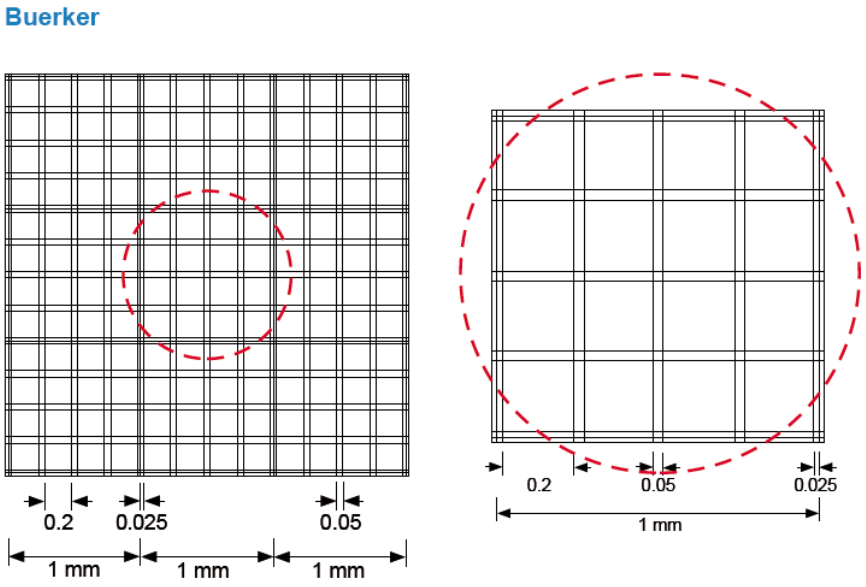

Figure 51: Visualized Counting grids on Buerker type counting chamber

The amount of $\frac{cells}{mL}$ can be calculated using Equation 3

$$\frac{cells}{mL} = \frac{cells_{counted}}{Height[mm] \cdot Width[mm] \cdot Depth[mm] \cdot \frac{1mm^3}{1000mL}} \tag{3}$$

Colony forming units

B.1 Growth

The optical density of Muri, Stranda, Hornindal, Sigmund and Idun was measured at 600 nm in order to find the growth rate and generation time. The full growth curve is given in Figure 52. The OD_{600} corrected for dilution is plotted as a function of time. The values are taken from Table 44 - 48. 250 μL yeast was inoculated in 30 mL 5 % ME medium in 250 mL shake flasks

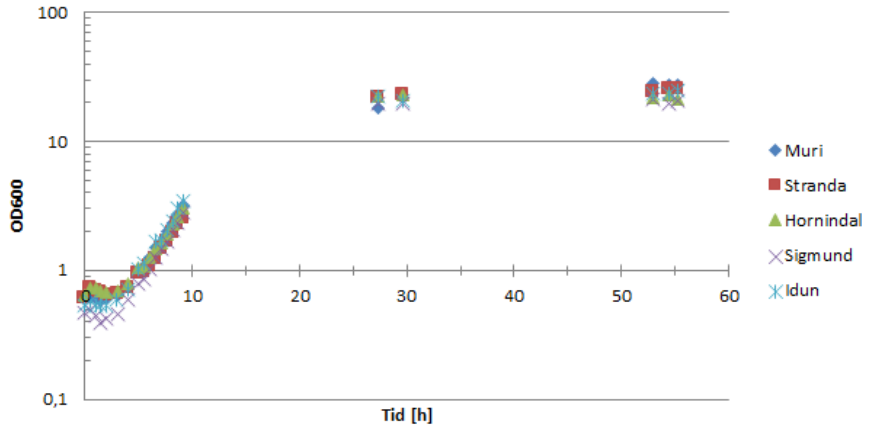

Figure 52: Optical density as a function of time for Muri, Stranda, Hornindal, Sigmund and Idun yeast for all entire growth

Table 44: Raw data of Optical density measurements for Muri in order to determine growth rate and generation time

Blank	OD_{600}	
1	0,143	0,097
2	0,183	
3	0,123	
4	0,128	
Avg.	0,1348	

Time	Time [hh:mm]	OD_{600}	$OD600_{600}$ Diluted	OD+Blank	OD-Avg. Blank	OD corrected for dilution	Time [h]
10:41	00:00	0,613		0,756	0,6212	0,6212	0
11:11	00:30		0,052	0,195	0,0602	0,602	0,5
11:41	01:00		0,052	0,195	0,0602	0,602	1
12:11	01:30		0,05	0,193	0,0582	0,582	1,5
12:41	02:00		0,052	0,195	0,0602	0,602	2
13:41	03:00		0,056	0,199	0,0642	0,642	3
14:41	04:00		0,066	0,209	0,0742	0,742	4
15:41	05:00		0,093	0,236	0,1012	1,012	5
16:11	05:30		0,098	0,241	0,1062	1,062	5,5
16:41	06:00		0,117	0,26	0,1252	1,252	6
17:17	06:36		0,101	0,284	0,1492	1,492	6,6
17:41	07:00		0,114	0,297	0,1622	1,622	7,17
18:11	07:30		0,152	0,335	0,2002	2,002	7,67
18:41	08:00		0,184	0,367	0,2322	2,322	8,17
19:11	08:30		0,22	0,403	0,2682	2,682	8,67
19:41	09:00		0,274	0,457	0,3222	3,222	9,17
13:59	03:18		0,379	0,502	0,3672	18,36	27,3
16:21	05:40		0,44	0,568	0,4332	21,66	29,67
15:37	04:56		0,506	0,603	0,4682	28,092	52,93
17:07	06:26		0,499	0,596	0,4612	27,672	54,43
18:00	07:19		0,495	0,592	0,4572	27,432	55,32

The color coded blank sample (non-sterile 5 % ME medium) was added to the measured diluted OD_{600} value. The average blank samples was then subtracted from all measurements before correcting for the dilution.

Example calculation

$$OD + blank = OD_{600}Diluted + Blank = 0,052 + 0,143 = 0,195$$

$$OD + blank - Blank.Avg0,195 - 0,1348 = 0,0602$$

$$OD_{corrected} = \frac{0,0602 \cdot V_{cuvette}}{V_{sample}} = \frac{0,0602 \cdot 1000}{100} = 0,602$$

Table 45: Raw data of Optical density measurements for Stranda in order to determine growth rate and generation time

				Blank	OD600		
				1	0,143	0,097	
				2	0,183		
				3	0,123		
				4	0,128		
				avg	0,1348		
Time	Time [hh:mm]	OD600	OD600 Diluted	OD+Blank	OD-avg Blank	OD corrected for dilution	Time [h]
10:43	00:00	0,603		0,746	0,6112	0,6112	0,00
11:13	00:30		0,065	0,208	0,0732	0,732	0,50
11:43	01:00		0,061	0,204	0,0692	0,692	1,00
12:13	01:30		0,059	0,202	0,0672	0,672	1,50
12:43	02:00		0,056	0,199	0,0642	0,642	2,00
13:43	03:00		0,058	0,201	0,0662	0,662	3,00
14:43	04:00		0,065	0,208	0,0732	0,732	4,00
15:43	05:00		0,087	0,23	0,0952	0,952	5,00
16:13	05:30		0,088	0,231	0,0962	0,962	5,50
16:43	06:00		0,1	0,243	0,1082	1,082	6,00
17:17	06:34		0,074	0,257	0,1222	1,222	6,60
17:43	07:00		0,098	0,281	0,1462	1,462	7,17
18:13	07:30		0,121	0,304	0,1692	1,692	7,67
18:43	08:00		0,149	0,332	0,1972	1,972	8,17
19:13	08:30		0,178	0,361	0,2262	2,262	8,67
19:43	09:00		0,207	0,39	0,2552	2,552	9,17
14:01	03:18		0,45	0,573	0,4382	21,91	27,30
16:23	05:40		0,468	0,596	0,4612	23,06	29,67
15:37	04:54		0,44	0,537	0,4022	24,132	52,93
17:07	06:24		0,464	0,561	0,4262	25,572	54,43
18:00	07:17		0,465	0,562	0,4272	25,632	55,32

Table 46: Raw data of Optical density measurements for Hornindal in order to determine growth rate and generation time

				Blank	OD600		
				1	0,143	0,097	
				2	0,183		
				3	0,123		
				4	0,128		
				avg	0,1348		
Time	Time [hh:mm]	OD600	OD600 Diluted	OD+Blank	OD-avg Blank	OD corrected for dilution	Time [h]
10:44	00:00	0,64		0,783	0,6482	0,6482	0,00
11:14	00:30		0,064	0,207	0,0722	0,722	0,50
11:44	01:00		0,064	0,207	0,0722	0,722	1,00
12:14	01:30		0,061	0,204	0,0692	0,692	1,50
12:44	02:00		0,06	0,203	0,0682	0,682	2,00
13:44	03:00		0,061	0,204	0,0692	0,692	3,00
14:44	04:00		0,07	0,213	0,0782	0,782	4,00
15:44	05:00		0,097	0,24	0,1052	1,052	5,00
16:14	05:30		0,099	0,242	0,1072	1,072	5,50
16:44	06:00		0,118	0,261	0,1262	1,262	6,00
17:20	06:36		0,098	0,281	0,1462	1,462	6,60
17:44	07:00		0,114	0,297	0,1622	1,622	7,17
18:14	07:30		0,143	0,326	0,1912	1,912	7,67
18:44	08:00		0,18	0,363	0,2282	2,282	8,17
19:14	08:30		0,214	0,397	0,2622	2,622	8,67
19:44	09:00		0,255	0,438	0,3032	3,032	9,17
14:03	03:19		0,465	0,588	0,4532	22,66	27,32
16:24	05:40		0,462	0,59	0,4552	22,76	29,67
15:37	04:53		0,397	0,494	0,3592	21,552	52,93
17:07	06:23		0,421	0,518	0,3832	22,992	54,43
18:00	07:16		0,39	0,487	0,3522	21,132	55,32

Table 47: Raw data of Optical density measurements for Sigmund in order to determine growth rate and generation time

				Blank	OD600		
				1	0,143	0,097	
				2	0,183		
				3	0,123		
				4	0,128		
				avg	0,1348		
Time	Time [hh:mm]	OD600	OD600 Diluted	OD+Blank	OD-avg Blank	OD corrected for dilution	Time [h]
10:45	00:00	0,461		0,604	0,4692	0,4692	0,00
11:15	00:30		0,041	0,184	0,0492	0,492	0,50
11:45	01:00		0,036	0,179	0,0442	0,442	1,00
12:15	01:30		0,031	0,174	0,0392	0,392	1,50
12:45	02:00		0,034	0,177	0,0422	0,422	2,00
13:45	03:00		0,037	0,18	0,0452	0,452	3,00
14:45	04:00		0,051	0,194	0,0592	0,592	4,00
15:45	05:00		0,071	0,214	0,0792	0,792	5,00
16:15	05:30		0,077	0,22	0,0852	0,852	5,50
16:45	06:00		0,094	0,237	0,1022	1,022	6,00
17:21	06:36		0,078	0,261	0,1262	1,262	6,60
17:45	07:00		0,1	0,283	0,1482	1,482	7,17
18:15	07:30		0,121	0,304	0,1692	1,692	7,67
18:45	08:00		0,156	0,339	0,2042	2,042	8,17
19:15	08:30		0,189	0,372	0,2372	2,372	8,67
19:45	09:00		0,232	0,415	0,2802	2,802	9,17
14:05	03:20		0,404	0,527	0,3922	19,61	27,33
16:25	05:40		0,401	0,529	0,3942	19,71	29,67
15:37	04:52		0,395	0,492	0,3572	21,432	52,93
17:07	06:22		0,365	0,462	0,3272	19,632	54,43
18:00	07:15		0,38	0,477	0,3422	20,532	55,32

