Practical Strategies for Using Kveik Brewing Yeasts at Industrial Scale

For

The Brewers' Company

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Abstract / Executive Summary

Kveik yeast are a subspecies of *Saccharomyces cerevisiae,* historically used by rural Norwegian brewers to brew traditional ales for personal and ceremonial consumption. These yeasts have remained largely unexplored until recent times, where their potential for use in mainstream beer production has begun to be realised. The advantages of kveik yeast are that they ferment rapidly at high temperatures and, under these conditions, produce a relatively neutral flavour contribution when compared to contemporary brewers' yeasts. However, a full understanding of the properties and potential for kveik strains for the production of a range of beers, as well as practicalities with regard to yeast handling have yet to be achieved.

This study aimed to investigate several aspects of fermentation and yeast handling in relation to kveik yeast. Firstly, the fermentation performance of kveik strains was compared to contemporary brewer's yeast at a range of temperatures and wort gravities. This was achieved through assessment of fermentation progression, as well as the development of ethanol and key flavour compounds. The data obtained demonstrate that kveik isolates are able to ferment successfully at comparatively high temperatures and at high gravity. Similar to conventional brewing yeasts, they exhibit an individual capacity to attenuate worts and are able to create unique products based on strain-specific flavour profiles. A detailed analysis of beer volatiles indicated that kveik strains produce broadly similar spectra of flavour compounds to contemporary brewing yeasts. Furthermore, when fermentation conditions are manipulated kveik strains can produce beers which are comparable to both ale and lager type products, suggesting a high degree of versatility. Surprisingly, it was found to be easier to match lager-type beer styles than ale-type products. This data holds promise for conducting rapid fermentations while flavour-matching current brands, in addition to the application of kveik yeasts for novel product streams. From a practical perspective, kveik strains were shown to exhibit typical flocculation characteristics that were similar to conventional brewing yeasts. In addition, the data generated provides further evidence that kveik strains are particularly robust, providing reassurance that they can be handled in a similar fashion to conventional strains. Specifically, kveik strains maintained viability during extended storage, and were tolerant to practices such as acid washing. It is anticipated that the data presented here will be of value to brewers of all sizes, and encourage a wider use of kveik yeasts throughout the brewing industry.

Introduction

Kveik strains belong to a unique subspecies of *Saccharomyces cerevisiae* which originate from isolated farms, towns, or valleys in Norway. These yeast cultures were traditionally passed down between generations within families or close farming communities for decades (perhaps centuries). The strains were also often used indiscriminately for both brewing and the baking of bread (Garshol, 2020), and in Norwegian, the word kveik is a catch-all phrase derived from the meaning 'to bring a spark to' or 'bring life to' (Geithung, 2019). When referring to the topic of brewing, 'kveik' is used as a direct term for 'yeast,' and the brewing community has adopted this word to describe this category of yeasts, even though it is a broad group, and individual strains are unique.

Until recently, kveik strains were only used within local Scandinavian communities and their application in commercial brewing was largely unexplored. Due to the nature of their origins and use, kveik cultures often comprise multiple strains (often as many as 4-5), although single-strain isolates have also been identified. Some cultures may also include bacteria (predominantly LAB species); this is somewhat unusual since most modern breweries exclusively aim to utilize pure yeast cultures. It should be noted that it is difficult to discern precisely where each yeast originated, as their lineage is often described only by word-of-mouth, with little to no documentation (Garshol, 2020). However, recent genetic studies have shown that kveik cultures originate from the 'Beer 1 clade' defined by Gallone et al. (2016) (Figure 1) and are closely related to German wheat beer (hefeweizen) yeasts (Preiss et al., 2018). This indicates that kveik cultures are likely to share a common ancestor with British, American, and some Belgian and German ale strains. During the same series of studies, sections of kveik DNA were identified to possess genetic material outside the Beer 1 clade, suggesting an alternative theory that kveik strains may have additional genetic links to a currently unidentified wild yeast strain (Figure 2). Interestingly, a common trait of wild yeasts, as well as hefeweizen and Belgian yeasts is the genetic capacity to produce phenolic compounds (referred to as **p**henolic **o**ff **f**lavour positive, or simply POF positive), notably the clove-like constituent 4-vinyl guaiacol. Despite some degree of genetic similarity, it appears that Kveik strains have lost this characteristic, possibly due to selection of POF negative isolates. Thus, kveik cultures are anecdotally referred to as 'clean' yeasts, much like the majority of yeast strains routinely employed in the brewing industry.

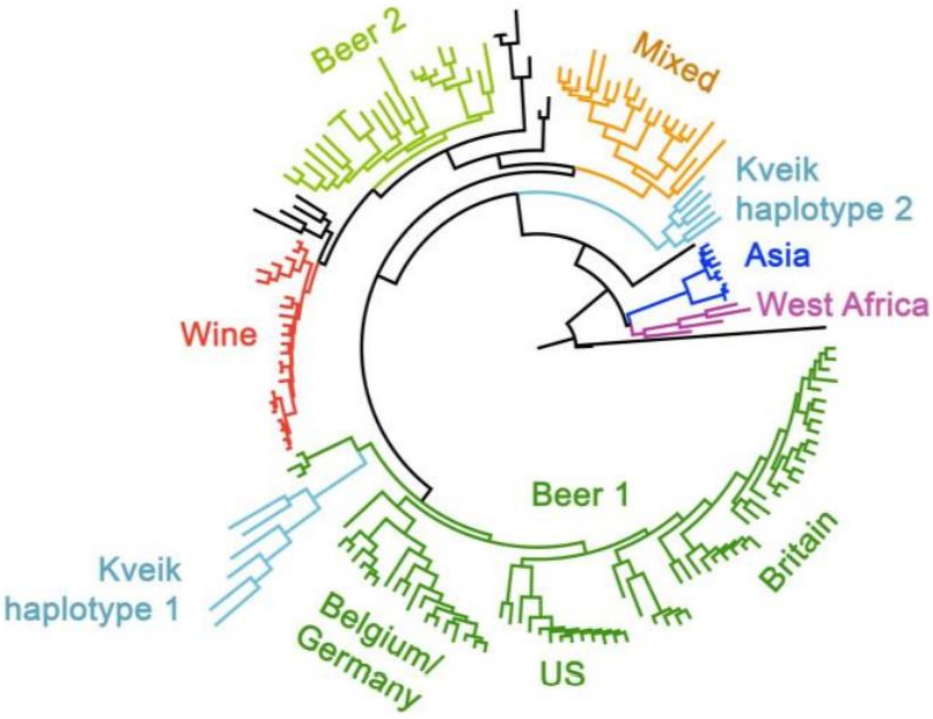


Figure 1. Simplified dendrogram indicating the relationship between kveik and other domesticated yeasts, obtained via whole genome sequencing (Garshol & Preiss, 2018; Preiss et al., 2018).

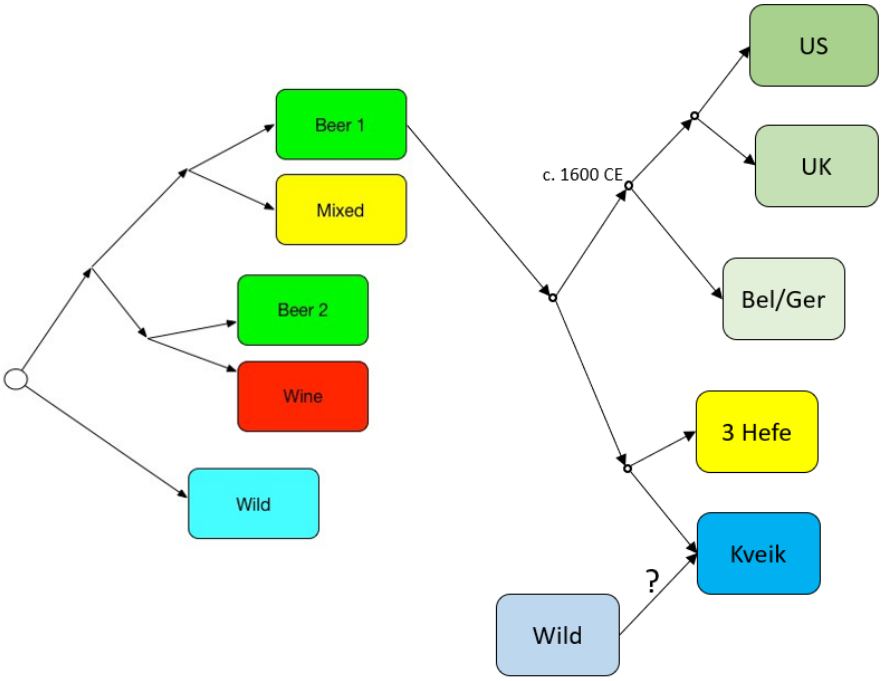


Figure 2. Suggested kveik lineage, adapted from Garshol (2017) and Preiss et al. (2018).

Kveik strains also display other properties associated with conventional brewing strains. They are efficient at fermenting malt-based sugars and can tolerate high levels of alcohol, and exhibit good flocculation properties (Preiss et al., 2018). Furthermore, these yeasts do not have diastatic power and are not super attenuators (Garshol & Preiss, 2018). Since these traits are not typical attributes of wild yeast, it can be surmised that these combined characteristics are indicative of domestication (Gallone et al., 2016).

Although many properties are shared with conventional brewing yeasts, an interesting hallmark of kveik strains is their ability to thrive at temperatures that exceed those typically applied in industry. Kveik strains are often used for fermentations above 30˚C and some have the capacity to thrive above 40˚C which is a strong indication of general thermotolerance (Garshol, 2021). When such temperatures are applied, rates of fermentation are exceedingly fast, enabling kveik strains to reach attenuation in 1-2 days (Garshol & Preiss, 2018). The reason for this deviation from 'standard' brewing properties is likely derived from the fact that, historically, brewers did not have efficient cooling equipment. The brewer would therefore need to either wait for the wort to cool from boiling to pitching temperature before retiring to bed (Garshol, 2021), or pitch the yeast 'warm' (Figure 3). Since a longer period of cooling could also increase the likelihood of wild yeast or bacterial infection (Hornsey, 2003), the latter was likely considered best practice and repeating this during extended serial repitching, could have led to development of thermotolerance over multiple generations. Interestingly, it is well established that standard brewing practices dictate that 'cold' environments are applied, at least from the perspective of the yeast cell. These conditions are employed to restrict the rate of yeast metabolism and to control flavour production. When conventional strains are used at high temperatures, the range of flavour compounds that are produced can be excessive, imbalanced and yield an unpalatable product. However, anecdotal evidence suggests that this is not always the case with kveik strains, and that they can be employed successfully at higher temperatures than normal to create beer quickly without the usual flavour issues described above.

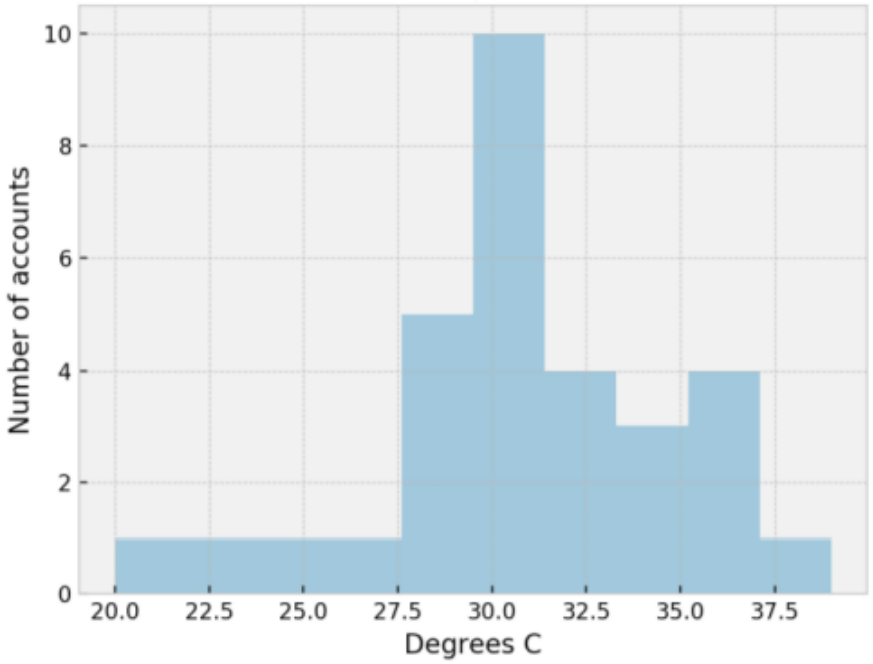


Figure 3. Wort temperature at pitching, as reported by traditional kveik brewers (Garshol, 2021).

The precise reasons why kveik yeast can be used successfully at high temperatures are currently unknown, however the capacity to survive under such conditions it is likely to be reflective of general stress tolerance. In a prior study (Isbell, 2022; Isbell & Powell, 2023), kveik yeast strains were investigated for their tolerance to industrially relevant stress factors. To achieve this, the capacity of strains to resist stress factors derived from ethanol, osmotic and oxidative challenges, as well as temperature, were compared to a selection of ale and lager yeasts currently used within the brewing industry. Kveik and conventional yeast strains were evaluated for biomass production under conditions incorporating oxidative stress (hydrogen peroxide), osmotic stress (sorbitol), alcohol stress (ethanol), and high temperature stress (heat). A 'heat map' illustrating a broad summary of this data can be found in Figure 4. All kveik and non-kveik brewing yeast isolates varied in their tolerance capacity when subjected to the various stressors used in the experiments. However, when compared to the conventional (non-kveik) strains, the kveik yeasts all exhibited greater capacity to tolerate each of the stress factors applied. It is worth noting that the kveik strains analysed involved multiple isolates derived from mixed cultures; in such instances individual isolates were distinct and exhibited varying levels of visual growth when exposed to the same concentration

of a given stressor. Collectively, it appeared that the kveik strains 3996, 3997, and 3998 (originating from a three-strain culture: 399x), were the most tolerant to the various stressors employed, while strains 4410, 4411, 4412, and 4413 (derived from a four-strain slurry: 441x), were the most sensitive. However, as mentioned above, despite this variation all of the kveik yeasts showed enhanced tolerance compared to the conventional (i.e. non-kveik) strains analysed.

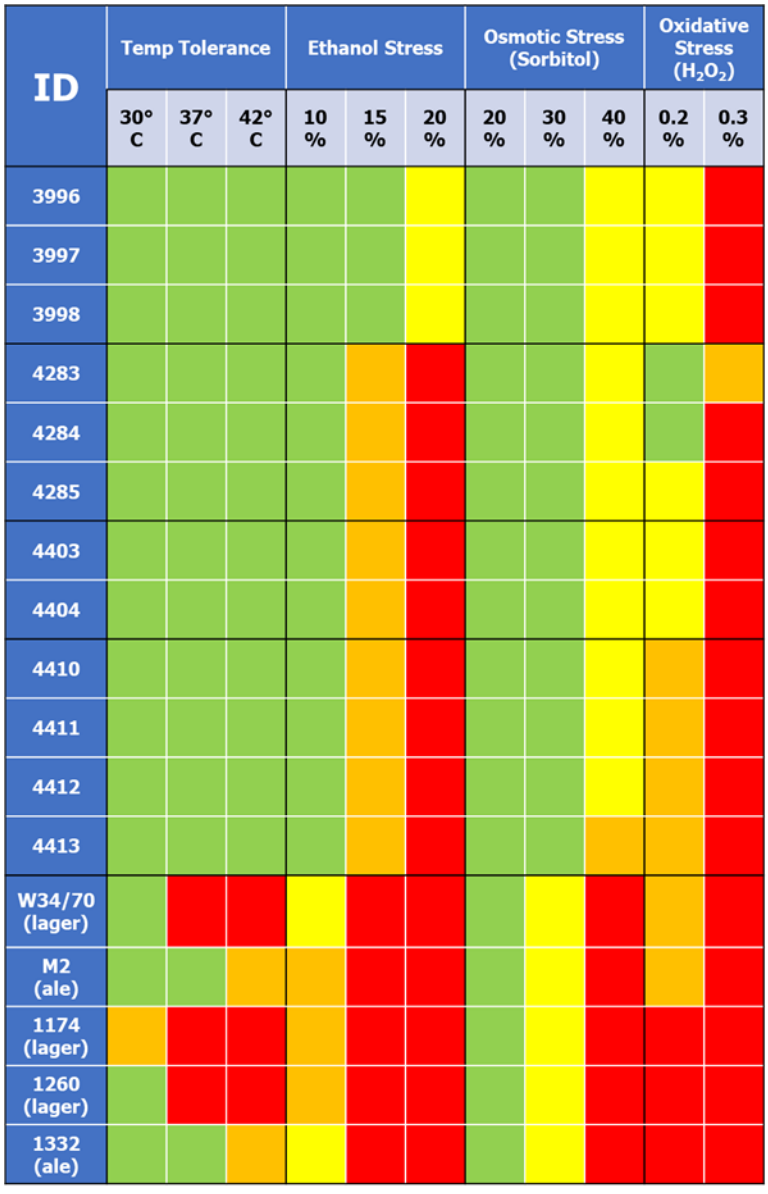


Figure 4. The capacity of kveik and conventional yeast strains to tolerate industrially relevant stress factors. Each 'cell' indicates tolerance where green reflects the capacity to survive and red indicates that strains could not withstand the respective environmental conditions.

A further intriguing aspect of kveik production is how the yeast culture was traditionally handled and stored. Complex rings of wood are traditionally used to store yeast, as shown in Figure 5a. These rings, called 'gjærkans,' are coated with the yeast after fermentation and hung up to dry. Once dried, they can be stored until the next brew, at which point they are lowered into the cooled wort to activate the yeast and allow fermentation to ensue. A similar but alternative design is a block of wood called a 'kveikstokk,' which has many holes drilled within the structure (Figure 5b). These two designs create a large surface area to which the yeast can adhere (Garshol, 2020). An alternative traditional practice for yeast harvesting was to skim the foam during active fermentation (high kräusen) or from the bottom of the fermenter after transferring the beer to the final container. This yeast would then spread out onto a sheet to dry. After the advent of refrigeration in the late 19th century, the flaked dry yeast would then be stored in a household freezer or refrigerator until the next use, which may be several months later (Garshol & Preiss, 2018).



Figure 5. Kveik 'ring' (left) belonging to Sigmund Gjernes from Voss, Norway (Garshol, 2020) and a 'yeast log' (right) displayed at Voss Folkemuseum in western Norway (Laitinen, 2020).

Irrespective of these traditional practices, it is evident that usage of kveik strains in modern-day fermentation systems requires a more structured and controlled process. This is especially true given that mainstream commercial beers should be consistent in nature and free of unwanted microbial contamination. Given that the yeast strain employed in fermentation plays a vital role in generating the final flavour and aroma profile of beer, its performance must also be reliable and consistent. Although the commercial availability of kveik yeasts has become more widespread in recent years, little is known about the physiology of kveik yeasts, how they can be handled, and their potential for use in brewing for mainstream beer production. This includes important yeast characteristics such as flocculation capacity, attenuation limits, rate of fermentation, and general robustness in an industrial setting. In particular, the ability of yeast to reliably withstand the multitude of stress factors experienced during yeast handling (i.e. storage) is unknown. Finally, key properties such as flavour generation in relation to brewing parameters remain anecdotal at best, and the possibility for creating 'standard' beers of desired specifications in a shorter period of time than normal remains largely unexplored.

Aims

Over the past 5 years there has been increasing interest in the use of alternative brewing yeast strains to create novel products. Much focus has centred on strains that create novel flavours, or related attributes that yield a product with unique characteristics. However, recently kveik yeasts have attracted particular attention, due specifically to their enhanced performance during fermentation (rather than their sensorial attributes *per se*). These yeasts originate from Norwegian farmhouse brewing and form a sub-category within the *S. cerevisiae* species. They are of particular interest to brewers because of their ability to ferment rapidly at higher temperatures (30-40°C), without producing an imbalance of desirable flavour compounds, or any significant off-flavours. Because of this, there has been interest in how to employ these yeasts to rapidly create beers in a shorter period of time than when using more traditional brewing strains. The potential for using kveik yeast commercially has grown to the extent that some smaller craft brewing companies now already exclusively use these strains for production of key brands. Despite this, little is known about kveik yeast in terms of their cellular physiology and/or why they continue to function well at high temperatures. Furthermore, from a practical brewing perspective, individual brewers typically employ strains under conditions initially optimised for traditional brewing strains, and there is a lack of knowledge with regard to the optimum conditions for their use, and how they should be best handled.

In this project we aim to perform a functional analysis of kveik yeast strains in order to provide learnings that can be directly transferred to industry and their use in commercial beer production. Furthermore, from a scientific perspective, we aim to benchmark these yeasts against traditional brewing strains to provide a greater understanding of yeast cell physiology and the yeast stress response. It should be noted that this research is relevant for brewers of all sizes, with the ambition being to enable the creation of true-to-type beers within a shorter time frame, reflecting a viable and contemporary strategy for maximising process efficiencies across the industry.

Materials and Methods

Yeast Strains

In this study, a number of kveik strains isolated from a range of sources and performance were evaluated and benchmarked against two widely utilised contemporary yeasts: W34/70 (Frohberg type lager) and Nottingham ale (Table 1). W34/70 and Nottingham ale were chosen since they are widely used on a global basis for the production of a range of ale and lager type produces respectively. Five kveik strains were investigated, including two commercially available strains known as Lutra and Voss (Table 1). The other three cultures were derived from stocks deposited with the NCYC (National Collection of Yeast Cultures, UK). All the strains were pure isolates, except for the strain referred to here as titled 399x. This yeast represents a blend of the isolates 3996, 3997, and 3998 and is equivalent to the original 3-strain mixed culture (originally deposited as NCYC 3995). However, it should be noted that in this instance 399x was prepared using equal ratios of each of the component strains to ensure reproducibility across independent experiments, which may otherwise have been adversely impacted by differences in population dynamics arising due to varying growth rates of individual isolates within the master culture.

Strain	Type	Source
Lutra	Kveik	Omega Yeast
Voss	Kveik	Lallemand
399X (Blend)	Kveik	NCYC
4283	Kveik	NCYC
4403	Kveik	NCYC
Nottingham Ale	Ale	Lallemand
W34/70	Lager (Frohberg type)	Fermentis

Table 1. Yeast strains used in this study.

Fermentation Protocol

Irrespective of the wort preparation procedure (see below) all worts were prepared using standard English 2-row base malt with a hop addition to create wort of approximately 20IBU's. The University of Nottingham Research Pilot Brewery was used to create standard gravity worts of 16°P and 12°P (through dilution). The high gravity worts were created using a Grainfather G30 brewing system at an initial 24°P which was then used to prepare the 20°P wort through dilution.

For initial analysis of yeast fermentation characteristics, small scale fermentations were carried out in triplicate using 150mL glass hypo-vials in accordance with the method of Quain et al. (1985). Each glass hypo-vial contained a magnetic stirrer and was autoclaved prior to use at 121°C, 15 psi for 15 min with a foam bung and aluminium foil sealing the aperture. Aliquots of the all-malt hopped worts (100 mL) described above were distributed into each vial (gravity adjusted using sterile water where required), pitched with yeast (adjusted for 1 million cells/mL per degree Plato) and sealed using rubber septa and metal crimps. To allow for the liberation of carbon dioxide produced during fermentation, a gas outlet port or Bunsen valve (constructed from a Durham tube and silicon tubing) was inserted into the septa via a sterile needle (Figure 6). Fermentations were stirred at 200 rpm on a flatbed magnetic stirring plate in a static incubator at a predetermined temperature as specified. Typically, 12°C was selected as a representative temperature for traditional lager beers, 18°C as a temperature reflective of the production of some contemporary lagers and traditional ales; elevated temperatures of 24°C (typical for some kveik fermentations) and ultra-high conditions at 36°C were also applied for comparative purposes. Fermentation progression was monitored by tracking CO_2 production over time by measuring weight loss twice per day. Fermentations were deemed to have reached attenuation when two consecutive readings were identical.

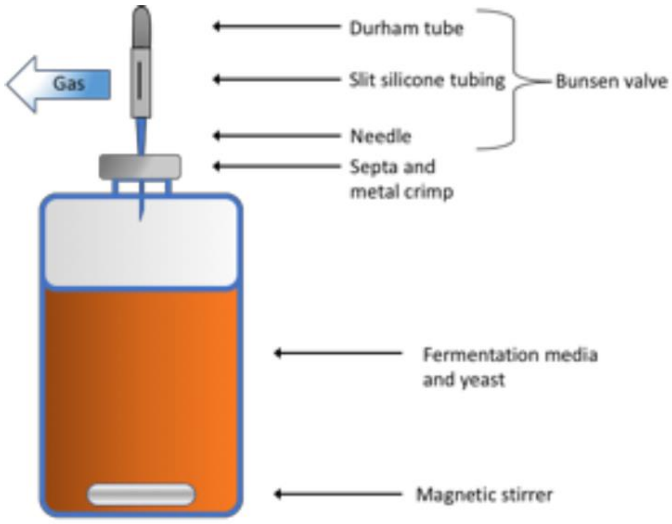


Figure 6. Representative fermentation vessel set-up.

For the experiments related to yeast health, specifically the impact of storage and acid washing on viability, larger fermenters were used. In these instances, a standard 15°P wort was used for the initial fermentations, conducted in stirred 2L Duran vessels with a 1L working volume. These were used primarily to obtain an increased volume of yeast biomass for subsequent analysis.

Flavour Production and Analysis

In this study, all beer samples were analysed for flavour production at attenuation. Flavours were quantified using headspace gas chromatography and concentrations of key esters and higher alcohols. A trace 1300 series Gas Chromatograph coupled with a Single-Quadrupole Mass Spectrometer (Thermo Fisher Scientific, UK) was used for analysis of volatile aroma compounds. Samples were incubated under constant shaking at 50°C for 5 min. A 50/30 µm DVB/CAR/PDMS SPME Fibre (Supelco, Sigma Aldrich, UK) was used to extract volatile aroma compounds from the sample headspace (extraction for 5 min then desorption for 15 min). The inlet temperature was set at 200°C and a split mode was used due to the connection of GC-Olfactory, and the constant carrier pressure was at 124.11 kPa. Separation was carried out using a ZB-WAX Capillary GC Column (length 30 m, inner diameter 0.25 mm, and film thickness

1 µm; Phenomenex Inc., UK). Column temperature was held initially at 40°C for 2 min, increased by 6°C/min to 240°C and held for 5 min. 'Full scan' mode was used to detect the volatile compounds (mass range from 20 to 300 AMU). Volatiles were identified by comparison of each mass spectrum against either the spectra from authentic compounds or spectra derived from reference libraries (NIST/EPA/NIH Mass Spectral Library, version 2.0, Faircom Corporation, USA). The relative abundance of each volatile compound present in the headspace was determined from the GC peak area found in the beer sample (average of 3 replicates) and quantified based on relative concentration against the internal standard, 3-heptanone.

Based on the raw data, TraceFinder 5.1 software (Thermo Fisher Scientific, UK) was utilised to obtain concentrations of specific volatile constituents. The 11 compounds present in the highest quantity with matching scores from the MS database were used in the comparative study. For comparison and presentation, results were logarithmically normalised.

Yeast Viability Analysis

To determine the proportion of live and dead cells within a population, the methylene blue staining method traditionally employed in brewing laboratories was conducted according to the method of Pierce (1970). Methylene blue (Sigma, UK) was dissolved in 2 % (w/v) sodium citrate (Fisher Scientific, UK) solution to a final concentration of 0.01 % (w/v). Yeast cell suspensions were diluted with sterile RO water to reach a working solution of approximately 1.0×10^7 cells/mL . Aliquots of 0.5 mL yeast suspension were mixed with 0.5 mL methylene blue solution and gently agitated before incubation for 5 min at room temperature. An Aber CountStar (Aber Instruments Ltd, Aberystwyth) was used to determine the total number of yeast cells and viability at 10- and 100-fold dilutions. Viability is expressed as the percentage of live cells within the original population.

Yeast Storage Analysis Protocol

To emulate the process of yeast handling, each yeast strain was initially used to ferment 15°P wort (2L Duran-style vessels; 1L working volume, stirred, in triplicate) until completion. Once attenuation had been reached, yeast slurries were recovered by decanting beer from the

vessel and transferring the yeast with a portion of beer to create a slurry of approximately $1x10^9$ cells/mL into a sterile container in a cold room (2- 4°C) statically (i.e. without agitation or mixing). For analysis of viability, yeast was roused briefly using a magnetic stirrer until yeast was fully in suspension (typically 1-2 min). Viability was measured using an ABER CountStar in conjunction with methylene blue staining each day for a period of 8 days.

Yeast Acid Washing Protocol

To emulate the process of brewery yeast handling and acid washing, each yeast strains was initially used to ferment a 15°P wort (2 Litre Duran-style vessel, in triplicate). Once attenuation had been achieved, yeast slurries were recovered by decanting beer from the vessel and transferring the yeast into a fresh container with a proportion of beer to create approximately $1x10^9$ cells/mL. At this stage, viability was determined using methylene blue staining in conjunction with an ABER CountStar to confirm that they were typical and representative of yeast post-fermentation. Slurries were then chilled to 2-4°C for one day to replicate a brief period of yeast storage, before acidulation with phosphoric acid (85%) to a pH of 2.0-2.2 for 2h. During this time period, slurries were kept in a cold state (2-4°C) with continuous but gentle agitation using a magnetic stirrer. The viability of each culture was measured at the point of acid addition and again after two hours of acid washing.

Flocculation Assay (Helms Test)

Flocculation was determined using a modified Helm's assay according to the method of Bendiak (1995) with adjustments as suggested by D'Hautcourt and Smart (1999). Flocculation was estimated by expressing the optical density of experimental assays as a percentage of deflocculated control samples (Section 2.8.1.3), where 100% denotes complete flocculation and 0% indicates the absence of flocculation.

Yeast strains were cultivated in YPD aerobically at 25°C by shaking at 120 rpm for 72 hours. To prepare control samples, cells were recovered by centrifugation (15,000rpm 1 min), washed in sterile deionised water and resuspended to achieve a final cell concentration of $1X10^8$ cells/mL. Aliquots of yeast (10mL) were transferred to Falcon tubes (15 replicates). For experimental samples, the same procedure was followed for cell cultivation. However, once

cells had been grown, they were washed once in 'washing buffer' consisting of calcium sulphate (0.51g/litre). Samples were then recovered by centrifugation (15000 rpm for 1 min) and resuspended in suspension buffer (calcium sulphate (0.51g/litre), sodium acetate (6.8g/litre), glacial acetic acid (4.05g/litre) and ethanol (4%)) at a final cell concentration of $1X10^8$ cells/mL.