Table 48: Raw data of Optical density measurements for Sigmund in order to determine growth rate and generation time

				Blank	OD600		
				1	0,143	0,097	
				2	0,183		
				3	0,123		
				4	0,128		
				avg	0,1348		
Time	Time [hh:mm]	OD600	OD600 Diluted	OD+Blank	OD-avg Blank	OD corrected for dilution	Time [h]
10:47	00:00	0,53		0,673	0,5382	0,5382	0,0
11:17	00:30		0,049	0,192	0,0572	0,572	0,5
11:46	00:59		0,045	0,188	0,0532	0,532	1,0
12:16	01:29		0,045	0,188	0,0532	0,532	1,5
12:46	01:59		0,045	0,188	0,0532	0,532	2,0
13:46	02:59		0,051	0,194	0,0592	0,592	3,0
14:46	03:59		0,063	0,206	0,0712	0,712	4,0
15:46	04:59		0,093	0,236	0,1012	1,012	5,0
16:16	05:29		0,105	0,248	0,1132	1,132	5,5
17:23	06:36		0,117	0,3	0,1652	1,652	6,6
17:46	06:59		0,124	0,307	0,1722	1,722	7,2
18:16	07:29		0,159	0,342	0,2072	2,072	7,7
18:46	07:59		0,194	0,377	0,2422	2,422	8,2
19:16	08:29		0,253	0,436	0,3012	3,012	8,7
19:46	08:59		0,294	0,477	0,3422	3,422	9,2
14:06	03:19		0,456	0,579	0,4442	22,21	27,3
16:25	05:38		0,425	0,553	0,4182	20,91	29,6
15:37	04:50		0,434	0,531	0,3962	23,772	52,9
17:07	06:20		0,431	0,528	0,3932	23,592	54,4
18:00	07:13		0,454	0,551	0,4162	24,972	55,3
18:00	07:15		0,38	0,477	0,3422	20,532	55,32

B.2 Cell count

Colony Forming Units Muri and Sigmund yeast cells were grown in 250 mL shake flasks. 30 μL inoculum volume of either Muri and Sigmund was transferred to a marked 250 mL shake flasks with 30 mL 5 % ME medium. 100 μL was plated on agar plates. Counted cells are given in Table 49 for Muri and Table 50 for Sigmund.

Table 49: Counted CFU at a given dilution for Muri

	Dilution		
Ident	10^{-5}	10^{-6}	10^{-7}
muri910	203	23	
muri1114	521	87	
muri1309	380	52	10
muri1514	437	103	25

Table 50: Counted CFU at a given dilution for Sigmund

	Dilution				
Ident	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}
sig912	4				
sig112		23			
sig1315		205			
sig1509			69		
sig1709				113	
sig1909					337

The raw data for the OD measurement of Muri and Sigmund are given in Table 51 and 52.

Table 51: Average counted CFU of Muri at the measured OD

Ident	CFU/ml (avg)	OD
muri910	$2.04 \cdot 10^8$	4.16
muri1114	$6.96 \cdot 10^8$	6.02
muri1309	$6.33 \cdot 10^8$	7.86
muri1514	$1.32 \cdot 10^9$	9.54

Table 52: Average counted CFU of Sigmund at the measured OD

Ident	CFU/ml (avg)	OD
sig912	$4.00 \cdot 10^7$	2.33
sig112	$2.30 \cdot 10^9$	4.16
sig1315	$2.05 \cdot 10^{10}$	6.66
sig1509	$6.90 \cdot 10^{10}$	9.12
sig1709	$1.13 \cdot 10^{12}$	11.97
sig1909	$3.37 \cdot 10^{13}$	14.03

Cell Counting Chamber Muri and Sigmund yeast cells were grown in 250 mL shake flasks. 30 μL inoculum volume of either Muri and Sigmund was transferred to a marked 250 mL shake flasks with 30 mL 5 % ME medium. The raw data for the OD measurement of Muri and Sigmund are given in Table 53 and 54. The data are processed the same way described in the **Example calculation** described for Table 44, some columns are omitted.

Table 53: Corrected OD measurements for Muri to determine the relationship between Optical Density and Cell count. 150 μL yeast sample was diluted to 3000 μL 5 % ME medium

Time	Time [h]	OD_{600}	OD_{600}(diluted)
17:00	0	0,376	0,094
08:05	15,1	3,08	0,154
09:10	16,2	4,16	0,208
10:03	17,1	4,74	0,237
11:09	18,2	6,02	0,301
12:00	19	6,86	0,343
13:00	20	7,86	0,393
14:00	21	8,78	0,439
15:05	22,1	9,54	0,477
16:00	23	9,92	0,496
14:33	46,5	23,4	0,39

Table 54: Corrected OD measurements for Sigmund to determine the relationship between Optical Density and Cell count. 100 μL Sigmund sample was diluted to 3000 μL 5 % ME medium

Time	Time [h]	OD600	OD_{600}(diluted)
19:30	0,0	0,195	0,0065
09:08	13,6	2,325	0,0775
10:08	14,6	3,195	0,1065
11:05	15,6	4,155	0,1385
12:09	16,7	5,355	0,1785
13:07	17,6	6,66	0,222
14:00	18,5	7,77	0,259
15:01	19,5	9,12	0,304
16:00	20,5	10,74	0,358
17:00	21,5	11,97	0,399
18:00	22,5	13,305	0,4435
19:00	23,5	14,025	0,4675
13:37	66,1	30,75	0,5125

Samples around the exponential growth region (at times 9:10, 11:09, 13:00 and 15:05 for Muri, times 9:20, 11:10 13:10, 15:10, 17:00 and 19:00 for Sigmund) were taken. Muri And Sigmund samples were diluted to 10^{-1} and 10^{-2} (100 μL sample + 900 μL dH_2O. A drop of 10^{-1} and 10^{-2} each Muri and Sigmund using a pipette was placed onto a hemocytometer and counted in a microscope. Figure 53 shows the snapshot taken for the 13:10 10^{-1} sample.

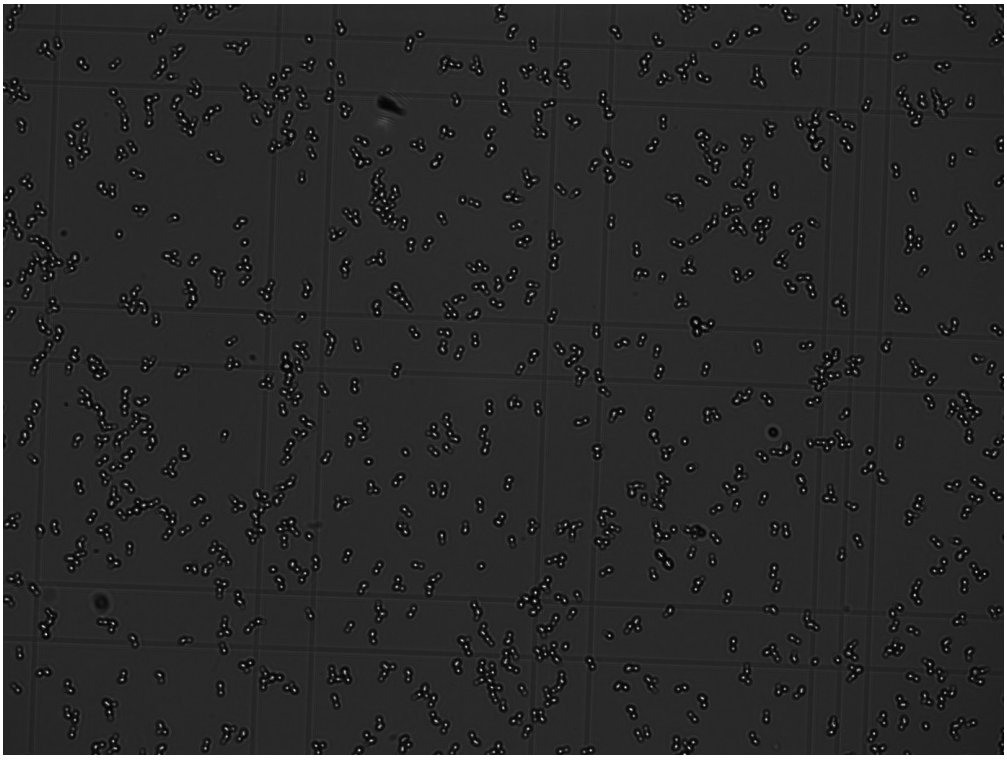

Figure 53: Cells counted for Sigmund at time 13:10 in order to determine the relationship between OD and cell count

The average counted yeast cells of 6 squares at the different times and different dilutions are given in Table 55 for Muri, and Table 56 for Sigmund.

Table 55: Average cell count for Muri yeast at 10^{-1} and 10^{-2} dilution counted in a Buerker counting chamber

	Counted cells		
	10^{-1}	10^{-2}	OD_{600}
Muri 09:10	83	13	4,16
Muri 10:09	170	13	6,02
Muri 13:00	209	20	7,86
Muri 15:05	245	25	9,54

Table 56: Average cell count for Sigmund yeast at 10^{-1} and 10^{-2} dilution counted in a Buerker counting chamber

	Counted cells		
	10^{-1}	10^{-2}	OD_{600}
Sig 09:08	25	3	2,33
Sig 11:05	64	5	4,16
Sig 13:07	117	8	6,66
Sig 15:01	140	12	9,12
Sig 17:00	157	15	11,97

Using Equation 3. The amount of $\frac{cells}{mL}$ can be calculated. Using the values from Table 55 and 56 the average cell count over the 10^{-1} and 10^{-2} dilutions were calculated. The calculated cell concentration values are given in Table 57 for Sigmund and 58 for Muri with their applicable OD.