After preparation of control and experimental samples, cell suspensions were agitated thoroughly for 30 seconds and subsequently inverted 5 times in 15 seconds for consistency. All tubes were then maintained statically at room temperature for 15 min to allow for flocculation to occur. Subsequently, the top 1mL of each sample was removed and added to 9mL deionised water. Absorbance was then measured at 600nm and the data obtained from the experimental samples was expressed as a percentage of the control sample:

$$\% \text{ Flocculation} = \frac{\text{Control Absorbance} - \text{Experimental Absorbance}}{\text{Control Absorbance}} \times 100$$

Results

Work Package 1 – How to achieve commercially acceptable products through the use of kveik yeasts

Novel kveik yeast strains were evaluated to identify the key physiological traits that allow for intensive performance at high temperatures. This information was used to identify differences between kveik yeast (5 isolates) and current production strains (2 isolates: W34/70 lager and Nottingham ale), for benchmarking purposes. A multi-faceted approach was then taken to identify the parameters within which kveik yeasts can successfully produce commercially viable beer, focusing primarily on the ability to ferment wort of a range of gravities and at a range of temperatures. The key assessment criteria are indicated below:

i. **How do kveik strains perform against conventional strains at low (12°P), high (16°P), very high (18°P) and ultra-high (22°P) gravity?**
ii. **What are the effects of low/high fermentation temperature on flavour production in kveik yeast?**
iii. **What temperatures can be applied to rapidly create acceptable ale-type beers?**
iv. **Is it possible to produce a lager-type product at elevated temperatures using kveik yeast?**

WP1 i-ii. Fermentation performance of kveik strains at elevated gravity and temperatures

When comparing fermentation efficiency, the **A**pparent **D**egree of **F**ermentation (ADF), which reflects the capacity to utilise wort sugars, provides useful information. Essentially, this describes the degree to which available sugars (extract) are consumed by yeast during fermentation and can be expressed as a percentage of the total available (Kunze, 2019). In this study, the five kveik strains (399X, 4283, 4403, Lutra, and Voss) and two conventional brewing yeasts (W34/70 lager and Nottingham ale), were fermented in triplicate using all-malt wort at gravities brewed to 12°P, 16°P, 20°P, and 24°P. Fermentations were also conducted at temperatures of 12°C, 18°C, 24°C, and 36°C, and the performance of each yeast was evaluated.

From Figure 7 it can be seen that at 12°P, all yeasts exhibited an increase in sugar utilisation based on attenuation (ADF) with an increase in temperature. The exception to this was the kveik Lutra strain, where ADF was observed to decrease at the highest temperature applied (36°C). It should be emphasised that this may be somewhat reflective of the fact that the Lutra strain was the best performing yeast at lower temperatures (Figure 7). Interestingly, the kveik strains 4283 and 4403 had the lowest attenuation capacity (ADF) in the study. The reasons for this are unknown and were somewhat unexpected, but it should be noted that these two yeasts exhibited amongst the highest flocculation (top 50%) relative to the other yeast strains (based on observation during fermentation; WP2), which may have been a contributing factor in this case. At the lowest temperature (12°C), the kveik strain Lutra had the highest level of attenuation, and at the highest temperature (36°C), Nottingham ale performed the best.

When the starting wort gravity was increased to 16°P, the yeast strains displayed a similar pattern of results with a trend towards increasing ADF as temperature increased. The kveik Lutra strain continued to be an exception to this trend, along with the lager yeast W34/70 in this instance. Both of these strains showed a lower ADF at 36°C, perhaps not unexpected for the lager strain (W34/70) since it is well recognised that 36°C is close to the limit at which lager strains can survive. As observed previously at 12°P, the more flocculant kveik strains 4283 and 4403 showed the lowest levels of attenuation at all temperatures, while the Nottingham ale strain continued to show the highest ADF at 36°C (Figure 8).

At an initial wort gravity of 20°P only three temperatures were applied: 12°C, 18°C, and 24°C. In this instance 36°C was omitted since these combined conditions resulted in poor fermentations for the commercial yeasts employed. At 20°P all of the yeast cultures continued to show a similar trend to previously, with increased attenuation (ADF) with temperature (Figure 9). At this starting gravity, the Lutra strain that was previously negatively affected by high temperatures continued to perform well, in contrast to previous data. However, the kveik strains 4283 and 4403 continued to exhibit the lowest attenuation rate as indicated by ADF (Figure 9). Interestingly, all of the strains were impacted when 24°P worts were used in conjunction with cold temperatures. In this instance, all strains except for the cold-tolerant lager (W34/70) showed low ADF values (Figure 10). However, as temperature increased, ADF was observed to be similar to those observed for the other experimental parameters; all yeasts showed an increase in ADF with temperature except for the kveik strain Lutra. It should be noted that Lutra performed the best at this starting gravity irrespective of the temperature employed (Figure 10).

A summary of the data can be found in Table 2, indicating that the kveik strain Lutra exhibited the highest ADF in 11 of the 14 parameters tested, indicating that it is particularly efficient and robust under a variety of conditions. Conversely, the lowest ADF% was shown by the two kveik strains 4283 and 4403. As noted above, these two strains were visually observed to flocculate efficiently and form a heavy sediment, even though vessels were stirred and not static. This may have contributed to a lower ADF, although it is equally possible that these two strains simply were less efficient at utilising complex sugars including maltose and maltotriose when compared to the other strains. Further analysis would be required to substantiate this hypothesis. Interestingly, analysis of ethanol production yielded data that did not exactly match the ADF results. Principally it can be seen that, although Lutra displayed the best capacity to utilise wort sugars (Table 2), the Nottingham ale yeast was more efficient at converting these into ethanol (Table 3). The precise reasons for this are unknown, especially since pitch rate and oxygen provision were consistent across strains/experiments. It is possible that the Lutra kveik strain was more efficient at producing biomass under these conditions or may simply be reflective of the good fermentation performance of Nottingham ale. Irrespective, it is widely accepted that individual yeast strains display their own unique

properties, and the data in general indicates that kveik yeast are broadly similar to the conventional yeasts examined in this study.

Additional analysis of fermentation performance indicated that both kveik strains and conventional yeasts performed in a similar fashion with respect to fermentation speed. The conventional yeasts Nottingham and W34/70 appeared to ferment more rapidly at all gravities at 12°C, 18°C and 24°C. However, at 36°C the kveik strains showed a significantly higher fermentation velocity, as expected. In the interest of brevity, this summary has not been detailed further here, but the data can be found in Appendix 1-4 for interest.

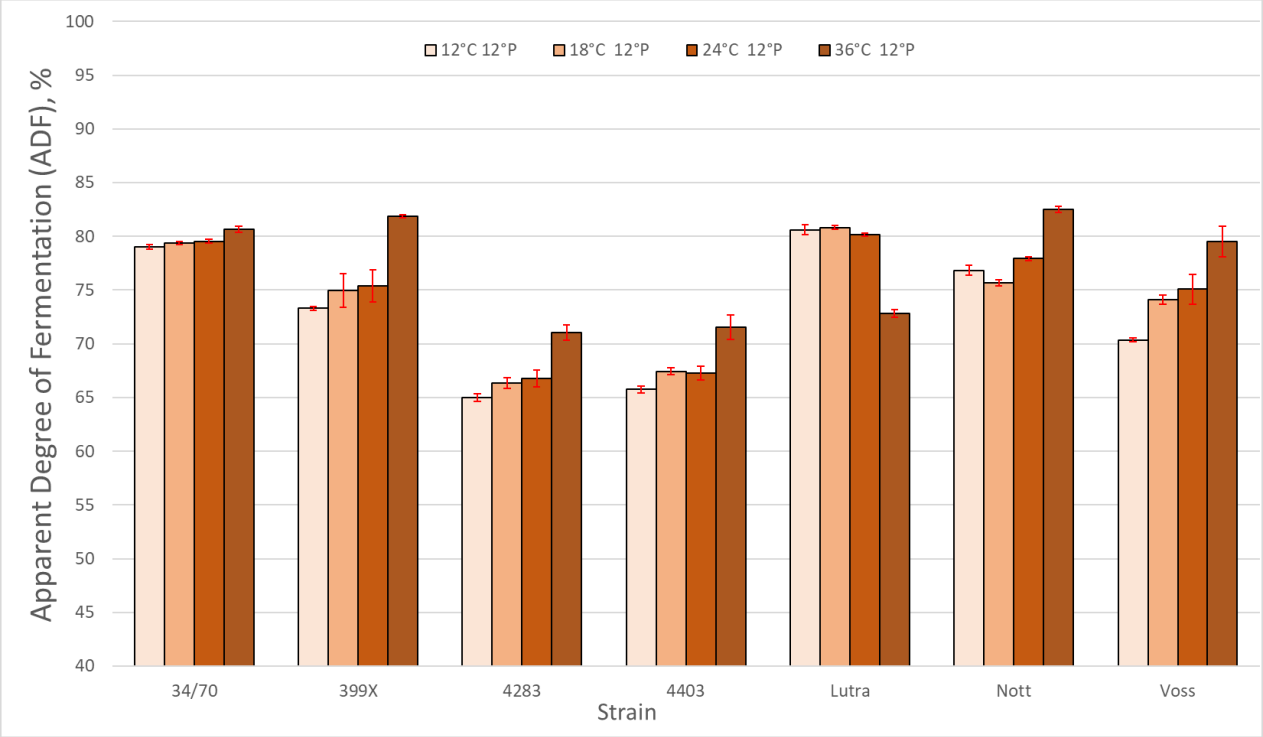


Figure 7. The capacity of yeast strains to assimilate wort sugars (ADF) from a base at 12° P wort at different temperatures (12-36°C).

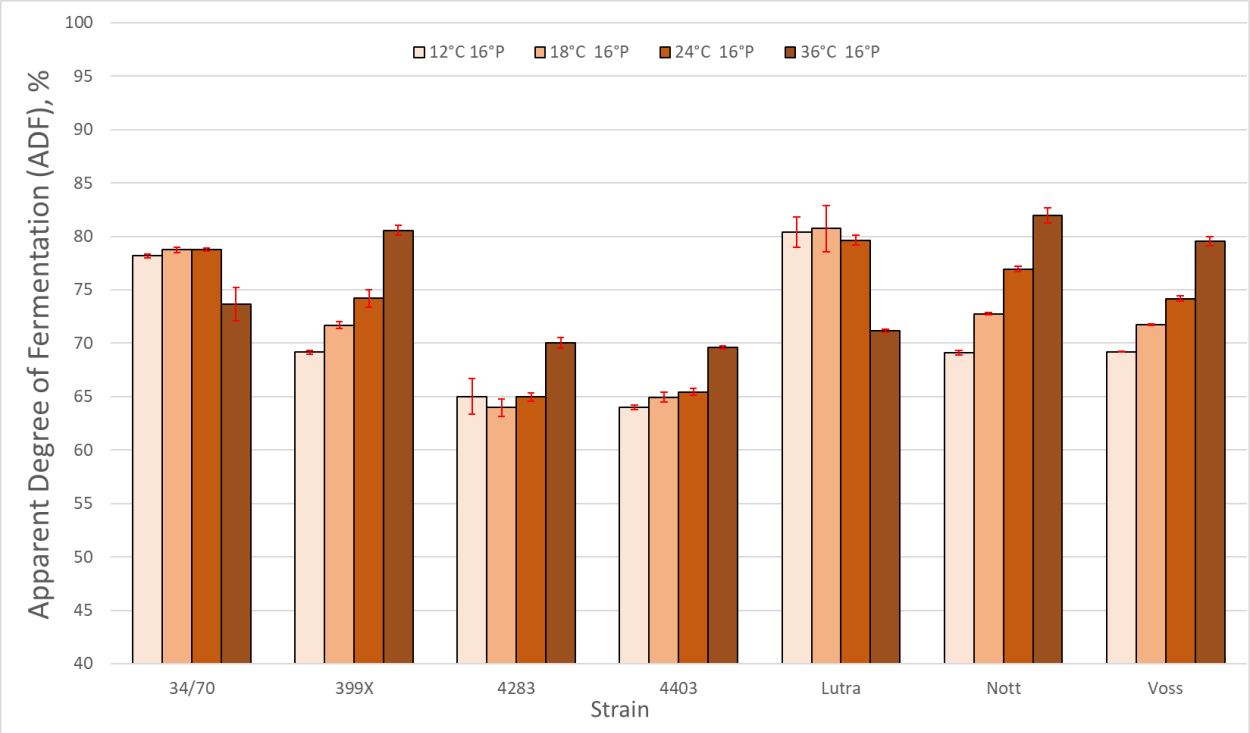


Figure 8. The capacity of yeast strains to assimilate wort sugars (ADF) from a base at 16° P wort at different temperatures (12-36°C).

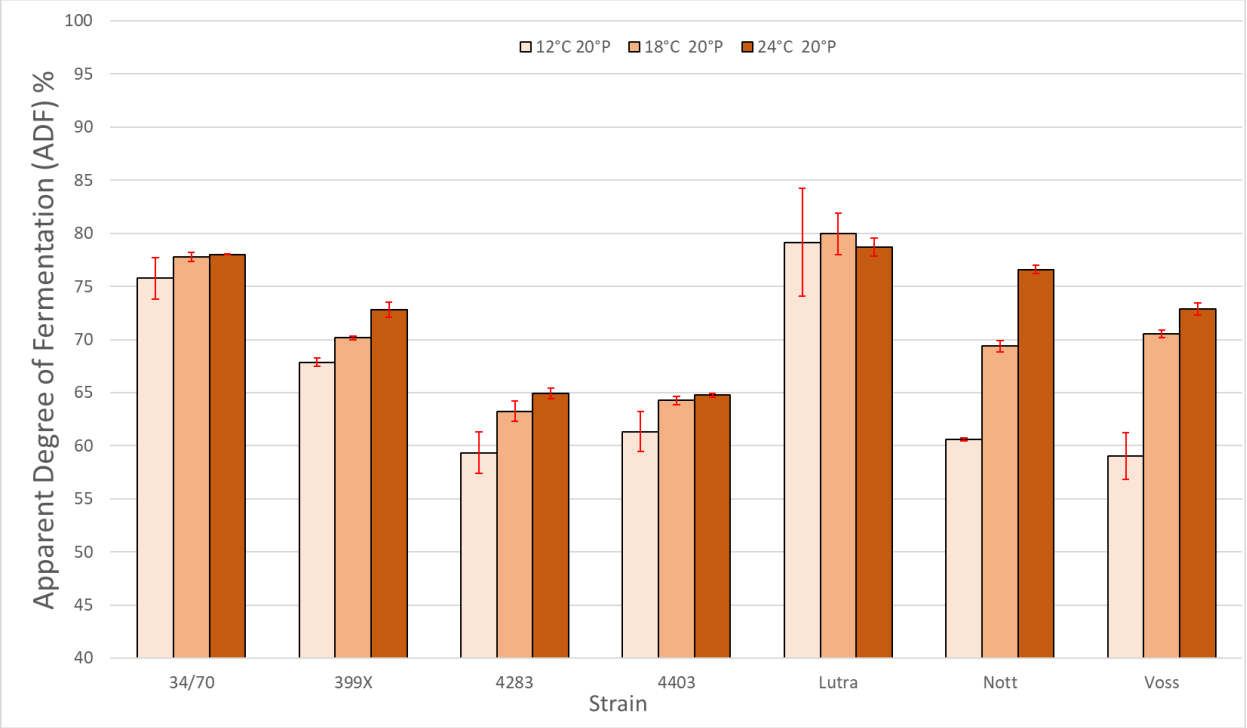


Figure 9. The capacity of yeast strains to assimilate wort sugars (ADF) from a base at 20° P wort at different temperatures (12-24°C).

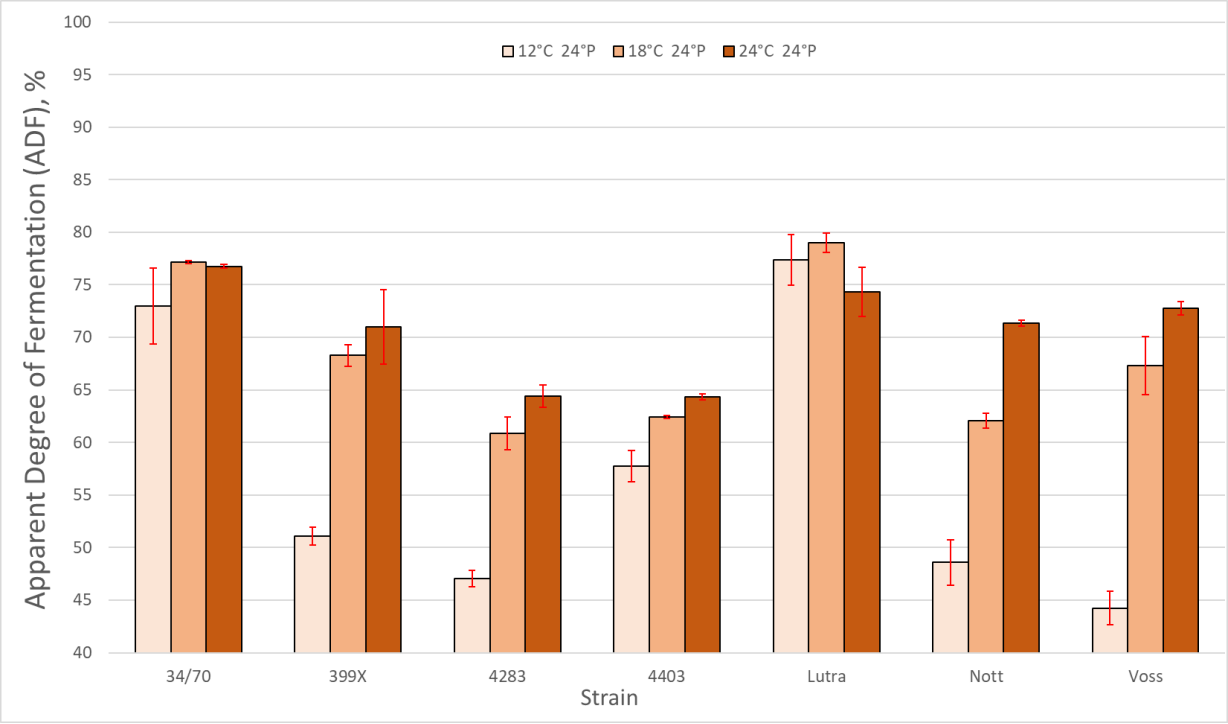


Figure 10. The capacity of yeast strains to assimilate wort sugars (ADF) from a base at 24° P wort at different temperatures (12-24°C).

Apparent Degree of Fermentation (ADF), % Highest ADF shown in Red and lowest ADF in Blue							
Condition	**34/70**	**399X**	**4283**	**4403**	**Lutra**	**Nott**	**Voss**
12°C 12°P	79.02	73.29	**65.00[2]**	65.74	**80.61[1]**	76.82	70.35
12°C 16°P	78.19	69.15	65.01	**63.97[2]**	**80.38[1]**	69.11	69.21
12°C 20°P	75.75	67.84	59.35	61.34	**79.14[1]**	60.58	**59.05[2]**
12°C 24°P	73.00	51.11	47.04	57.74	**77.35[1]**	48.59	**44.23[2]**
18°C 12°P	79.40	74.93	**66.34[2]**	67.45	**80.82[1]**	75.65	74.11
18°C 16°P	78.74	71.70	**63.97[2]**	64.93	**80.72[1]**	72.76	71.71
18°C 20°P	77.78	70.15	**63.23[2]**	64.27	**79.95[1]**	69.37	70.55
18°C 24°P	77.16	68.28	**60.86[2]**	62.42	**79.01[1]**	62.06	67.32
24°C 12°P	79.52	75.41	**66.75[2]**	67.28	**80.17[1]**	77.93	75.10
24°C 16°P	78.77	74.20	**64.96[2]**	65.44	**79.65[1]**	76.94	74.18
24°C 20°P	78.01	72.80	64.94	**64.77[2]**	**78.68[1]**	76.58	72.87
24°C 24°P	**76.76[1]**	71.00	64.41	**64.35[2]**	74.33	71.37	72.79
36°C 12°P	80.64	81.87	**71.04[2]**	71.53	72.85	**82.50[1]**	79.50
36°C 16°P	73.63	80.56	**70.04[2]**	69.59	71.18	**81.96[1]**	79.55

Table 2. Summary of ADF data presented in Figures 7-10. Highest ADF values are indicated in **red[1]**, while lowest ADF values are shown in **blue[2]**.

Ethanol Production % Highest Ethanol shown in Red and lowest ADF in Blue							
Condition	**W34/70**	**399X**	**4283**	**4403**	**Lutra**	**Nott**	**Voss**
12°C 12°P	**5.47[1]**	4.97	**4.57[2]**	4.61	5.14	5.43	4.89
12°C 16°P	7.32	6.48	**4.93[2]**	6.06	6.56	**7.48[1]**	6.59
12°C 20°P	9.42	7.87	7.17	7.72	**7.12[2]**	**9.75[1]**	7.16
12°C 24°P	10.19	7.36	6.97	8.07	7.03	**10.66[1]**	**6.57[2]**
18°C 12°P	**5.50[1]**	5.05	**4.53[2]**	4.55	5.13	**5.50[1]**	5.05
18°C 16°P	7.38	6.66	**5.96[2]**	6.04	6.76	**7.47[1]**	6.65
18°C 20°P	9.17	8.37	**7.47[2]**	7.60	8.31	**9.33[1]**	8.33
18°C 24°P	11.08	9.95	**8.74[2]**	9.02	9.11	**11.34[1]**	9.48
24°C 12°P	5.42	5.07	4.64	**4.60[2]**	5.32	**5.47[1]**	5.15
24°C 16°P	7.34	6.88	**6.13[2]**	**6.13[2]**	7.20	**7.39[1]**	6.92
24°C 20°P	**9.27[1]**	8.47	7.71	**7.69[2]**	8.89	9.19	8.66
24°C 24°P	**11.16[1]**	10.11	9.41	**9.31[2]**	10.07	10.63	10.53
36°C 12°P	**5.42[1]**	5.28	**4.60[2]**	4.77	5.30	4.74	5.31
36°C 16°P	6.67	7.10	**6.20[2]**	6.26	**7.26**	6.31	7.15

Table 3. Summary of ethanol production. Highest ethanol values are indicated in **red[1]**, while lowest ADF values are shown in **blue[2]**.

WP1 ii. Flavour production by kveik yeast strains

The yeast strain employed for fermentation is broadly responsible for the range of flavour compounds that are present in the final beer. Although raw materials clearly define the product type, different yeast strains have individual nutritional requirements and thus create a specific flavour profile when used to ferment a given wort. This is directly influenced by the action of yeast based on its nutritional preferences, the environmental conditions applied and the extent of yeast growth. Key factors include pitching rate, oxygenation level and, importantly, temperature. Specifically, temperature has a major impact on yeast metabolic activity and the speed of fermentation. Typically, within the brewing industry, 'cold' temperatures are applied to restrict yeast activity and to prevent an imbalance and/or excessive production of esters and higher alcohols. However, it is well reported that kveik yeast can be fermented 'hot' while maintaining relatively neutral flavour profiles. Furthermore, anecdotal evidence has indicated that kveik strains can be used at high gravity to good effect. However, precise studies on flavour production using kveik strains have not previously been performed. In this study, fermentations were conducted using range of wort gravities (as above) and flavour production was determined using GC-Mass Spectrometry in order to quantify the following flavour compounds: acetaldehyde, ethyl acetate, isobutyl acetate, 1-Propanol, ethyl butyrate, isobutanol, isoamyl acetate, isoamyl alcohol, ethyl hexanoate, and ethyl octanoate. This data was used directly to create spider diagrams for each strain under each set of conditions (Figures 11-17). It should be noted that full data is included in the Appendices for completeness.

It can be seen that each yeast created its own unique combination of flavour compounds, as expected. However, the flavour distribution was observed to vary depending on temperature irrespective of strain type; this was more noticeable for fermentations conducted using W34/70 lager (Figure 12) and Nottingham ale (Figure 16) than for the kveik strains (Figures 11; 13-15 and 17). Initial starting wort gravity also affected flavour generation, with more intensive worts yielding higher concentrations of all flavour compounds. This was not unexpected, but it is interesting to note that 399x, 4283, 4403 and Lutra produced flavour profiles at high gravity that were more similar to those at lower gravities. It should be noted that these results reflect the raw data obtained post-fermentation and beer was not cut to

the same gravity/ethanol content prior to analysis. However, the conclusions drawn here still remain valid in terms of flavour intensity in relation to wort gravity.

The data was also analysed to provide a broad indication of similarity/variation between flavour profiles across multiple criteria using Principle Component Analysis (CPA). For these analyses it should be noted that the data incorporates both starting wort gravity and fermentation temperature. In each instance, fermentation temperature is indicated by the number that succeeds the strain identifier. For example in Figure 18, Nott_36dC refers to the flavour data obtained when the ale yeast strain Notingham was used to ferment the respective wort (in this instance 12°P) at 36°C. Similarly, 'Lutra_24dC' refers to the flavour data obtained when the kveik strain Lutra was used to ferment wort at 24°C.

Based on the principal component analysis data volatile flavour compound production was compared separately for each of four different wort gravities. At the lowest gravity tested (12°P), it was shown that all of the kveik strains formed a cluster characterised by relatively high concentrations of acetaldehyde, but low levels of esters and higher alcohols relative to the conventional brewing strains (Nottingham ale and W34/70 lager) (Figure 18). This could be attributed to the fact that 12°C is a much cooler fermentation temperature than 'normal' for kveik strains and may have inhibited the effective conversion of acetaldehyde into ethanol. At 18°C and 24°C, all of the yeasts exhibited higher concentrations of flavour compounds (indicated by a shift to the right and the proximity of key esters and higher alcohols). Of particular note were the kveik strains 399X, Voss, and Lutra, which showed the highest levels of ethyl acetate, isoamyl acetate, isoamyl alcohol. The Nottingham ale strain showed high levels of isobutanol, isobutyl acetate, and 1-propanol relative to all the other yeasts (Figure 18). Interestingly, at 36°C, all yeasts were clustered together with an overall decrease in flavour expression, as indicated by a horizontal shift to the left.

When gravity was increased, similar patterns were observed with variation depending on temperature. Typically, the kveik strains clustered more closely with the lager yeast W34/70 than the Nottingham ale strain (Figures 19-21). This deviation became more pronounced at the highest gravities tested (20°P and 24°P; Figures 20 and 21 respectively), where Nottingham became a clear outlier, with intensive flavour production that was not seen in the kveik yeast or the lager strain investigated.

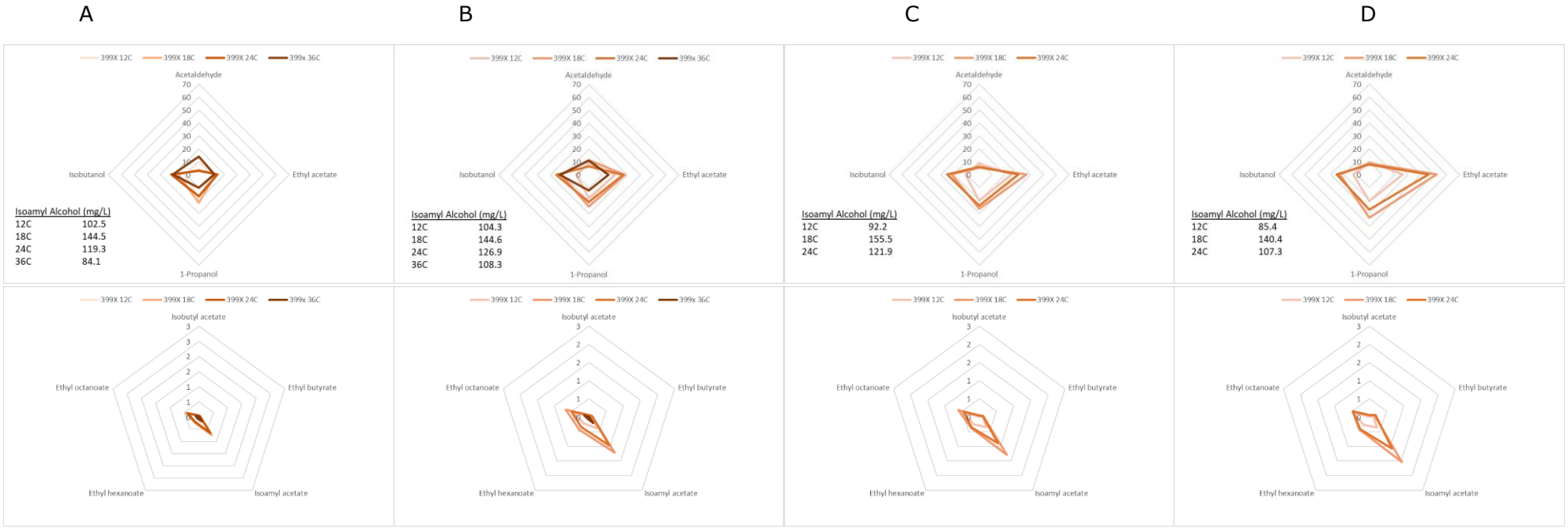


Figure 11. Production of flavour compounds by yeast strain 399X according to fermentation temperature and initial wort gravity. Gravity was adjusted to 12°P (A), 16°P (B), 20°P (C) and 24°P (D). Variation in flavour is shown on each figure where temperature is represented by individual lines as indicated. In each instance, data is expressed in mg/L (ppm) and values represents the mean of triplicate samples. Isoamyl alcohol data is shown numerically since concentrations were significantly higher than the other flavour compounds; scaling the figures accordingly did not allow for clear data presentation.

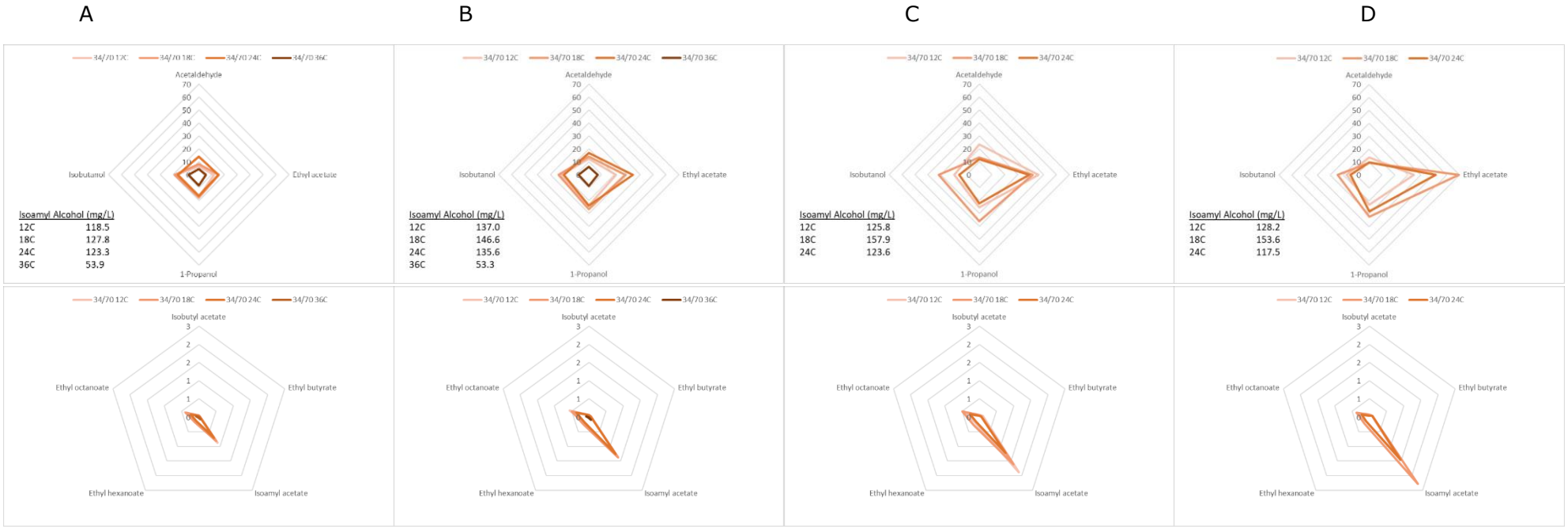


Figure 12. Production of flavour compounds by yeast strain W34/70 according to fermentation temperature and initial wort gravity. Gravity was adjusted to 12°P (A), 16°P (B), 20°P (C) and 24°P (D). Variation in flavour is shown on each figure where temperature is represented by individual lines as indicated. In each instance, data is expressed in mg/L (ppm) and values represents the mean of triplicate samples. Isoamyl alcohol data is shown numerically since concentrations were significantly higher than the other flavour compounds; scaling the figures accordingly did not allow for clear data presentation.