Table 57: Amount of $\frac{cells}{mL}$ of Sigmund to determine the relationship between the amount of cells and optical density

Ident	Cells (10^8) $[\frac{cells}{mL}/]$	OD_{600}
Sig 09:08	0,69	2,33
Sig 11:05	1,36	4,16
Sig 13:07	2,46	6,66
Sig 15:01	3,25	9,12
Sig 17:00	3,77	11,97

Table 58: Amount of $\frac{cells}{mL}$ of Muri to determine the relationship between the amount of cells and optical density

Ident	Cells (10^8) $[\frac{cells}{mL}/]$	OD_{600}
Muri 09:10	2,66	4,16
Muri 10:09	3,75	6,02
Muri 13:00	5,11	7,86
Muri 15:05	6,19	9,54

Example calculation Using the values from Table 56 and Equation 3, the cell concentration of Muri sample taken at 13:00 is calculated below.

$$\frac{cells}{mL} = \frac{Muri13:00(10^{-2})}{Height[mm] \cdot Width[mm] \cdot Depth[mm] \cdot \frac{1mm^3}{1000mL}}$$

$$\frac{20 \cdot 100}{0,2mm \cdot 0,2mm \cdot 0,1mm \cdot \frac{1mm^3}{1000mL}} = \frac{2000}{4 \cdot 10^{-6}mL} = 5,0 \cdot 10^8$$

B.3 Dry weight

Yeast cells were grown for roughly 48 hours, the Optical density was measured at 600 nm. 20 μL sample was diluted to 1000 μL using 5 % ME medium. The OD_{600} is given in Table 59

Table 59: Measured and corrected for dilution OD_{600} for yeast for the flocculation test

Yeast	OD_{600}	OD_{600} corrected for dilution
Muri	0,595	29,8
Stranda	0,735	36,8
Hornindal	0,545	27,3
Sigmund	0,727	36,4
Idun	0,703	35,2

For the flocculation test, the target amount of cells in order to perform the analysis correctly is $1 \cdot 10^8$ cells. It was assumed that the different strains of yeast have a similar cell count when using a hemocytometer, so the relationship between OD_{600} and cell count determined for Muri, The trendline on for the linear relationship between OD and cell concentration (Figure 15a in Chapter 4.1.2) applies for all strains.

Volume yeast required for a cell concentration of $1 \cdot 10^8$ cells in a 25 mL sample are given in Table 60

Table 60: Volume required for a 25 mL sample to have $1 \cdot 10^8$ cells of the different yeast strains

Yeast	Volume needed for $1 \cdot 10^8$ cells [mL]	How the pipette is adjusted ($\frac{Volume}{2}$) [μL]
Muri	1,46	731
Stranda	1,18	592
Hornindal	1,59	799
Sigmund	1,19	599
Idun	1,24	619

The total starting point of cells are calculated using a hemocytometer. The starting amount of cells are given in Table 61

Table 61: The starting amount of yeast cells counted in a hemocytometer used in the flocculation analysis

Yeast	Starting cell count ($\cdot 10^8$)
Muri	9,77
Stranda	2,02
Hornindal	3,92
Sigmund	3,46
Idun	2,50

50 μL yeast sample was diluted with 950 μL 250 mM NaCl pH 4.5 solution and the absorbance was measured. The measured OD_{600} for the flocculation are given in Table 62.

Table 62: Measured OD_{600} for Muri, Stranda, Hornindal, Sigmund and Idun

	OD_{600}				
Time	Muri	Stranda	Hornindal	Sigmund	Idun
0	[a]	[a]	0,154	0,100	0,095
15	0,171	0,064	0,099	0,095	0,105
30	0,142	0,064	0,078	0,091	0,092
60	0,133	0,054	0,059	0,089	0,09
120	0,086	0,032	0,033	0,091	0,097
180	0,083	0,027	0,025	0,083	0,089
240	0,082	0,022	0,018	0,081	0,089
300	0,089	0,023	0,012	0,084	0,086
360	0,087	0,017	0,012	0,074	0,086
420	[a]	0,016	0,012	0,074	[a]

[a] No data available

B.4 Microscopy of Kveik

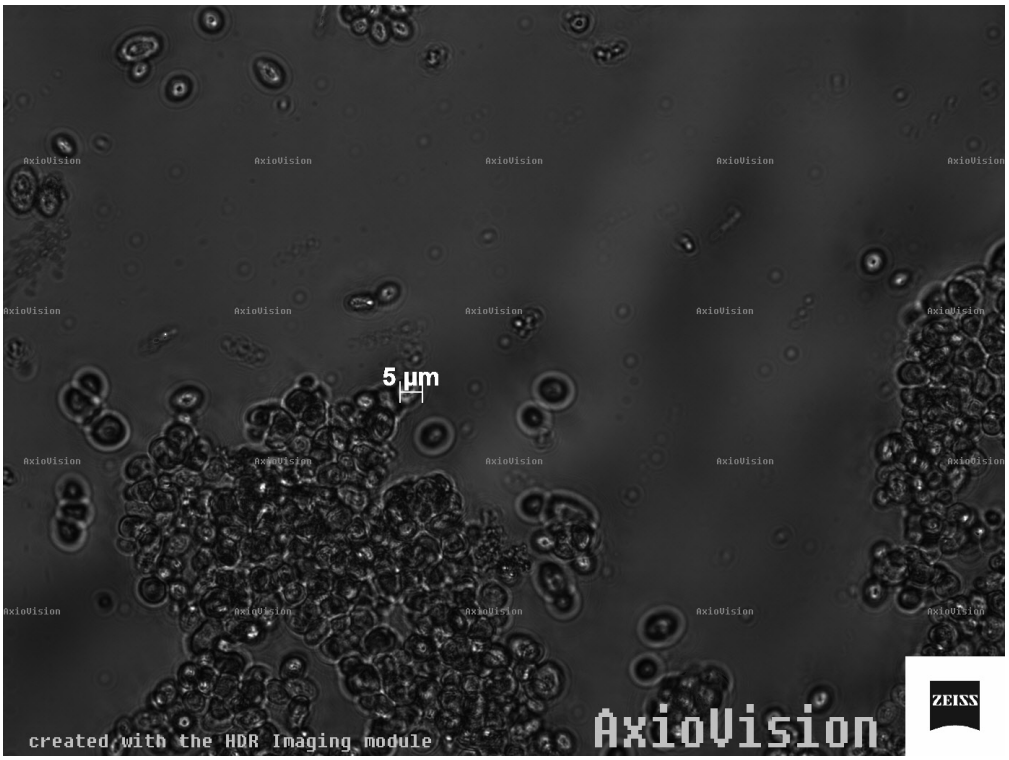

Figure 54: Microscopy of Rivenes at 40x zoom using AxioVision for Zeiss microscope

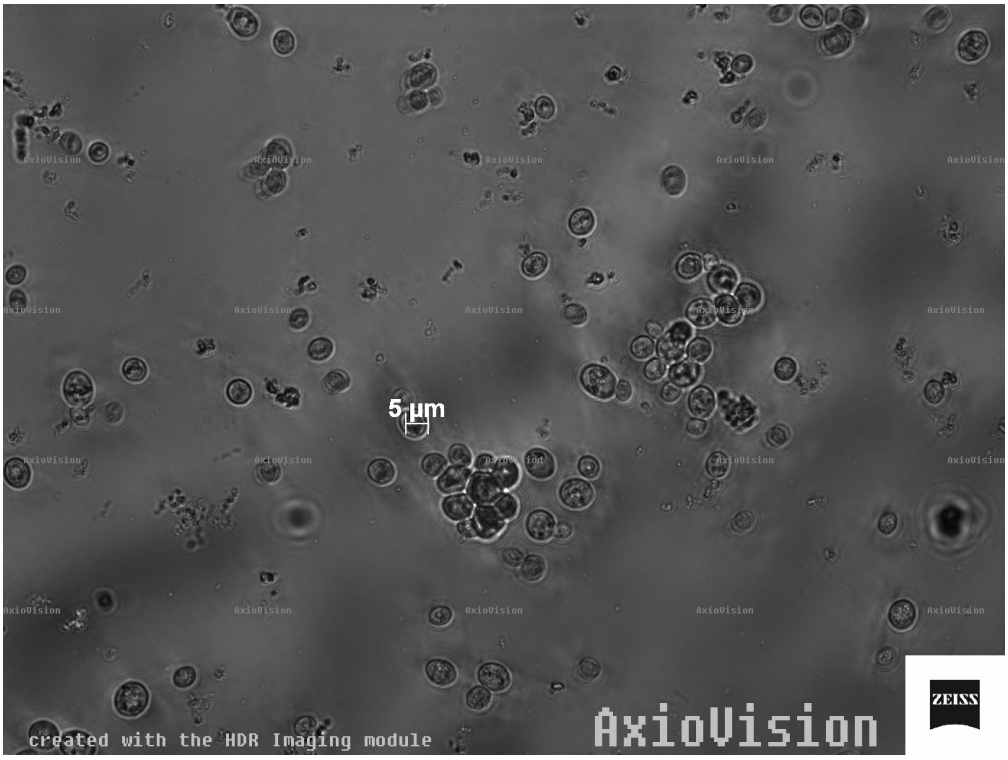

Figure 55: Microscopy of Raftevold at 40x zoom using AxioVision for Zeiss microscope

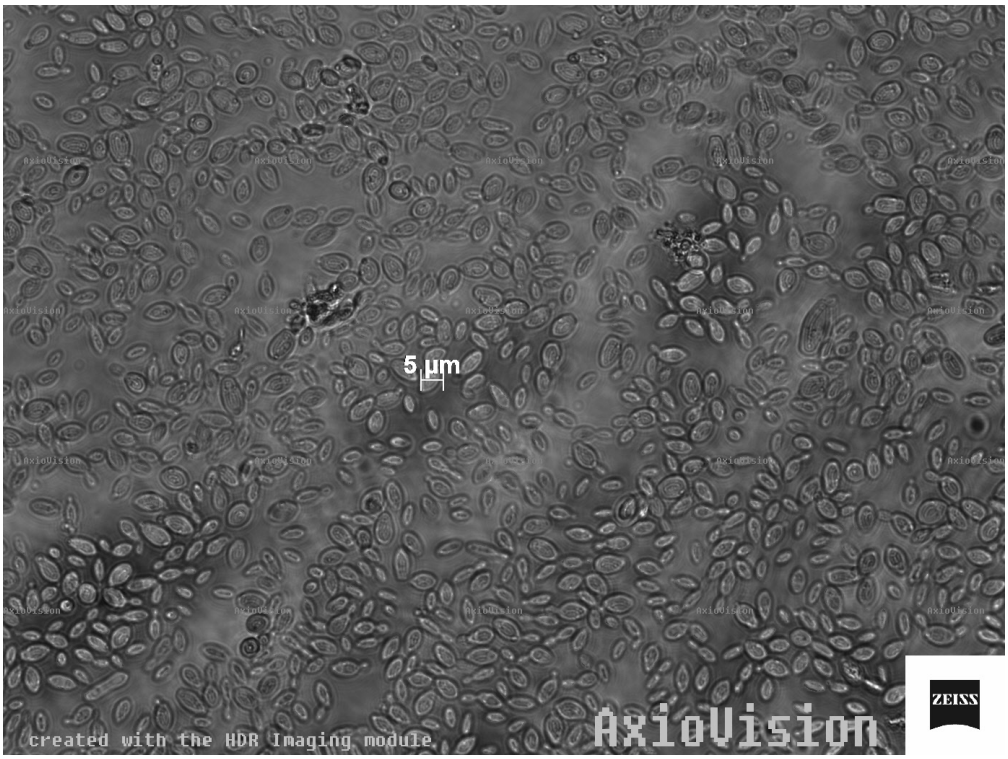

Figure 56: Microscopy of Lithuania 1 at 40x zoom using AxioVision for Zeiss microscope

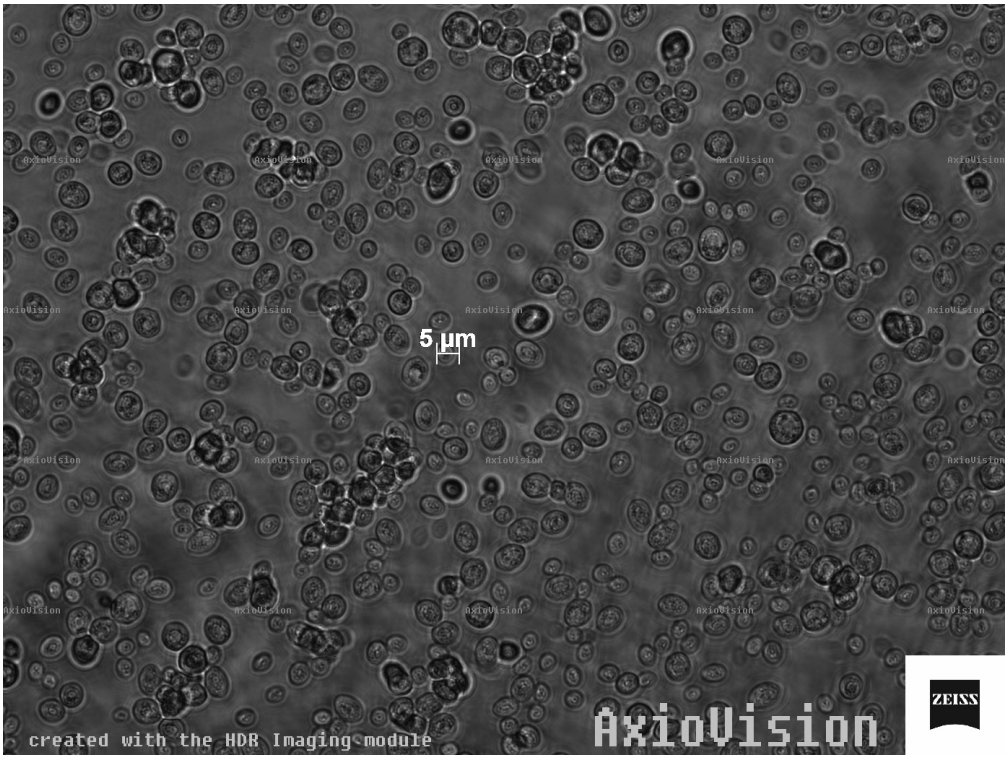

Figure 57: Microscopy of Lithuania 2 at 40x zoom using AxioVision for Zeiss microscope

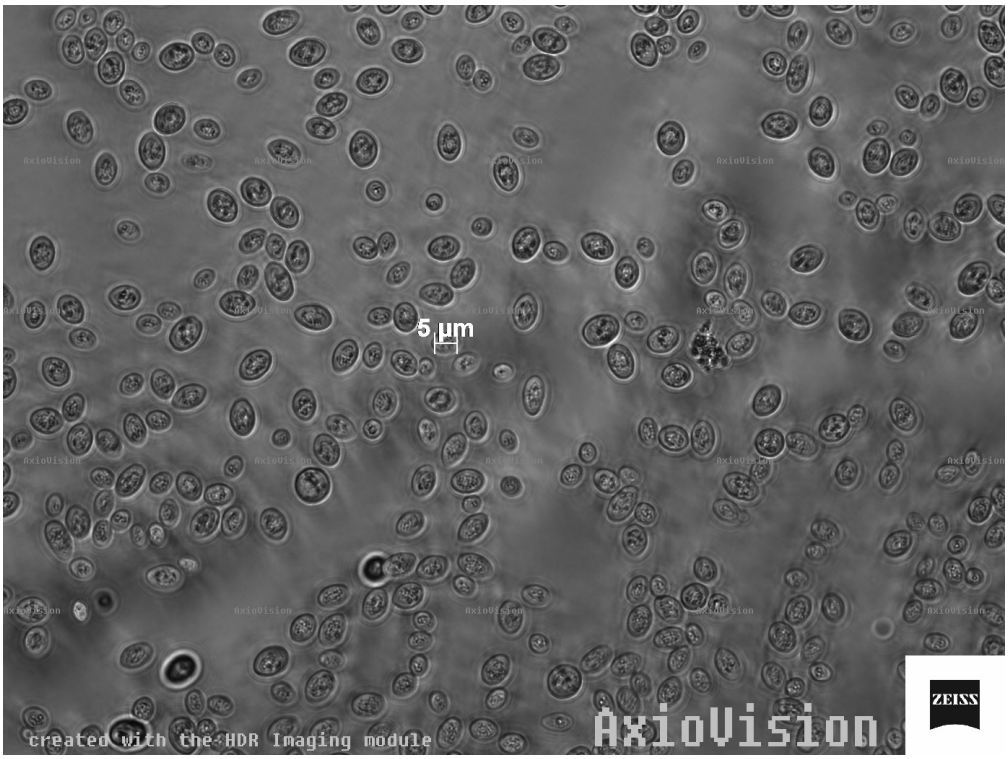

Figure 58: Microscopy of Lithuania 3 at 40x zoom using AxioVision for Zeiss microscope

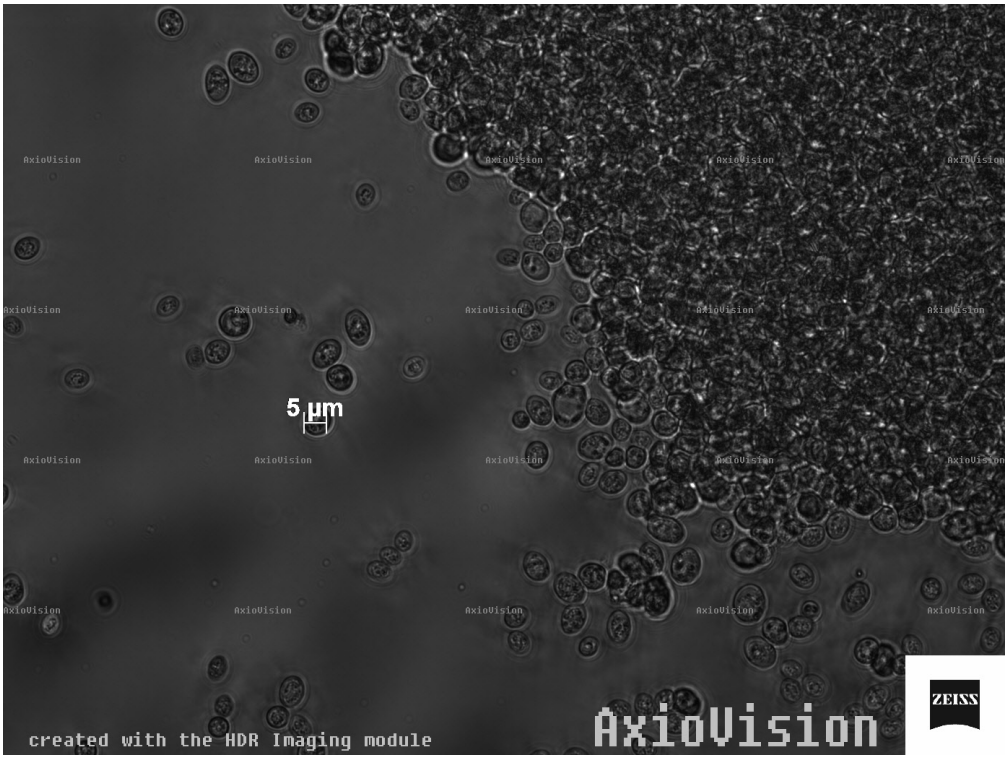

Figure 59: Microscopy of Lithuania 4 at 40x zoom using AxioVision for Zeiss microscope

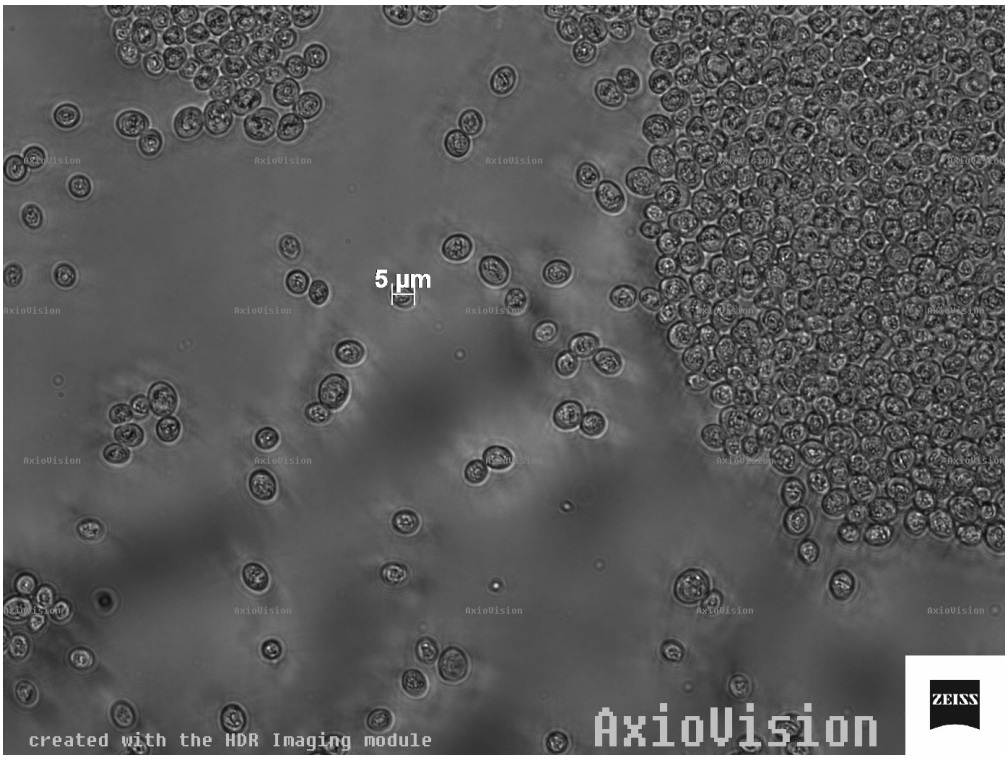

Figure 60: Microscopy of Gausemel at 40x zoom using AxioVision for Zeiss microscope