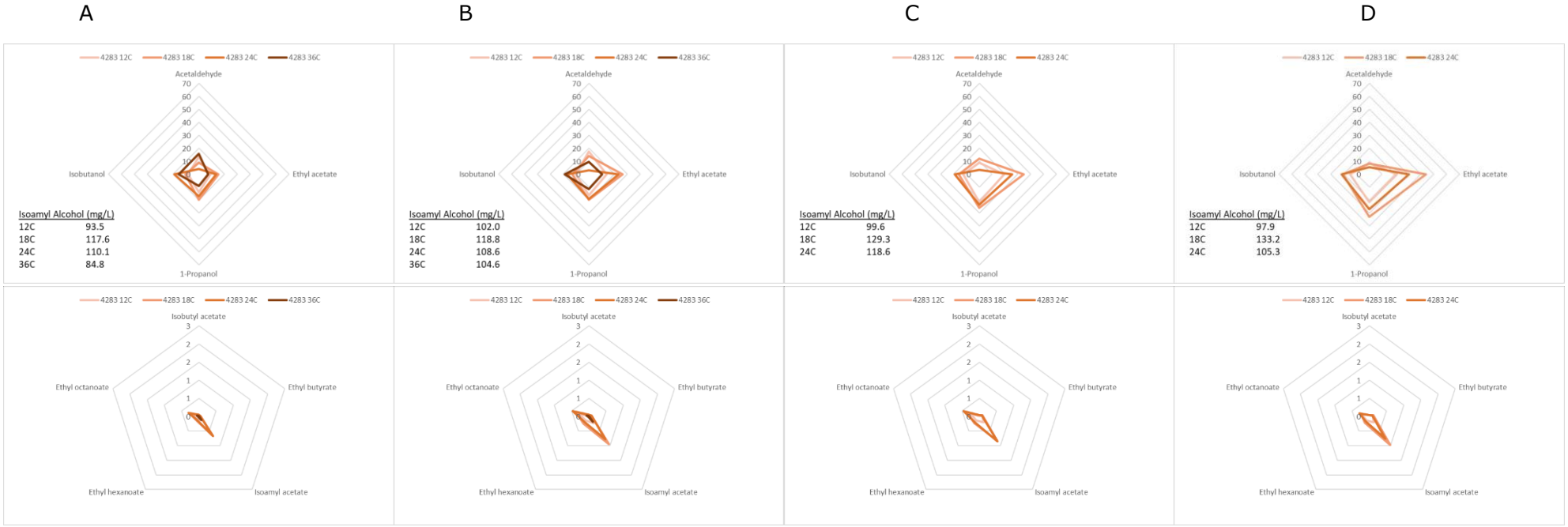


Figure 13. Production of flavour compounds by yeast strain 4283 according to fermentation temperature and initial wort gravity. Gravity was adjusted to 12°P (A), 16°P (B), 20°P (C) and 24°P (D). Variation in flavour is shown on each figure where temperature is represented by individual lines as indicated. In each instance, data is expressed in mg/L (ppm) and values represents the mean of triplicate samples. Isoamyl alcohol data is shown numerically since concentrations were significantly higher than the other flavour compounds; scaling the figures accordingly did not allow for clear data presentation.

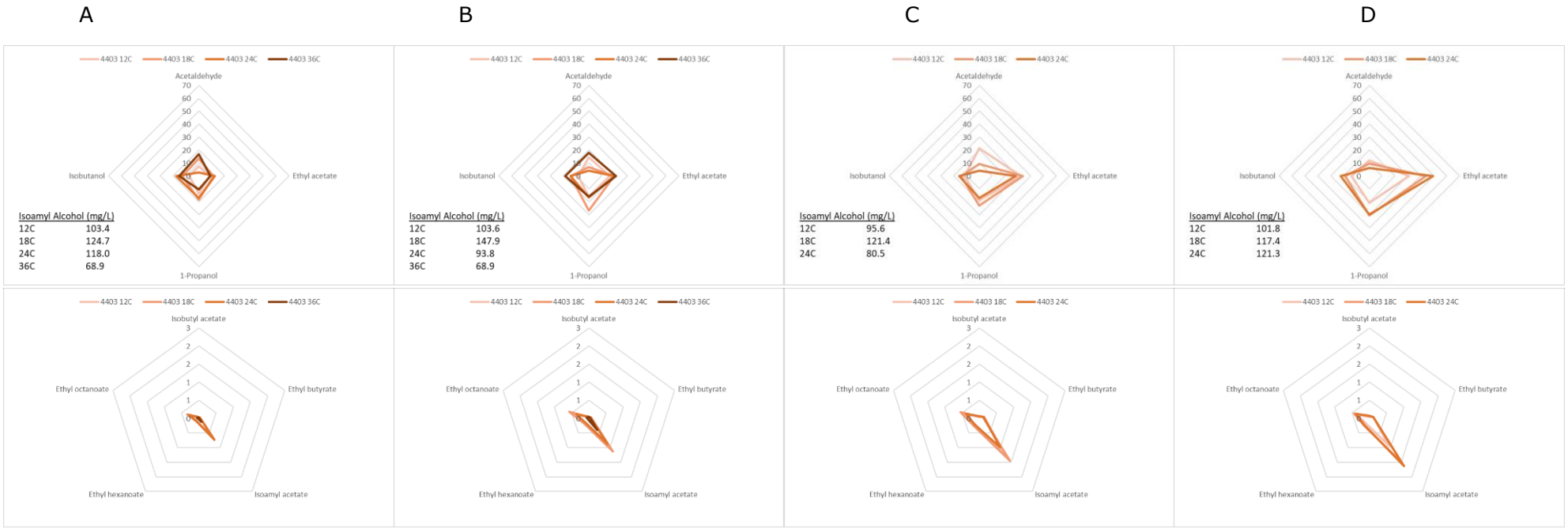


Figure 14. Production of flavour compounds by yeast strain 4403 according to fermentation temperature and initial wort gravity. Gravity was adjusted to 12°P (A), 16°P (B), 20°P (C) and 24°P (D). Variation in flavour is shown on each figure where temperature is represented by individual lines as indicated. In each instance, data is expressed in mg/L (ppm) and values represents the mean of triplicate samples. Isoamyl alcohol data is shown numerically since concentrations were significantly higher than the other flavour compounds; scaling the figures accordingly did not allow for clear data presentation.

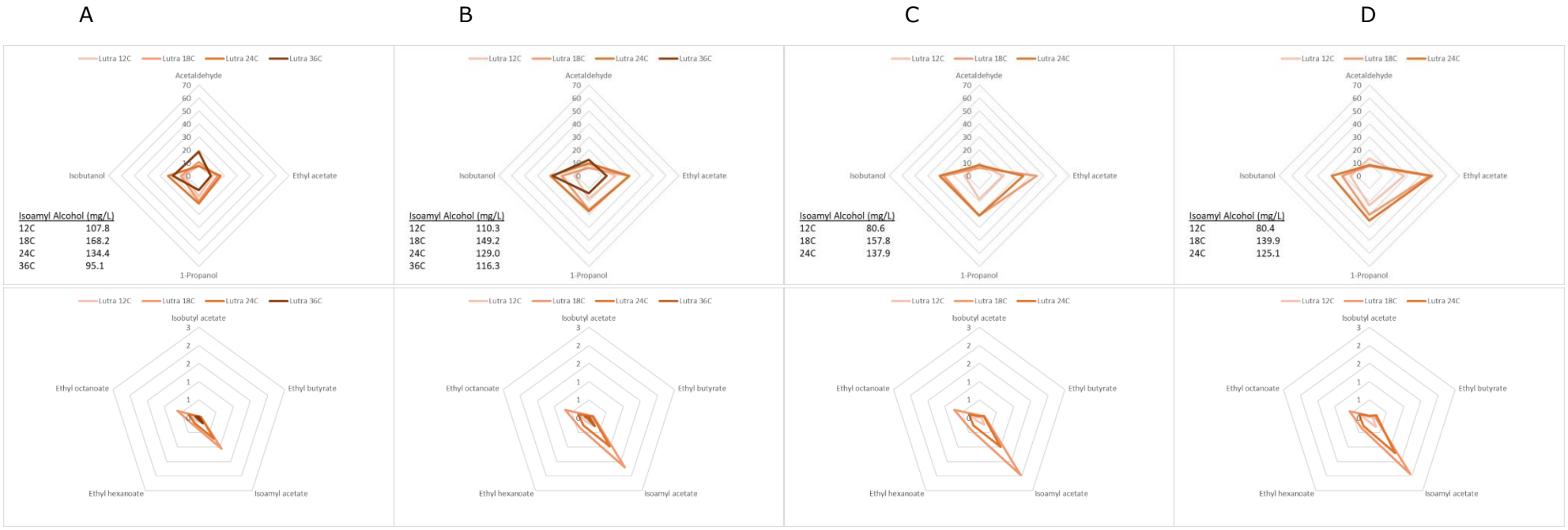


Figure 15. Production of flavour compounds by yeast strain Lutra according to fermentation temperature and initial wort gravity. Gravity was adjusted to 12°P (A), 16°P (B), 20°P (C) and 24°P (D). Variation in flavour is shown on each figure where temperature is represented by individual lines as indicated. In each instance, data is expressed in mg/L (ppm) and values represents the mean of triplicate samples. Isoamyl alcohol data is shown numerically since concentrations were significantly higher than the other flavour compounds; scaling the figures accordingly did not allow for clear data presentation.

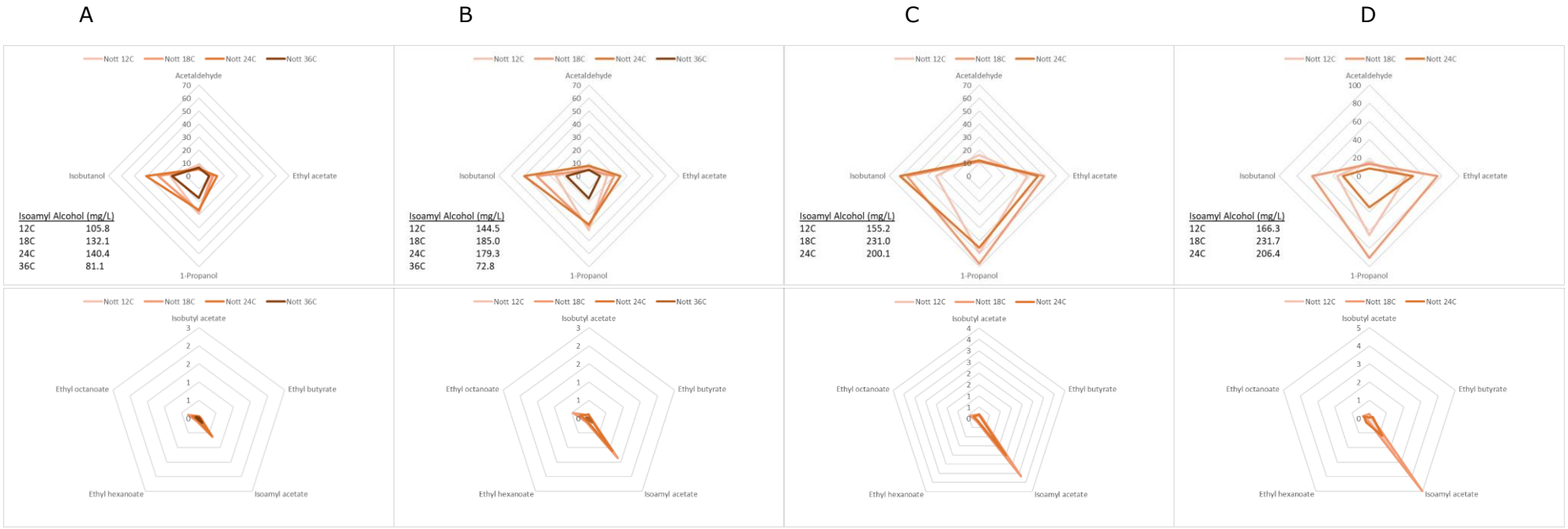


Figure 16. Production of flavour compounds by yeast strain Nottingham according to fermentation temperature and initial wort gravity. Gravity was adjusted to 12°P (A), 16°P (B), 20°P (C) and 24°P (D). Variation in flavour is shown on each figure where temperature is represented by individual lines as indicated. In each instance, data is expressed in mg/L (ppm) and values represents the mean of triplicate samples. Isoamyl alcohol data is shown numerically since concentrations were significantly higher than the other flavour compounds; scaling the figures accordingly did not allow for clear data presentation. N.B. The data shown for isobutyl acetate is scaled differently on this figure only, to account for the higher concentrations observed with Nottingham ale yeast.

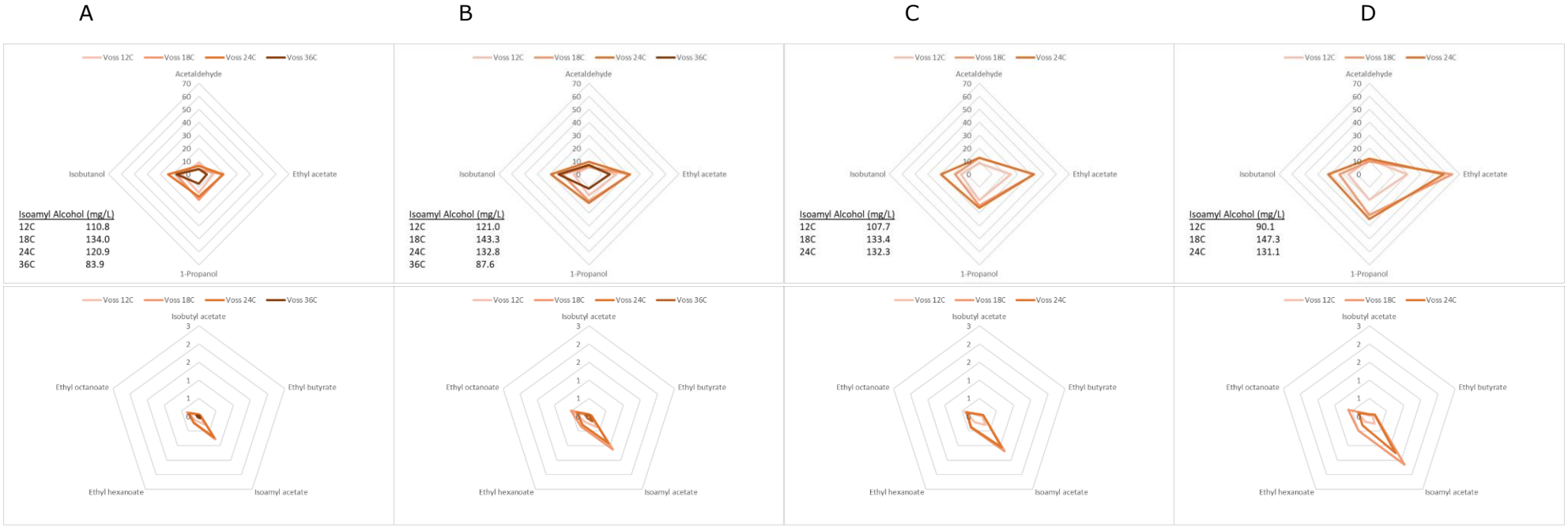


Figure 17. Production of flavour compounds by yeast strain Voss according to fermentation temperature and initial wort gravity. Gravity was adjusted to 12°P (A), 16°P (B), 20°P (C) and 24°P (D). Variation in flavour is shown on each figure where temperature is represented by individual lines as indicated. In each instance, data is expressed in mg/L (ppm) and values represents the mean of triplicate samples. Isoamyl alcohol data is shown numerically since concentrations were significantly higher than the other flavour compounds; scaling the figures accordingly did not allow for clear data presentation.