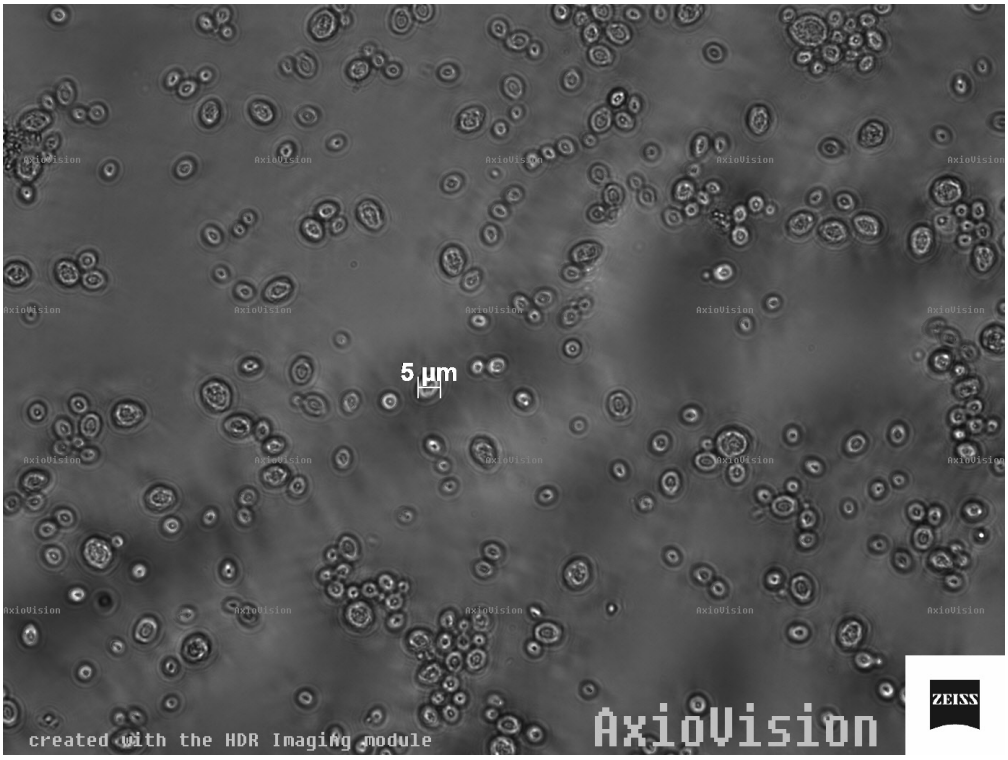

Figure 61: Microscopy of Finnish 1 at 40x zoom using AxioVision for Zeiss microscope

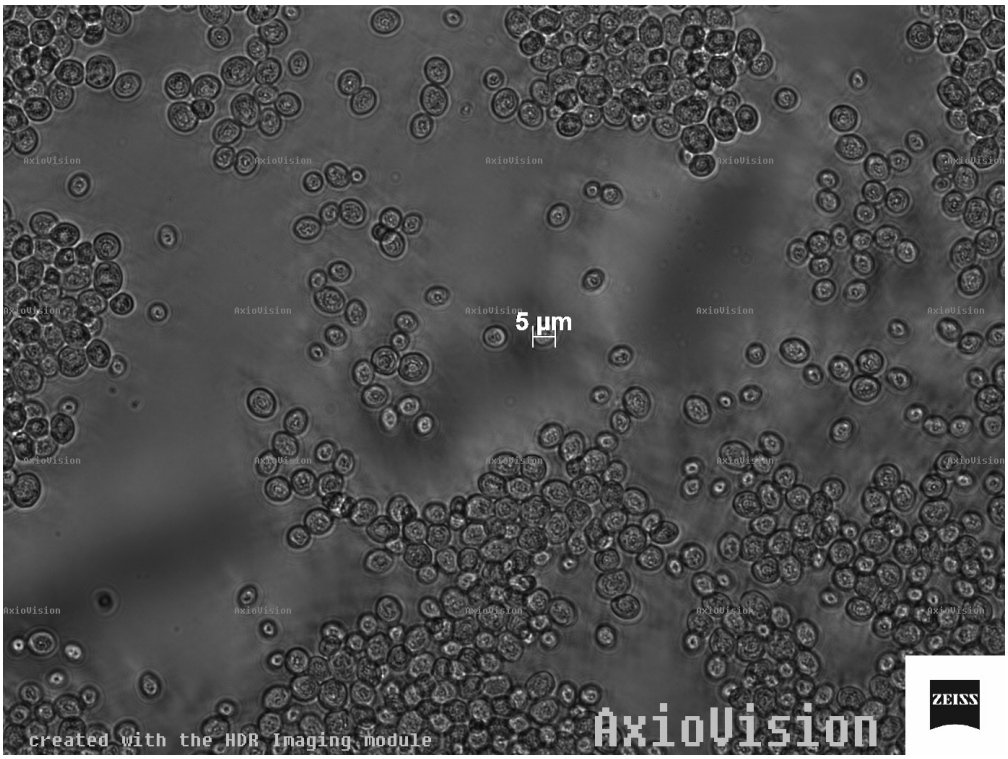

Figure 62: Microscopy of Wendelboe at 40x zoom using AxioVision for Zeiss microscope

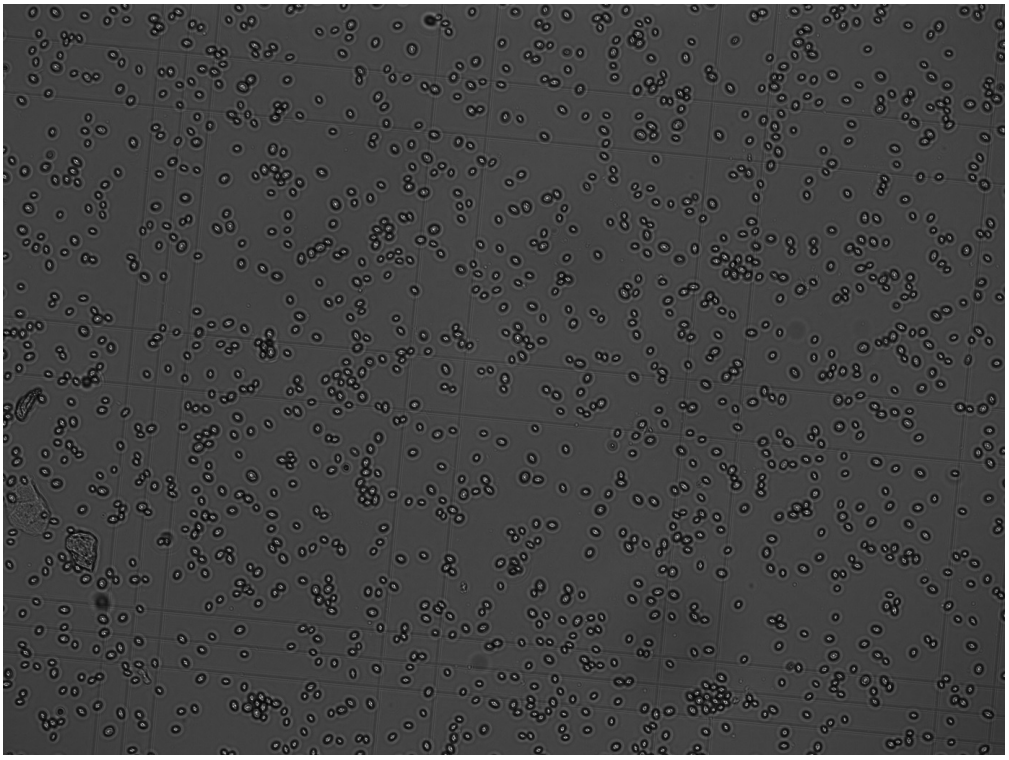

Figure 63: Microscopy of Hornindal at 10x zoom in a counting chamber using AxioVision for Zeiss microscope

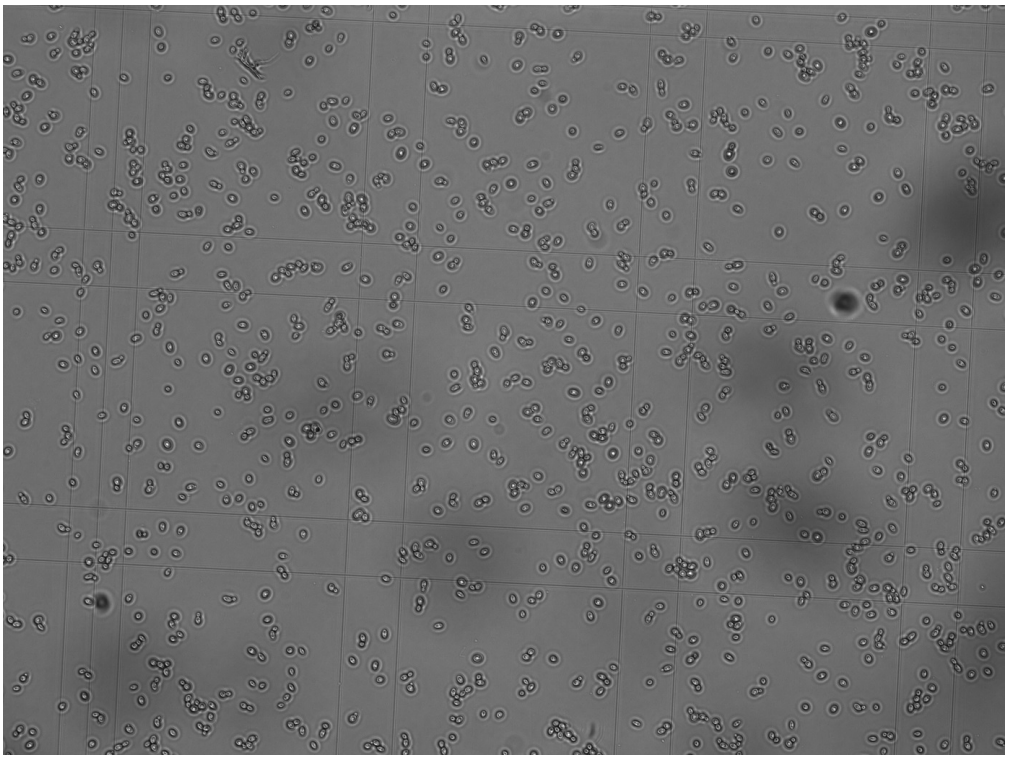

Figure 64: Microscopy of Idun at 10x zoom in a counting chamber zoom using AxioVision for Zeiss microscope