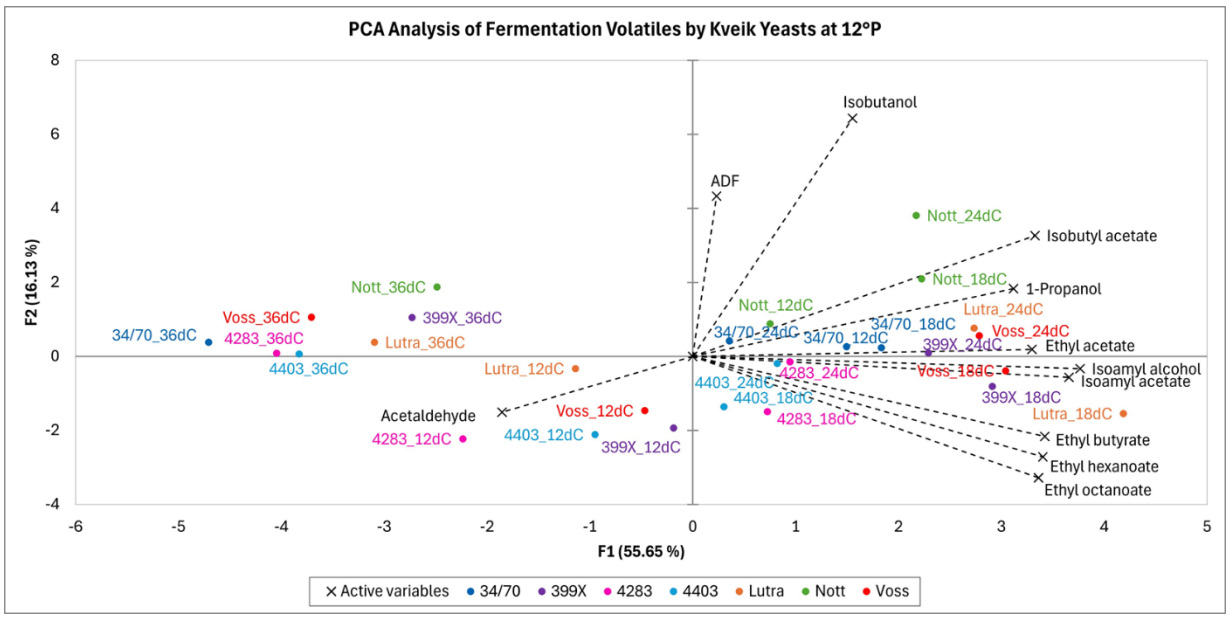


Figure 18. The relationship between starting wort gravity (12°P), fermentation temperature and flavour production based on PCA analysis. The relative intensity of key flavour compounds can be identified based on their proximity to each experimental condition. For example, kveik strain 4283 fermented at 12°C created relatively high levels of acetaldehyde.

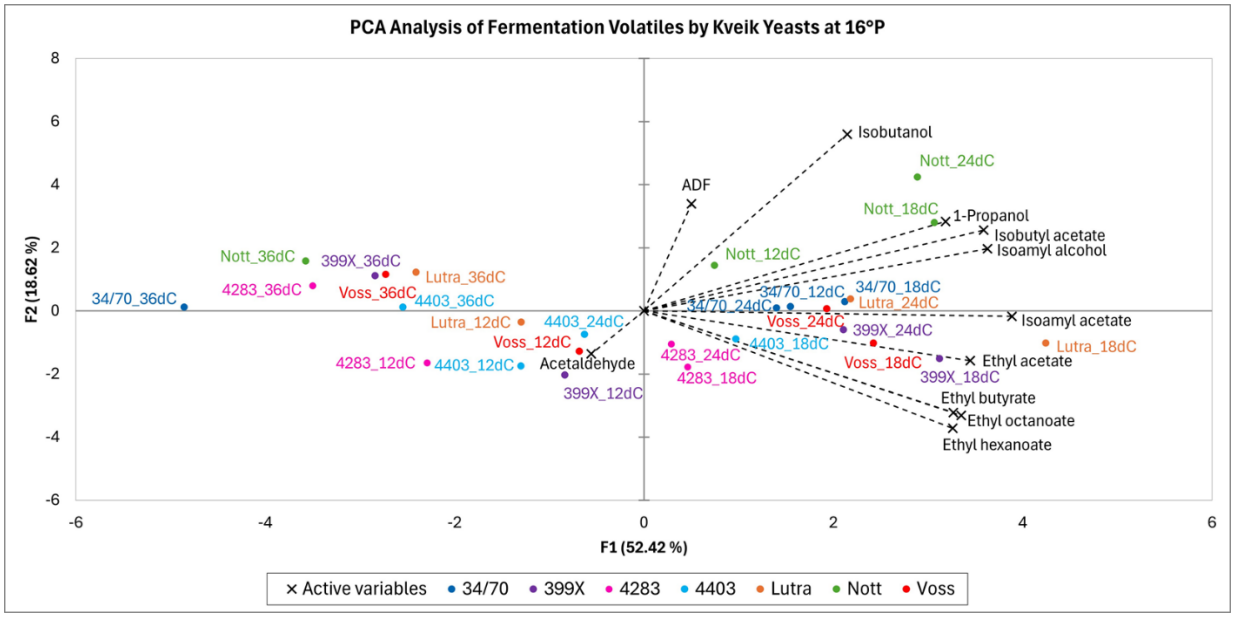


Figure 19. The relationship between starting wort gravity (16°P), fermentation temperature and flavour production based on PCA analysis. The relative intensity of key flavour compounds can be identified based on their proximity to each experimental condition. For example, Lutra yeast fermented at 18°C created relatively high levels of isoamyl acetate.

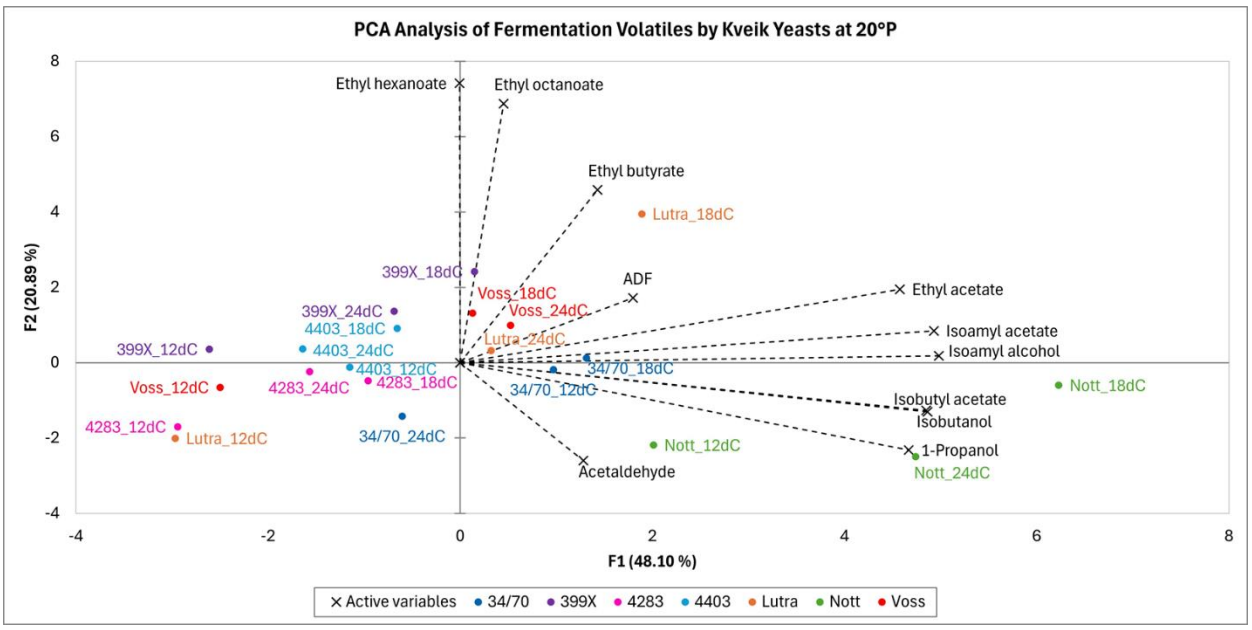


Figure 20. The relationship between starting wort gravity (20°P), fermentation temperature and flavour production based on PCA analysis. The relative intensity of key flavour compounds can be identified based on their proximity to each experimental condition. For example, Nottingham yeast fermented at 24°C created relatively high levels of 1-propanol.

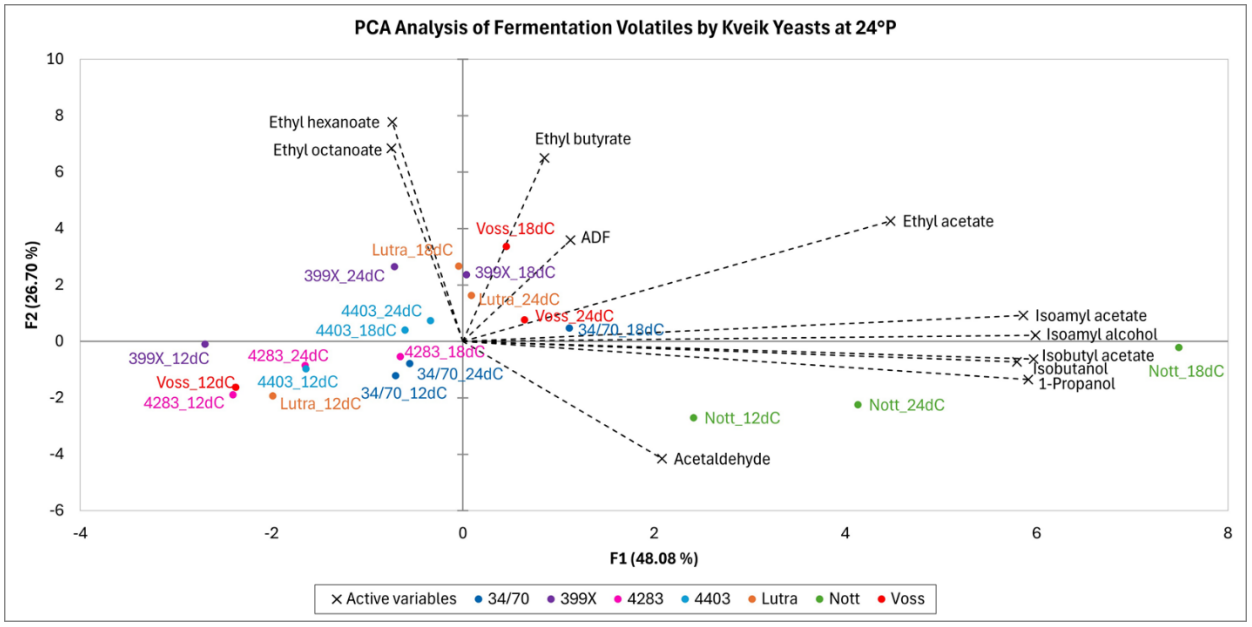


Figure 21. The relationship between starting wort gravity (24°P), fermentation temperature and flavour production based on PCA analysis. The relative intensity of key flavour compounds can be identified based on their proximity to each experimental condition. For example, Nottingham yeast fermented at 12°C created relatively high levels of acetaldehyde.

WP1 - iii. What temperatures can be applied to rapidly create acceptable ale-type beers?

Although kveik strains are currently assigned to be a sub-species of *S. cerevisiae* (ale yeast), it should be recognised that this genus is diverse, with practical applications for the creation of a range of products throughout the fermentation industry. A key artefact of kveik yeast is their ability to perform well at high temperature, thus allowing a product to be created more quickly than under standard conditions. Many brewers already employ this strategy to create acceptable ale-type products, however a comparative analysis of flavour production has not been performed to date. In order to determine the ability of kveik strains to produce beer flavour profiles that were comparable to standard ale type strains at high temperature, the flavour data generated previously (WP1) was used to create comparative flavour maps. Interestingly, it was challenging to identify conditions that allowed kveik yeast to exactly match the flavour profile observed for Nottingham ale (see also WP1. ii). At low and standard gravity (12°P and 16°P), Nottingham ale could be mapped against kveik strains (Figures 18-19), however at high gravities (20°P and 24°P) this yeast became an outlier and performed significantly differently to all of the other yeasts analysed (Figures 20-21). It should be recognised, and clearly acknowledged, that this analysis represents a relatively limited study in that only a single ale yeast was used. However, the data obtained was surprising as it had been expected that kveik strains would be similar to ale yeast. Consequently, it should be cautioned that this preliminary study suggests that kveik strains may perform differently to typical ale yeasts; in order to product-match current brands, more extensive research would be required to include other yeast strains as well as aspects of wort production and fermentation parameters such as pitching rate.

Irrespective of the above, at low gravity the kveik strains Lutra and Voss were able to generate beers that most closely matched Nottingham ale (Figure 22A). In this instance, the kveik yeast employed at 24°C (12°P and/or 16 °P) produced beers most similar to Nottingham at 12°P and 18°C, albeit the match was not perfect. Nottingham ale generated higher concentrations of 1-propanol, isobutanol and iso-amyl alcohol than either of the kveik yeast. As alluded to previously, this may simply be an artefact of strain specificity; the beers created were within acceptable limits expected for ale-type products. For comparative purposes, beer created with Nottingham at 24°C was also mapped against the kveik yeast; these beers displayed increasingly higher concentrations of isobutanol and iso-amyl alcohol (Figure 22A-B). It should be noted that the fermentations conducted at higher temperatures resulted in faster fermentations, as would typically be expected. For example, Nottingham at 12°P and 18°C reached attenuation in approximately 144h, compared to approximately 96h for Lutra and Voss at the same gravity but at 24°C, representing a time saving of approximately 2 days (Appendix 1-2; Note approximation only).

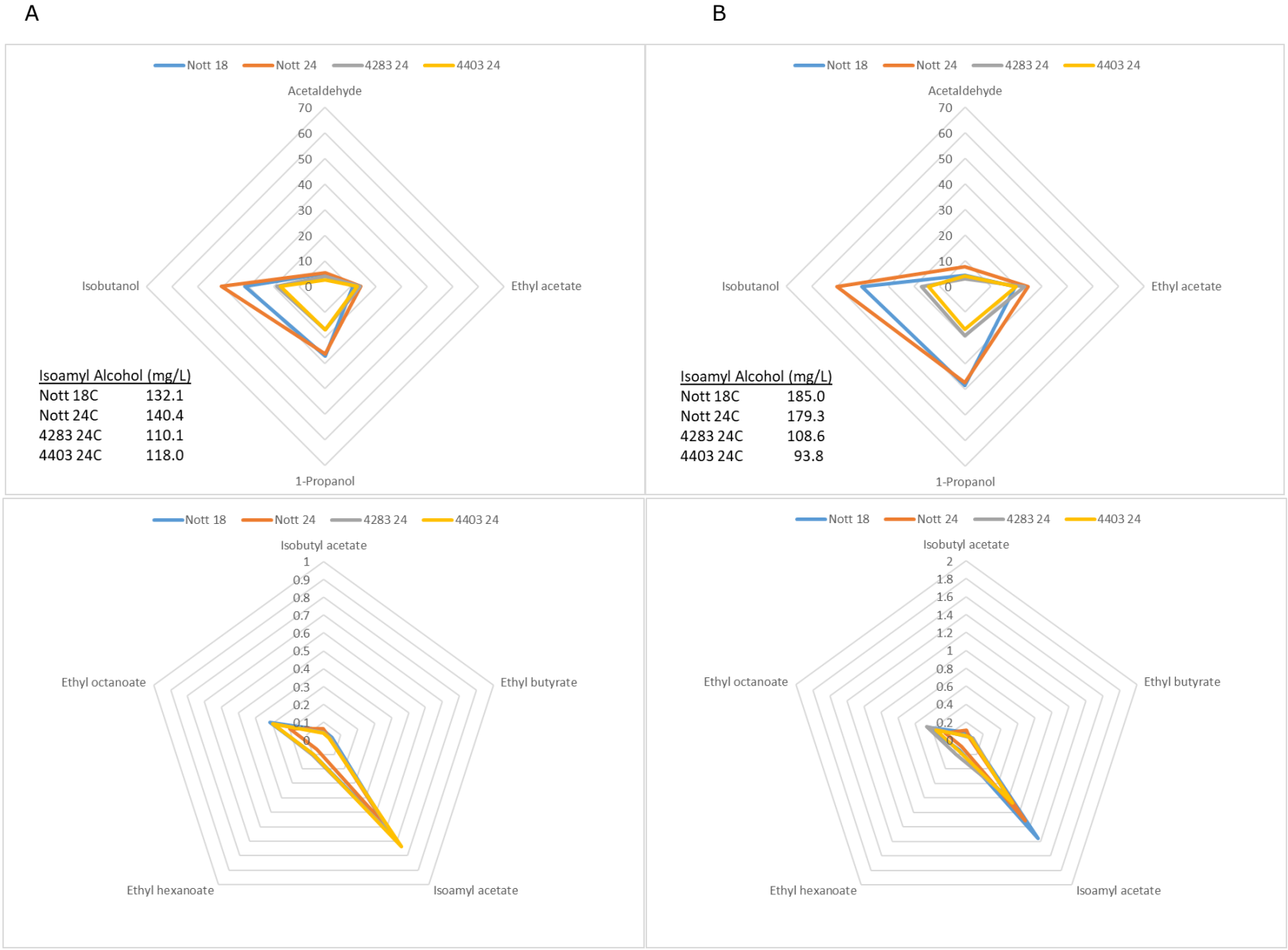


Figure 22. Production of flavour compounds by yeast strain Nottingham and Kveik strains 4283 and 4403 according to fermentation temperature and initial wort gravity. Gravity was adjusted to 12°P (A) and 16°P (B). Variation in flavour are indicated by the lines on each figure where temperature is represented by individual lines as indicated. Data is expressed in mg/L (ppm) in each instance and values represents the mean of triplicate samples.

WP1 - iv. Is it possible to produce a lager-type product at elevated temperatures using kveik yeast?

As noted previously (WP1-iii), it was initially expected that the kveik strains analysed would closely match the Nottingham ale yeast in terms of flavour production, however this was not observed to be the case. Consequently, in order to determine the ability of kveik strains to produce beer flavour profiles that were comparable to standard lager type strains at high temperature, the flavour data generated previously (WP1) was used to create a similar array of comparative flavour maps. Interestingly, it can be seen that the kveik strains analysed produced flavour profiles that were much closer to the *S. pastorianus* (lager) yeast W34/70 (Figure 23) than the ale yeast. It should be further emphasised that the current study involved only a single lager yeast for benchmarking purposes. However, the strain employed (W34/70) is extremely popular and this yeast (or close variants) are used for the majority of lager production worldwide. At low gravity (12°P and 16°P), W34/70 (fermented at 12°C and 18°C) created beer that was similar in flavour to both Lutra and Voss kveik strains when fermented at the equivalent gravities but at the higher temperature of 24°C. In this instance all of the flavour compounds analysed here were similar in intensity (Figure 23). This data indicates that it may be a relatively simple process to match lager-type products using kveik yeast at elevated temperatures, principally because of the relatively neutral flavour intensity observed. This data obtained was somewhat surprising, but also encouraging, as it had been expected that the profiles generated using kveik strains would be more unique. Consequently, it can be seen that there is significant potential to create acceptable lager-type products using kveik strains at elevated temperatures. For comparative purposes, W34/70 required approximately 168-192h to ferment 12°P wort at 12°C and 18°C, while the kveik strain was able to reach attenuation in 144h at 24°C (Appendix 1; Note approximation only). Similarly, at 16°P, W34/70 was able to ferment to completion in approximately 216h (12°C) and 192h (18°C) compared to approximately 168h for the Lutra and Voss kveik strains (Appendix 2; Note approximation only). For both wort gravities this represents substantial time savings in each instance. However, at higher gravities the flavour match became progressively more distant (data not shown) outweighing further savings in efficiencies. However, it should be noted that acceptable lager beers can still be created under these conditions (see WP1-ii; Figures 20-21). Furthermore, it is possible that with minor changes to wort production regimes and fermentation parameters such as oxygenation and pitching rates, some lager type brands beers could be re-created using kveik strains with significant savings to fermentation time and associated costs.

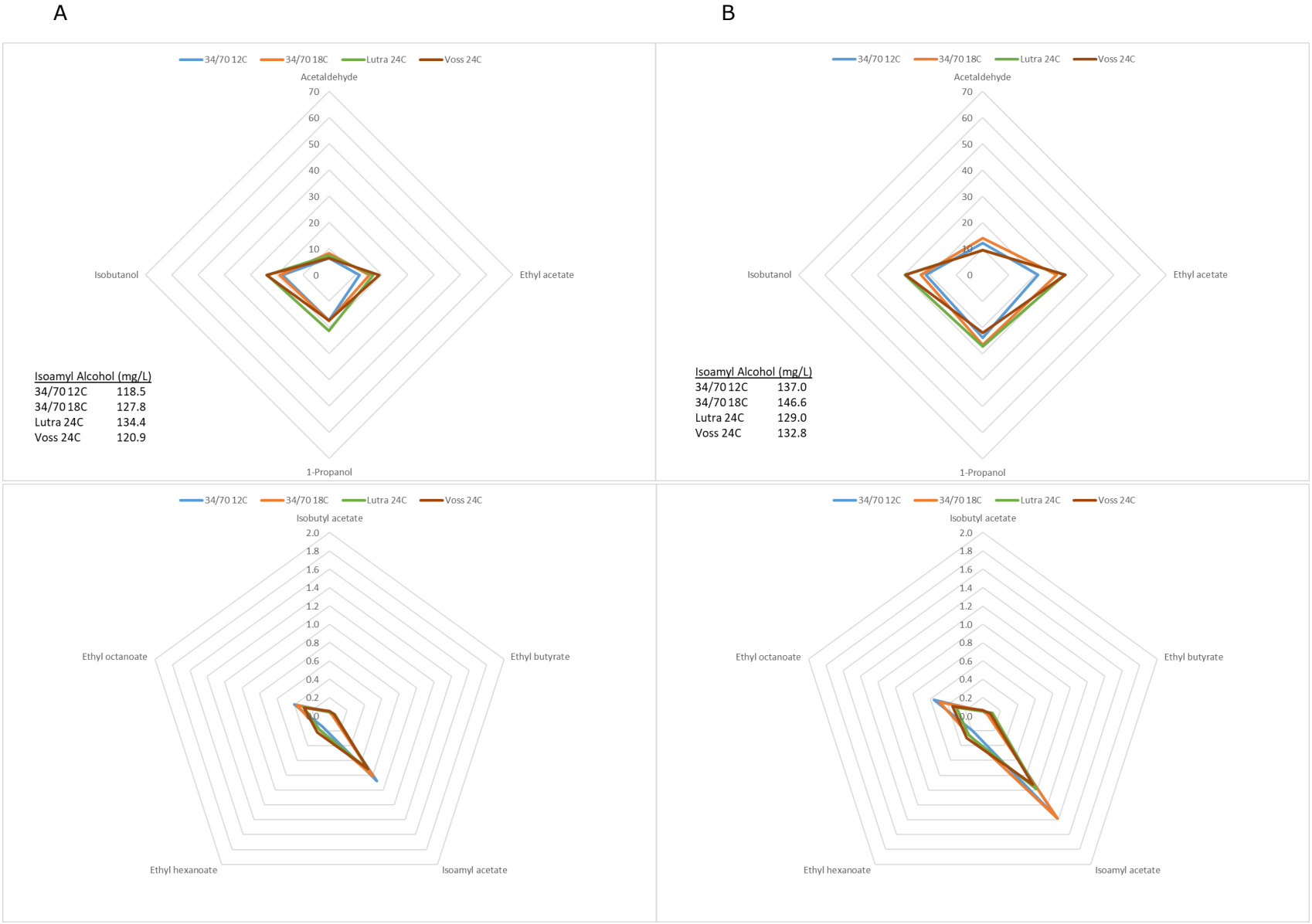


Figure 23. Production of flavour compounds by lager yeast strain 34/70 and kveik strains Lutra and Voss according to fermentation temperature and initial wort gravity. Gravity was adjusted to 12°P (A) and 16°P (B). Variation in flavour are indicated by the lines on each figure where temperature is represented by individual lines as indicated. Data is expressed in mg/L (ppm) in each instance and values represents the mean of triplicate samples.

Work Package 2 – Physiology of kveik strains and strategies for maintaining quality

Currently kveik yeast strains are handled in much the same way as traditional brewing yeasts. However, the potential for deviation from standard practices has not been explored. Given that kveik yeast are more tolerant of high temperatures there may be consequential impacts under cold conditions which have yet to be studied. Furthermore, the response of kveik strains to many standard brewery practices remains unknown. This is particularly the case for acid washing, which although not a recommended strategy, is still often employed especially within the craft sector. Finally, certain physiological aspects of kveik yeast have yet to be explored which can impact handling of yeast from an operational perspective, including flocculation properties. In order to assess the capacity of kveik yeast to be handled in a similar fashion to conventional yeasts, the key assessment criteria indicated below were applied:

i. **How do kveik yeast cultures respond to temperatures associated with cold storage?**
ii. **Can kveik yeast cultures be acid washed in the same way as conventional brewing strains?**
iii. **What are the flocculation characteristics of kveik yeast ?**

WP2 - i. The effect of cold storage on kveik yeast viability

Previous data has shown that kveik yeast are especially tolerant to heat and are able to ferment well at high temperatures (WP1). Although this characteristic is indicative of general stress tolerance, the effects of cold temperatures on kveik yeast have not currently been reported. This is particularly relevant to the brewing industry since brewing yeast cultures are typically stored cold (2-4°C) in yeast storage/collection vessels (yeast brinks) prior to use; a practice that is amplified during yeast handling (through serial repitching). It should be noted that although cold tolerance is important during storage, yeast are also subjected to starvation and, depending on the previous fermentation conditions, potentially also ethanol stress factors. Consequently, in order to ascertain whether kveik yeast display favourable storage properties, yeast cultures were assessed for viability over an extended period of time in cold storage. To emulate fermentation and brewery yeast handling protocol, each yeast strain was used to ferment a 15°P wort (in triplicate) and, once attenuation had been reached, the yeast was recovered and placed in a cold room (2- 4°C) without agitation. Viability was subsequently measured using an ABER CountStar in conjunction with methylene blue staining over a period of 8 days. It should be noted that this period exceeds that which would normally be applied, with most brewers imposing a limit of ~24-36h storage. It can be seen from Figure 24. that all of the yeast strains analysed showed a starting viability greater than 94%, deemed acceptable for yeast post-fermentation in most instances. The brewing strains Nottingham and W34/70 were observed to maintain viability during storage with viabilities of 94.7% and 95.8% respectively after 8 days. Similar data was observed for the kveik strains investigated; it should be noted that there was some variation in viability for strain 399x, with data indicating that viability dropped to ~90% (strain 399x; day 5). However, it is possible that this data value was erroneous, since the same strain gave a value of 98.8% on Day 6 and remained above 95% after Day 8. All of the other kveik strains maintained viability above 92%, and strains 4283 and Voss remained above 94% throughout, similar to the conventional strains W34/70 and Nottingham. Irrespective of some variation between yeasts, it should be emphasised that viability data values for all strains remained above 90% throughout, which is broadly indicative of good storage potential. Hence it be confirmed that kveik yeast strains can be maintained and handled in a similar way to conventional yeasts.

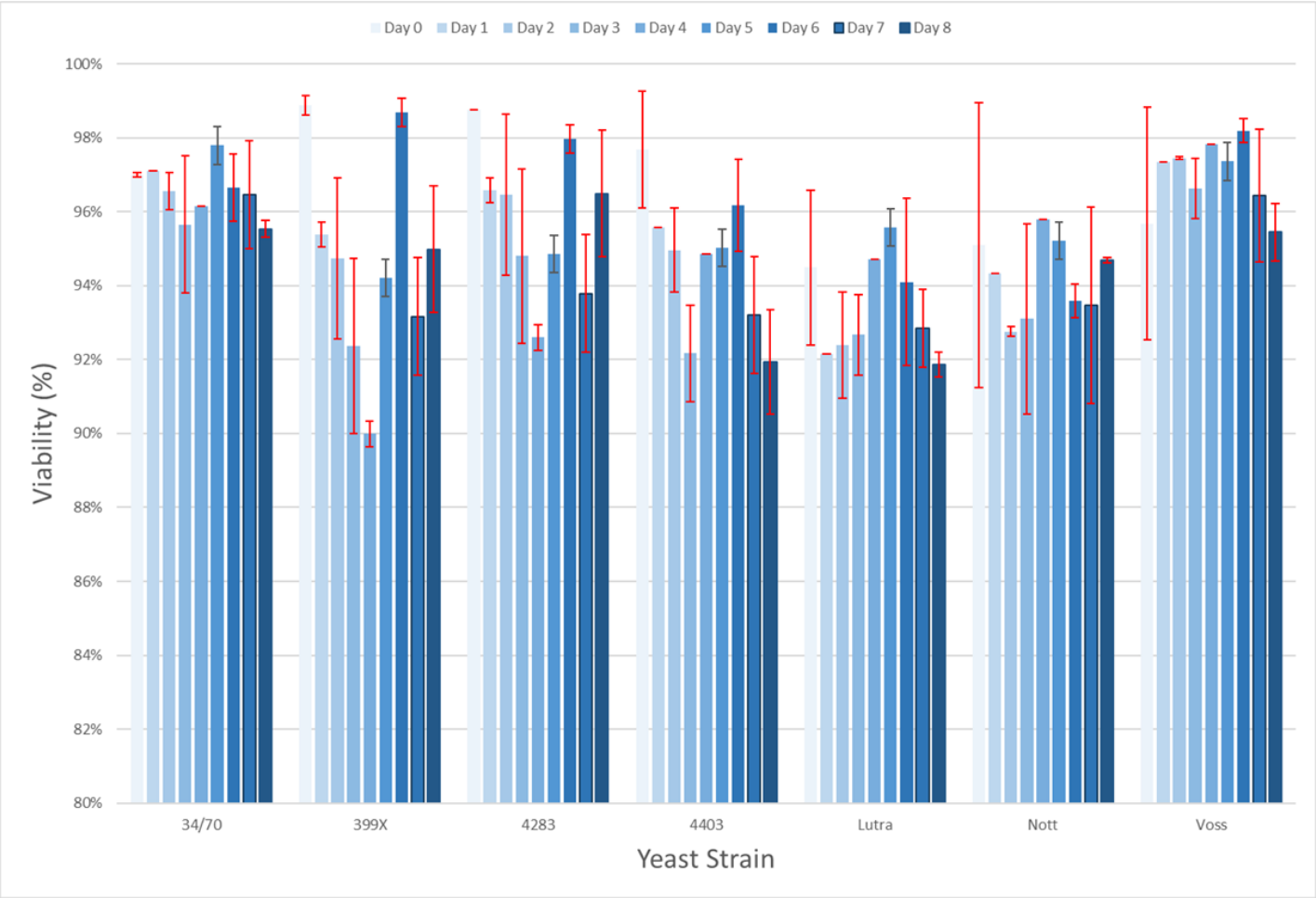


Figure 24. The relationship between storage time and yeast viability. All yeast strains were maintained statically at 2-4°C for the duration of the experiment. Methylene blue staining was used to determine live cell numbers, expressed as a percentage of the total population. Data represents the mean of triplicate samples with error bars indicating the standard deviation in each instance.