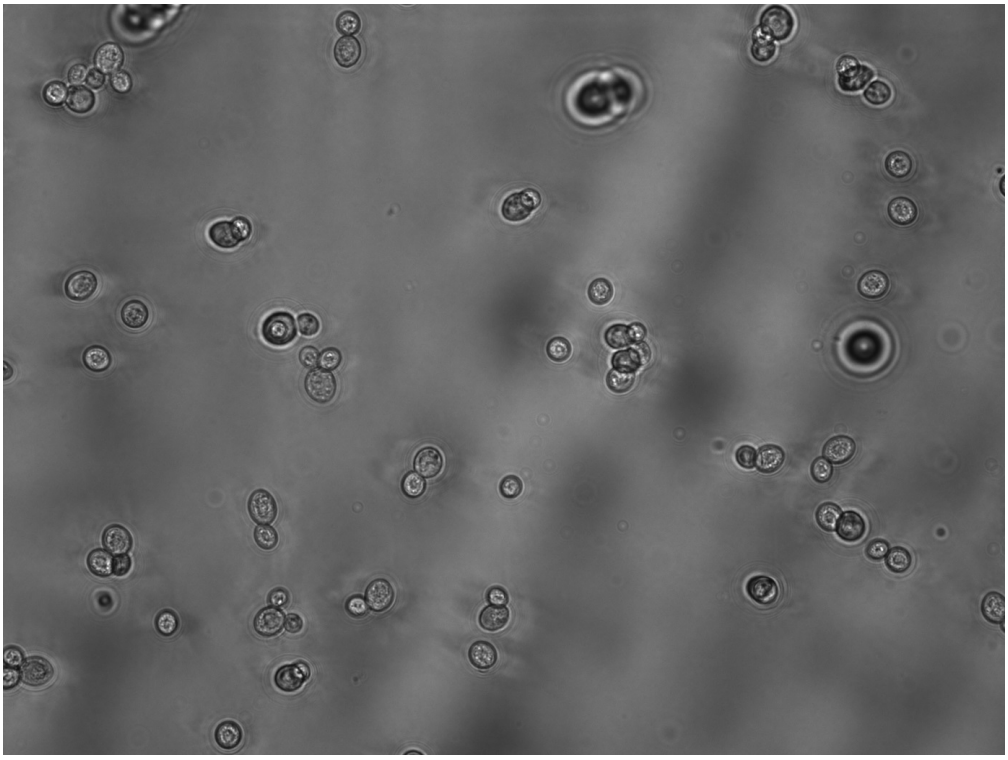

Figure 65: Microscopy of Sigmund at 40x zoom using AxioVision for Zeiss microscope

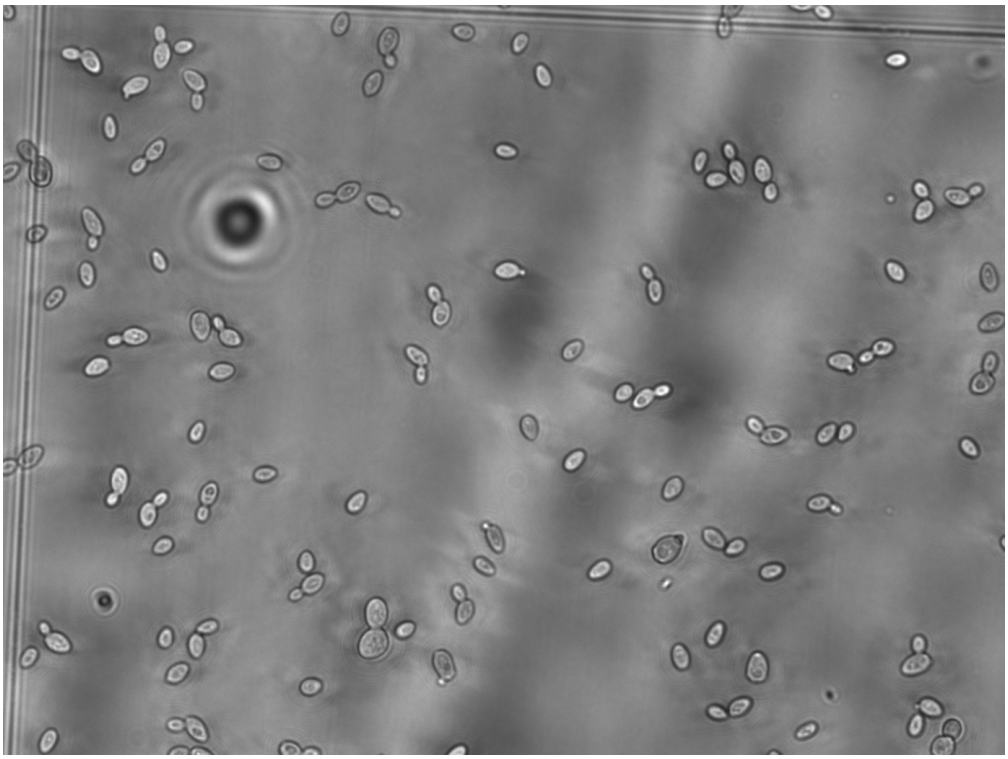

Figure 66: Microscopy of Muri at 40x zoom using AxioVision for Zeiss microscope

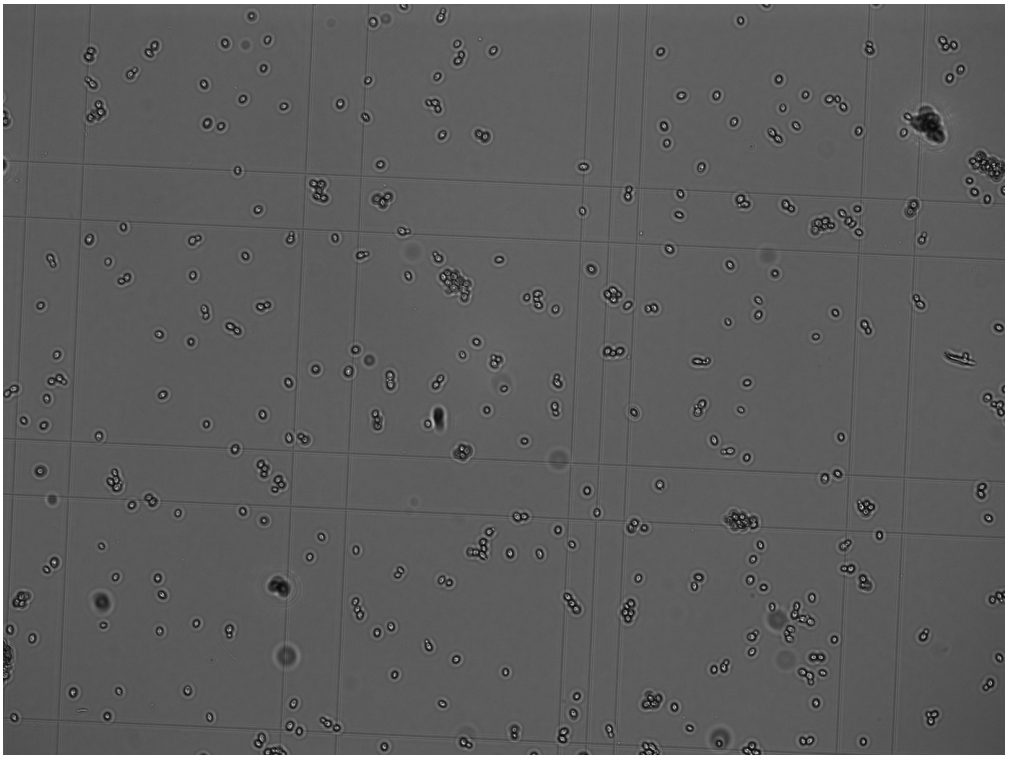

Figure 67: Microscopy of Stranda at 10x zoom in a counting chamber using AxioVision for Zeiss microscope

Appendix C Sensory analysis

In total, 17 assessors participated in the sensory analysis of pale ale from Experiment 1. 15 assessors participated in the Duo-Trio test, with 2 assessors taking part of a pilot run of the testing protocol (P3, parallel 2 and P16). The duo trio test was performed as described in Figure 68. The reference beer was placed in such a way that it was easily accessible for each participant.

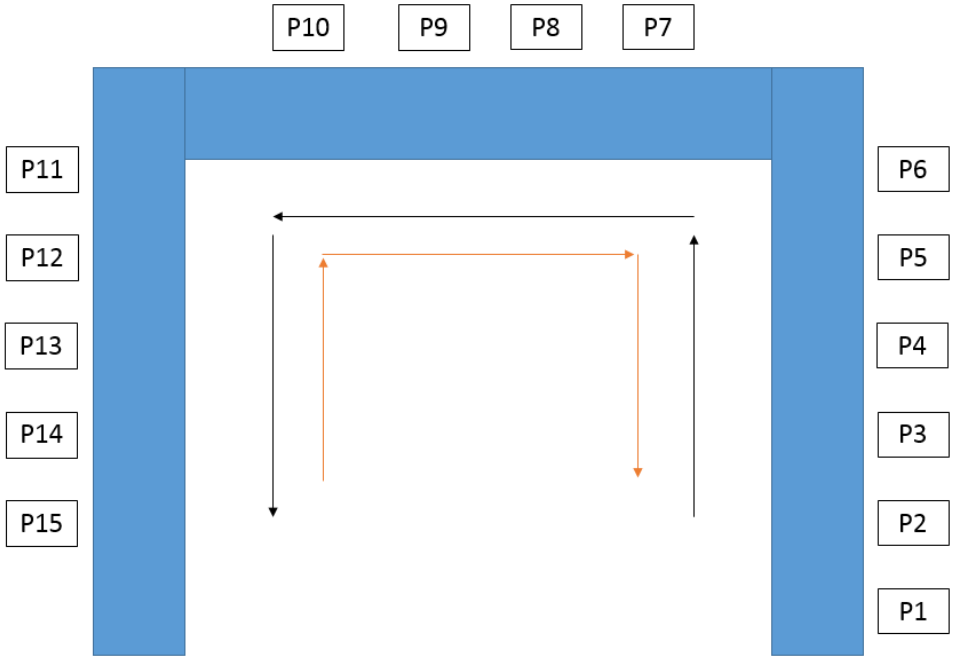

Figure 68: Setup of the Duo-trio test. Person P1 was served first following the black arrow, last being P15. P15 was served first using the orange arrow.

The sensory analysis had 5 rounds in total, and took around 45 minutes to complete. Each assessor filled in a score sheet, given in Figure 69

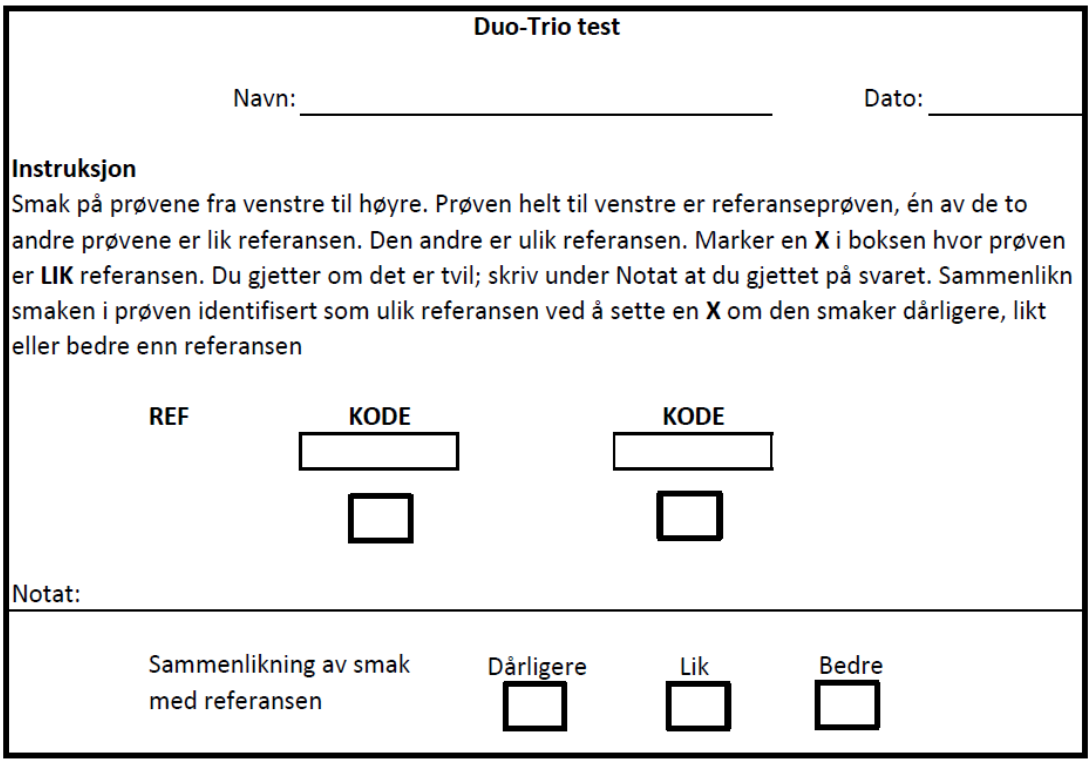

Figure 69: Duo-trio test score sheet filled in by each assessor, filled in a total of 5 times

The scores sheets were not gathered in after each round, however the participants did not have access to the beer that had been served in a previous round. Each 330 mL beer bottle had a 3-letter code which the assessors wrote down on the score sheet before tasting. The codes are given in Figure 70.

237	Muri		202	
631			769	
96			0	
858			940	
373	Stranda		553	
966			669	
874			370	
843			760	
465	Hdal		578	
247			373	WLP001
936			289	
336			297	
189	Hdal+K		30	
886			856	
384			685	
739			220	
524	Sigmund		506	
35			647	
106			289	
1			27	
814	Idun			
950				
575				
533				

Figure 70: Codes used to identify which code belonged to which sample

Due to beer limitation and personnel serving the samples, the letters were recycled, ideally each assessor would have one specific number. Orange arrow (Figure 68) were responsible to hand out WLP001 samples. Black arrow (Figure 68) were responsible to hand out Muri, Stranda, Hornindal, Sigmund and Idun so it would

be easier to divide the samples without repeating or mixing up samples. Hornindal + contaminant was not served as it was deemed too easy to differentiate, and the flavor itself was too unique, it was offered at the end for those who dared. Table 63 shows the result of the sensory analysis. A value of 1 was given to the assessor who recognized WLP001, and 0 for the assessor who couldn't.

Table 63: Result from the Duo-trio test. Which taste round, the ID of the person (Figure 68), the sample the person received and if they recognized WLP001 which was served together with the sample and reference and flavor comparison where the reference had a value of 3, <3 was better, >3 was worse.

Round	ID	Sample	Recognized WLP001	Comparison
1	P1	Muri	0	4
2	P1	Hornindal	1	5
3	P1	Stranda	1	4
4	P1	Sigmund	1	4
5	P1	Idun	1	5
1	P2	Muri	1	3
2	P2	Hornindal	1	4
3	P2	Stranda	1	4
4	P2	Sigmund	1	4
5	P2	Idun	1	5
1	P3	Muri	1	2
1	P3	Stranda	1	2
2	P3	Hornindal	1	3
2	P3	Muri	1	3
3	P3	Idun	1	2
1	P4	Muri	1	4
2	P4	Hornindal	0	2
3	P4	Stranda	1	4
4	P4	Sigmund	1	2
5	P4	Idun	1	2

Continued on next page

Table 63 – continued from previous page

Round	ID	Sample	Recognized WLP001	Comparison
1	P5	Muri	1	4
2	P5	Hornindal	0	3
3	P5	Stranda	1	1
4	P5	Sigmund	1	1
5	P5	Idun	1	4
1	P6	Muri	0	4
2	P6	Hornindal	0	2
3	P6	Stranda	1	3
4	P6	Sigmund	0	2
5	P6	Idun	1	4
1	P7	Muri	1	2
2	P7	Hornindal	1	3
3	P7	Stranda	1	1
4	P7	Sigmund	1	1
5	P7	Idun	1	4
1	P8	Muri	1	4
2	P8	Hornindal	1	2
3	P8	Stranda	0	3
4	P8	Sigmund	0	3
5	P8	Idun	1	4
1	P9	Muri	1	2
2	P9	Hornindal	0	4
3	P9	Stranda	1	2
4	P9	Sigmund	0	3
5	P9	Idun	1	4
1	P10	Muri	1	4
2	P10	Hornindal	1	4
3	P10	Stranda	1	3

Continued on next page

Table 63 – continued from previous page

Round	ID	Sample	Recognized WLP001	Comparison
4	P10	Sigmund	0	3
5	P10	Idun	1	4
1	P11	Muri	1	4
2	P11	Hornindal	1	5
3	P11	Stranda	1	4
4	P11	Sigmund	1	5
5	P11	Idun	1	5
1	P12	Muri	1	2
2	P12	Hornindal	1	2
3	P12	Stranda	1	1
4	P12	Sigmund	1	3
5	P12	Idun	1	3
1	P13	Muri	1	3
2	P13	Hornindal	1	2
3	P13	Stranda	1	
4	P13	Sigmund	1	3
5	P13	Idun	1	2
1	P14	Muri	0	3
2	P14	Hornindal	1	4
3	P14	Stranda	1	2
4	P14	Sigmund	1	2
5	P14	Idun	1	2
1	P15	Muri	1	4
2	P15	Hornindal	1	4
3	P15	Stranda	1	3
4	P15	Sigmund	0	3
5	P15	Idun	1	4
3	P3, parallel 2	Stranda	1	3

Continued on next page

Table 63 – continued from previous page

Round	ID	Sample	Recognized WLP001	Comparison
4	P3, parallel 2	Hornindal	0	3
4	P3, parallel 2	Sigmund	1	2
5	P3, parallel 2	H+K	1	1
5	P3, parallel 2	Idun	1	3
6	P3, parallel 2	Sigmund	1	4
1	16	Muri	1	3
2	16	Stranda	1	2
3	16	Idun	1	2
4	16	H+K	1	1
5	16	Hornindal	1	4
6	16	Sigmund	1	2

The minimum number of correct responses needed are given in Table 64, this is taken from ISO 10399 standard [29].

Table 64: Minimum number of correct responses needed to conclude that a perceptible difference exists based a duo-trio test

n	α					n	α				
	0.2	0.1	0.05	0.01	0.001		0.2	0.1	0.05	0.01	0.001
6	5	6	6	-	-	26	16	17	18	20	22
7	6	6	7	7	-	27	17	18	19	20	22
8	6	7	7	8	-	28	17	18	19	21	23
9	7	7	8	9	-	29	18	19	20	22	24
10	7	8	9	10	10	30	18	20	20	22	24
11	8	9	9	10	11	32	19	21	22	24	26
12	8	9	10	11	12	36	22	23	24	26	28
13	9	10	10	12	13	40	24	25	26	28	31
14	10	10	11	12	13	44	26	27	28	31	33
15	10	11	12	13	14	48	28	29	31	33	36
16	11	12	12	14	15	52	30	32	33	35	38
17	11	12	**13**	14	16	56	32	34	35	38	40
18	12	13	13	15	16	60	34	36	37	40	43
19	12	13	14	15	17	64	36	38	40	42	45
20	13	14	15	16	18	68	38	40	42	45	48
21	13	14	15	17	18	72	41	42	44	47	50
22	13	14	15	17	19	76	43	45	46	49	52
23	15	16	16	18	20	80	45	47	48	51	55
24	15	16	17	19	20	84	47	49	51	54	57
25	16	17	18	19	21	88	49	51	53	56	59

For the sensory analysis (N = 17) there needs to be 13 correct responses to conclude that there is a perceptible difference on a 95 % confidence level. Equation 4 can be used to calculate a one-sided lower confidence interval on the proportion of the population that can perceive a difference between the samples. Where x is the number of correct responses and n is the total number of assessors. $z_a = 1.64$ for 95 % confidence level.

$$s_d = \left[2 \cdot \left(\frac{x}{n} - 1\right)\right] - 2 \cdot z_a \cdot \sqrt{\left(\frac{n}{x}\right) \cdot \frac{\left[1 - \left(\frac{n}{x}\right)\right]}{n}} \quad (4)$$

Appendix D Genetic analysis

Measured OD for DNA isolation is given in Table 65. 50 μL sample was diluted in 1000 μL using 5 % ME medium. The OD of the Muri, Stranda, Sigmund, Hornindal and Idun were not measured when DNA was isolated, they were just allowed to grow for 48 h until reaching max cell count using Figure 13 as reference.

Table 65: Measured OD, diluted and real after 72 h incubation for some yeast strains

Yeast	OD	OD
Rivenes	0.778	15.56
Raftevold	0.791	15.82
Lit 4	1.376	27.52
Lit 1	0.995	19.9
Lit 3	1.195	23.9
Lit 2	1.295	25.9
Fin 1	0.961	19.22
Wendelboe	1.331	26.62
Gausemel	1.131	22.62

DNA was isolated from yeast in Table 65 as well as Muri, Stranda, Hornindal, Sigmund and Idun. The DNA concentration of Muri, Stranda, Hornindal, Sigmund and Idun was measured with Nanodrop in order to have 1 ng/μL of the DNA template for the PCR reaction. The the concentration of DNA and 260/280 and 260/230 ratios are given in Table 66

Table 66: Concentration of DNA using Nanodrop after DNA isolation

Yeast	DNA conc [ng/μL]	260/280	260/230
Muri	13.6	2.08	0.49
Stranda	13.2	1.82	0.49
Hornindal	15.2	1.87	0.58
Sigmund	11.6	1.69	0.55
Idun	13.9	1.79	0.53

10 ng/μL stock DNA solutions were made from the data in Table 66. The average DNA concentration was used to make stock solutions of Rivenes, Raftevold, Lit 1-4 and Fin. PCR was ran on the isolated DNA, an agarose gel was used to check the PCR product. Figure 71 and 72 shows the gel images of isolated Kveik strain PCR product.

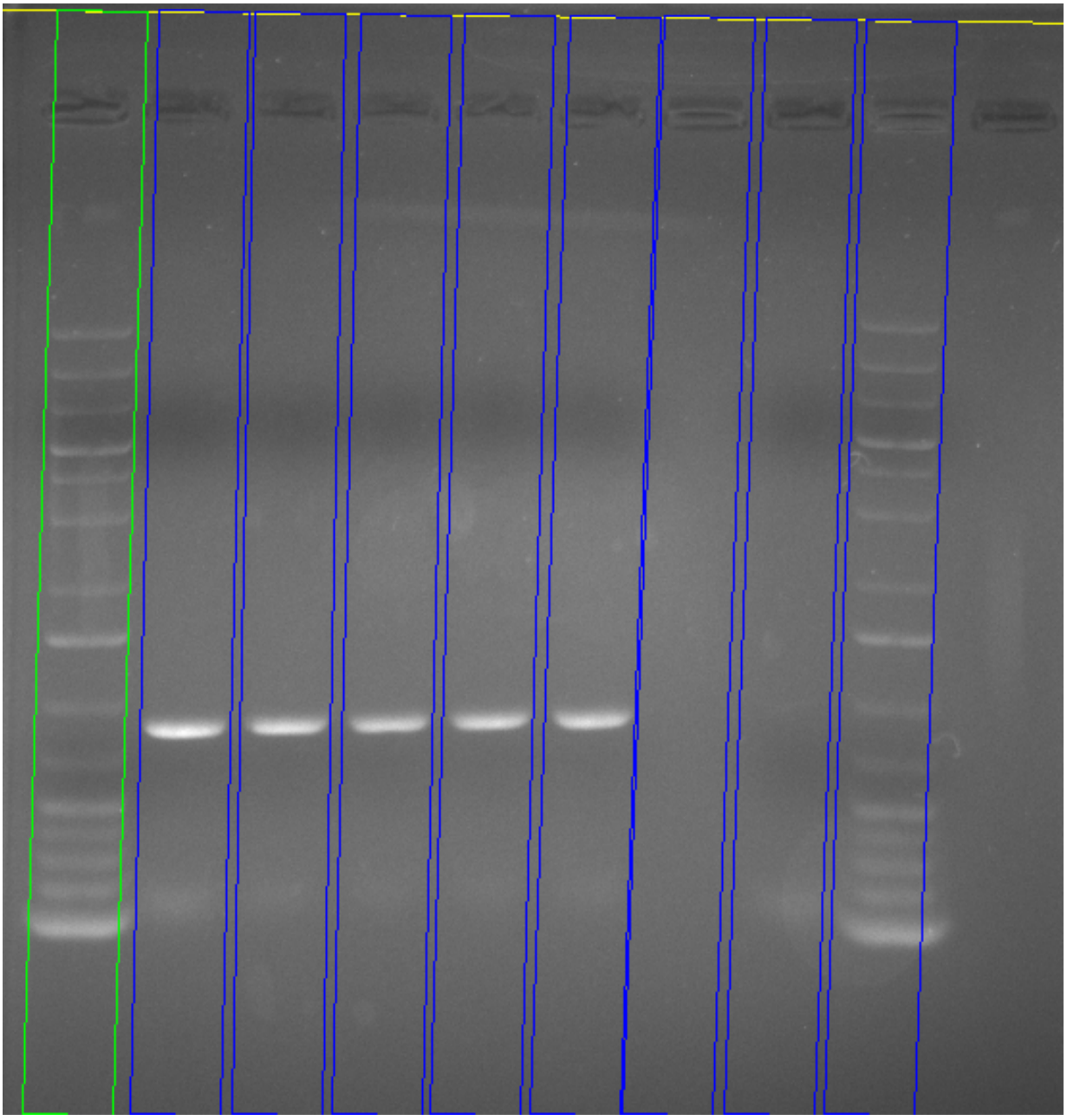

Figure 71: Agarose gel showing the PCR product from left to right Muri, Stranda, Hornindal, Sigmund and Idun

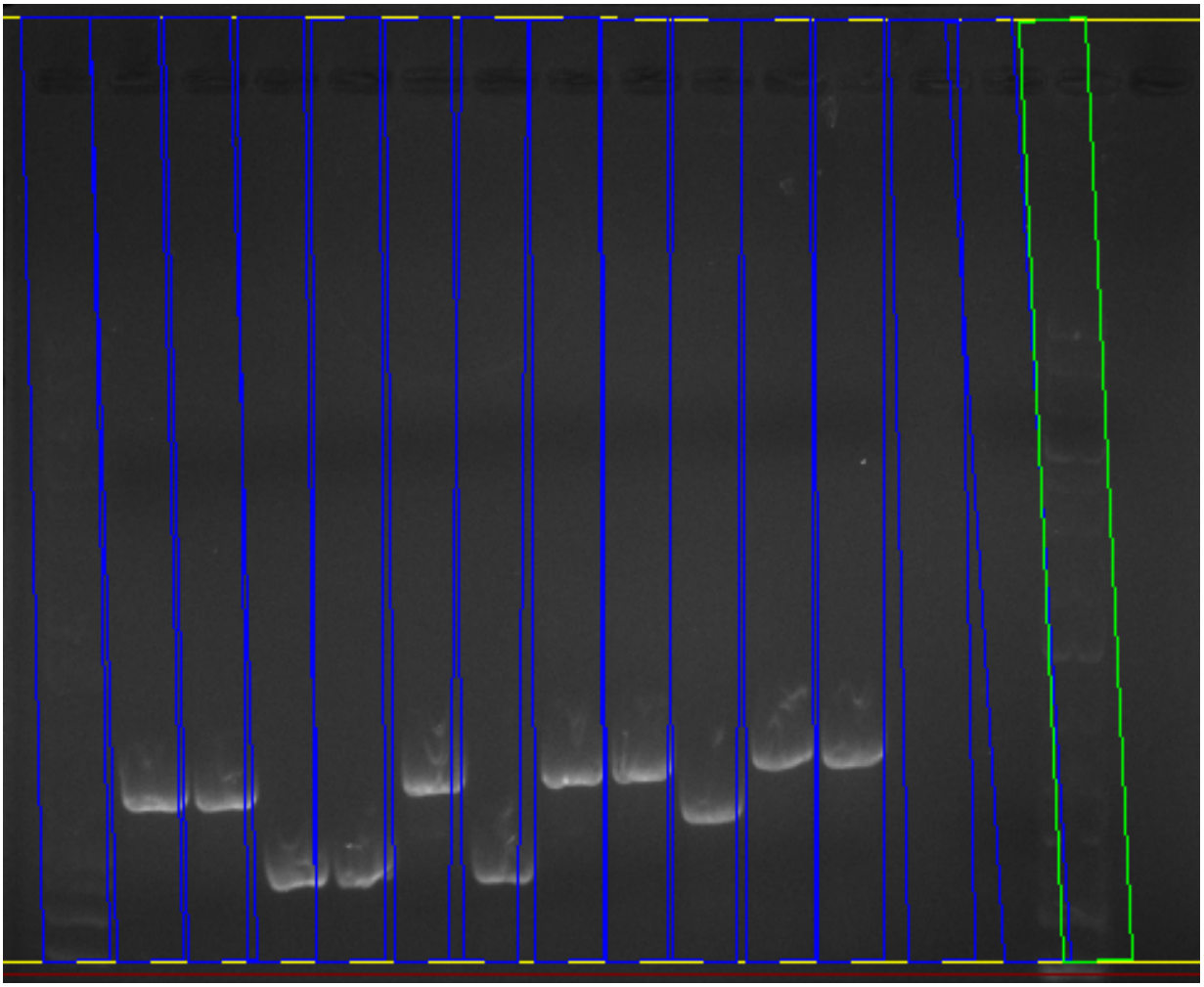

Figure 72: Agarose gel showing the PCR product from left to right Rivenes, Raftevold , raspberry 1, raspberry 2, Lit 4, Lit 1, Lit 2, Lit 3, Fin 1, Wendelboe, Gausemel

After agarosegel check, the PCR product was purified. The DNA concentration of purified PCR product was measured using NanoDrop because of a requirement by GATC-Biotech, the concentration of DNA and 260/280 and 260/230 ratios are given in Table 67

Table 67: Concentration of DNA and quality of purified PCR product to be sent in to GATC for sequencing.

Yeast	DNA conc [ng/μL]	260/280	260/230
Muri	62.5	1.88	2.7
Stranda	67.8	1.97	2.75
Hornindal	64.0	1.93	2.76
Sigmund	60.1	1.89	2.69
Idun	57.8	1.89	2.69
Rivenes	46.8	1.96	3.16
Raftevold	59.3	1.73	2.45
Lit 4	7437	1.86	1.93
Lit 1	44.5	2.01	2.70
Lit 2	62.7	1.85	2.74
Lit 3	65.6	1.88	2.80
Fin 1	72.2	1.92	2.60
Wendelboe	64.0	1.85	2.81
Gausemel	69.8	1.88	2.80

The Sanger sequencing data for the forward and reverse primers are given below, a consensus region was made in clone manager when aligning the forward (denoted -f) with the reverse primer (denoted -r)

Lit 2-f

GGAGACTCGTAGTGACTGCGGAGGATCATTAAAGAATTTAATAATTTTGAAATGGATTTT
TTTGTTTTGGCAAGAGCATGAGAGCTTTTACTGGGCAAGAAGACAAGAGATGGAGAGTCC
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CCAAACGGTGAGAGATTTCTGTGCTTTTGTTATAGGACAATTAAAACCGTTTCAATACAA
CACACTGTGGAGTTTTCATATCTTTGCAACTTTTTCTTTGGGCATTCGAGCAATCGGGGC
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CGTCATTTCCTTCTCAAACATTCTGTTTGGTAGTGAGTGATACTCTTTGGAGTTAACTTG
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AGTTTGACCTCAAATCAGGTAGGAGTACCCGCTGAACTTAAGCATATCATAAGACCGGAA
GAAAAAGAACAATTTGTTTTTCTAT

Lit 2-r

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Gausemel-f

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AAAAACTAAC

Gausemel-r

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GG

Hornindal-f

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Hornindal-r

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AAAAAAA

Idun-f

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AAAAGAAAAAGAAG

Idun-r

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Lit 3-f

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Lit 3-r

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Fin 1-f

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GCAGACAGAGAGACAATACAACCGCCCTCCCCCTGGCTAACAGTACTCTTTAAGTTTGAC
CTCAAATCAGGTAGGAATACCCGCTGAACTTAAGCATATCAAAAACCCGGAAGGAAAGGG
GGGGGGGGGGCGCGG

Fin 1-r

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Muri-f

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Raftevold-f

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GCCCCCCCCCCGGGGGGACCCCCGAAATAAAAAAAAAAGAAGGAAAAAACGTAATTTTTTT
TTTTTTTTTTTATTACGC

Raftevold-r

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Rivenes-f

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Rivenes-r

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G

Sigmund-f

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Sigmund-r

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Lit 1-f

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Lit 1-r

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Stranda-f

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Stranda-r

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Wendelboe-f

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Wendelboe-r

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Lit 4-f

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Lit 4-r

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