WP2 - ii. The effect of acid washing on kveik yeast viability

Although maintaining brewery hygiene should be a high priority for brewers of all sizes, it is acknowledged that, in some instances, low levels of contamination can occur. The best practice in such scenarios is to discard the yeast culture, rigorously clean to remove contamination, and investigate the source of contamination so that it can be eliminated. However, in some instances a yeast culture can be 'acid washed' when production demands insist that it must be used. It is recognised that this is approach is far from ideal and should be used as a last resort. However, some brewers do successfully acid wash on a periodic or routine basis. Broadly speaking if this is done correctly then bacteria can be eliminated without any adverse effects on the health of conventional brewing yeasts. However, the effect of acid washing on kveik strains remains to be investigated.

In order to determine the ability of kveik to withstand conditions associated with acid washing, all yeast strains were used to ferment a 15°P wort. After attenuation, yeast slurries were recovered, and viability was determined using methylene blue staining in conjunction with an ABER CountStar. Slurries were then chilled to 2-4°C for one day to replicate a brief period of yeast storage, before acidulation with phosphoric acid to a pH of 2.0-2.2 for 2h. During this time period, slurries were kept in a cold state (2-4°C) with continuous gentle agitation (stirring). Viability was measured at the point of acid addition and after two hours of acid washing. It can be seen from Figure 25 that all of the yeast strains investigated showed a high viability at the end of fermentation (greater than 94% in each instance) except for Lutra with an average of 93.8%. A similar trend was observed at the point of acidulation, with all strains showing a broadly similar viability. After 2h of acid washing viability remained consistent in each instance. This data indicates that all of the strains in the experiment were not negatively impacted by the acid washing procedure based on viability analysis. Although it should be noted that fermentations post-acid washing were not conducted, the data implies that kveik strains can be acid washed using a standard industry protocol in a similar fashion to conventional strains without any loss in quality.

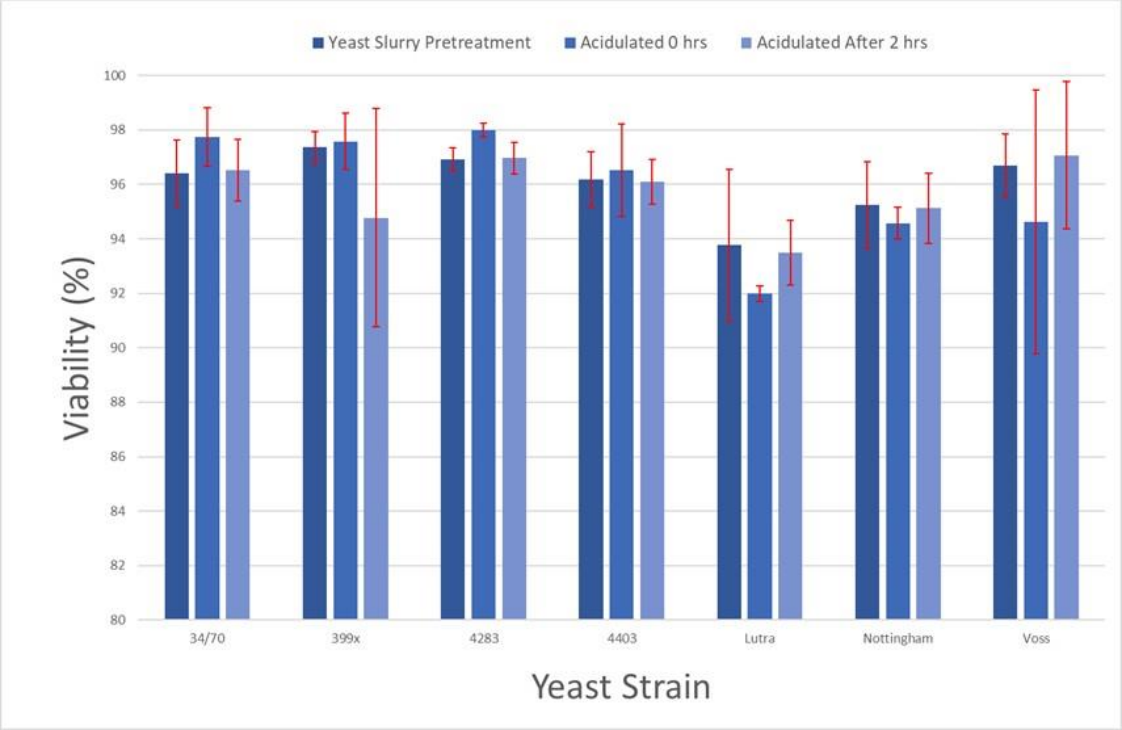


Figure 25. The relationship between acid washing (pH 2-2.2) and yeast viability. All yeast strains were gently agitated at 2-4°C for the duration of the experiment (2h total). Methylene blue staining was used to determine live cell numbers, expressed as a percentage of the total population. Data represents the mean of triplicate samples with error bars indicating the standard deviation in each instance.

WP2 - iii. The flocculation characteristics of kveik yeast

Yeast flocculation capacity is an intrinsic and essential aspect of brewing yeast physiology. The ability to form clumps (flocs) acts to allow cells to separate from the medium (wort/beer), resulting in a 'bright' product while also facilitating removal and recovery of the yeast crop. In this respect, brewing strains are relatively unusual in that they display flocculation characteristics that exceed those of wild strains, as well as yeasts used in other industrial settings. While anecdotal evidence suggests that flocculation characteristics of kveik strains are broadly similar to conventional yeasts, quantitative analysis data does not currently exist. Consequently, the flocculation properties of kveik strains relative to brewing yeasts does not currently exist. To rectify this, each strain was assessed for flocculation using the Helm's as described above. In brief, deflocculated 'control' samples were compared to flocculent experimental samples and the capacity to form flocs was assessed based on the ability to separate from the medium was determined spectrophotometrically. It can be seen from Figure 26. that the Nottingham ale strain was the most flocculent yeast and the kveik strains Lutra and Voss were comparatively the least flocculent, along with the lager strain W34/70. All of the kveik strains showed flocculation characteristics typical of conventional brewing strains, with variation within the limits of what would normally be expected.

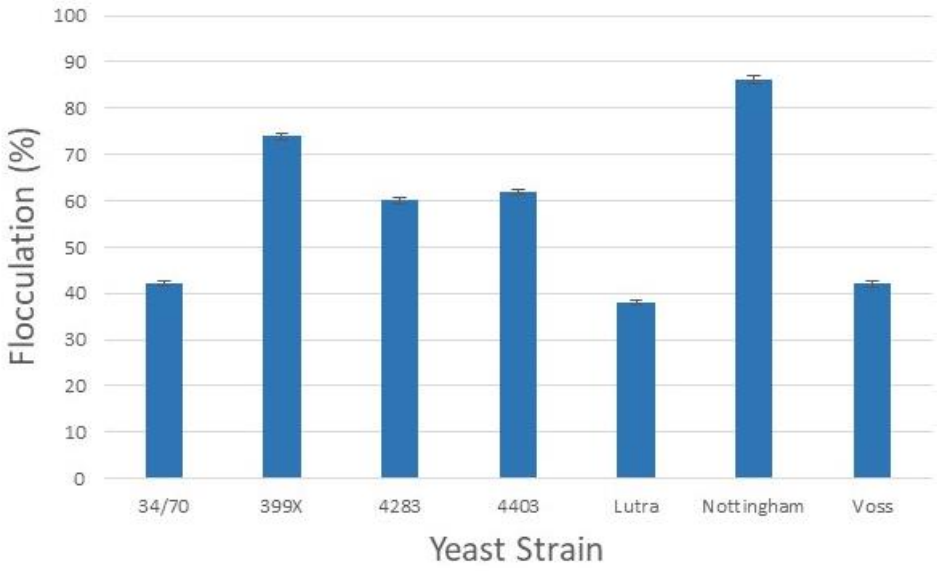


Figure 26. Flocculation characteristics of kveik and conventional yeast strains. Flocculation was determined using the Helm's assay and date reflects the average of triplicate analyses with error bars indicating the standard deviation in each instance.

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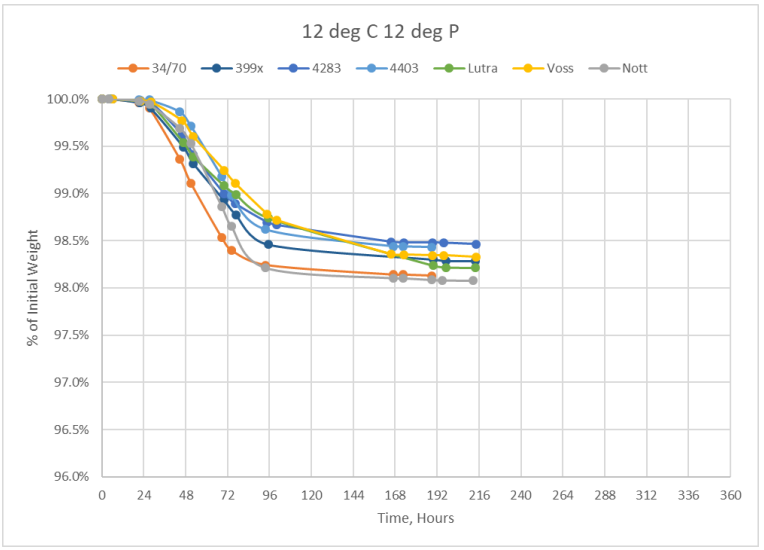
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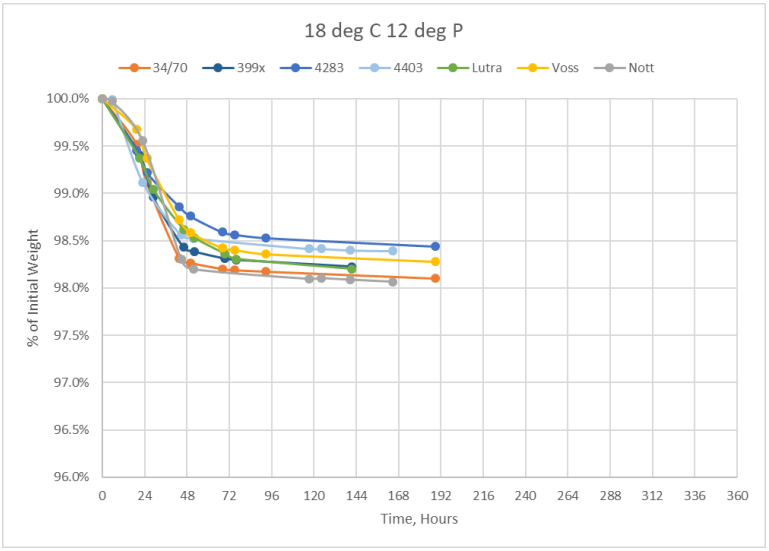
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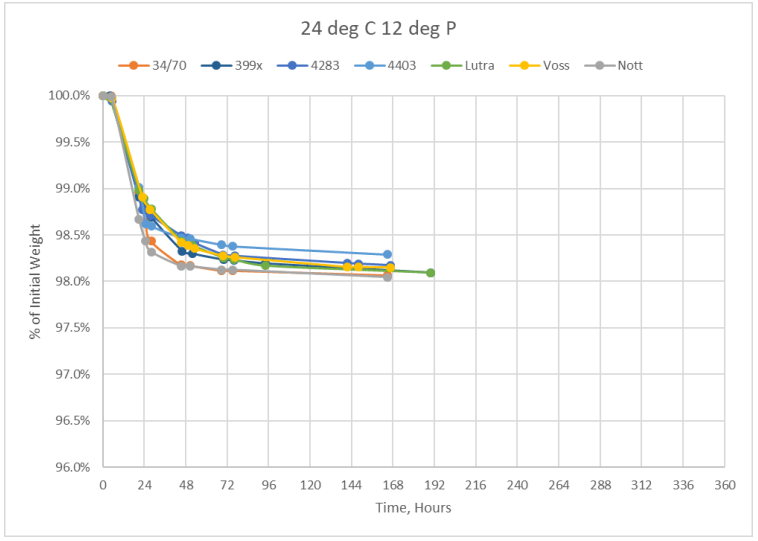
Appendices

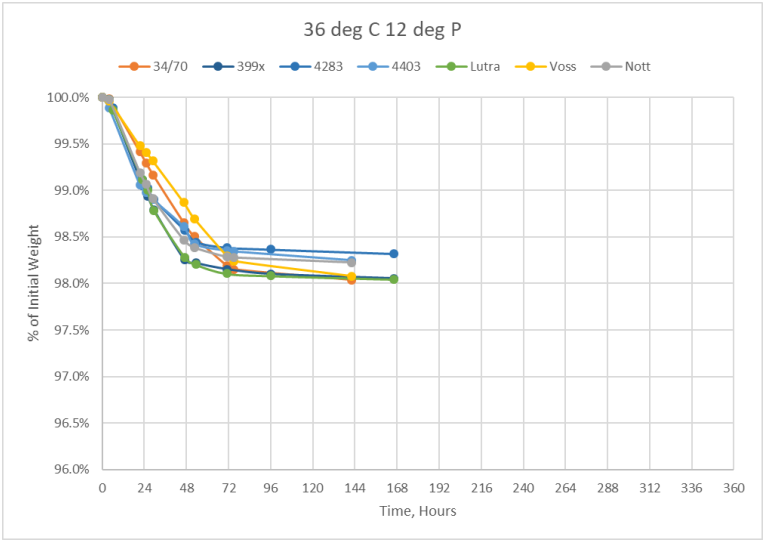
1. Fermentation profiles of yeasts strains at 12°P. Fermentation temperatures applied were 12, 18, 24 and 36°C.
2. Fermentation profiles of yeasts strains at 16°P. Fermentation temperatures applied were 12, 18, 24 and 36°C.
3. Fermentation profiles of yeasts strains at 20°P. Fermentation temperatures applied were 12, 18 and 24°C.
4. Fermentation profiles of yeasts strains at 24°P. Fermentation temperatures applied were 12, 18 and 24°C.
5. Fermentation profiles of yeast strain W34/70 at 12, 16, 20 and 24°P. Fermentation temperatures applied include 12, 18, 24 and 36°C.
6. Fermentation profiles of yeast strain 399X at 12, 16, 20 and 24°P. Fermentation temperatures applied include 12, 18, 24 and 36°C.
7. Fermentation profiles of yeast strain 4283 at 12, 16, 20 and 24°P. Fermentation temperatures applied include 12, 18, 24 and 36°C.
8. Fermentation profiles of yeast strain 4403 at 12, 16, 20 and 24°P. Fermentation temperatures applied include 12, 18, 24 and 36°C.
9. Fermentation profiles of yeast strain Lutra at 12, 16, 20 and 24°P. Fermentation temperatures applied include 12, 18, 24 and 36°C.
10. Fermentation profiles of yeast strain Nottingham at 12, 16, 20 and 24°P. Fermentation temperatures applied include 12, 18, 24 and 36°C.
11. Fermentation profiles of yeast strain Voss at 12, 16, 20 and 24°P. Fermentation temperatures applied include 12, 18, 24 and 36°C.
12. Headspace Gas Chromatography (GC) data by temperature and wort gravity.
13. Publications submitted or in preparation
14. List of presentations
15. Examples of presentation slides

Appendix 1. Fermentation profiles of yeasts strains at 12°P. Fermentation temperatures applied were 12, 18, 24 and 36°C.

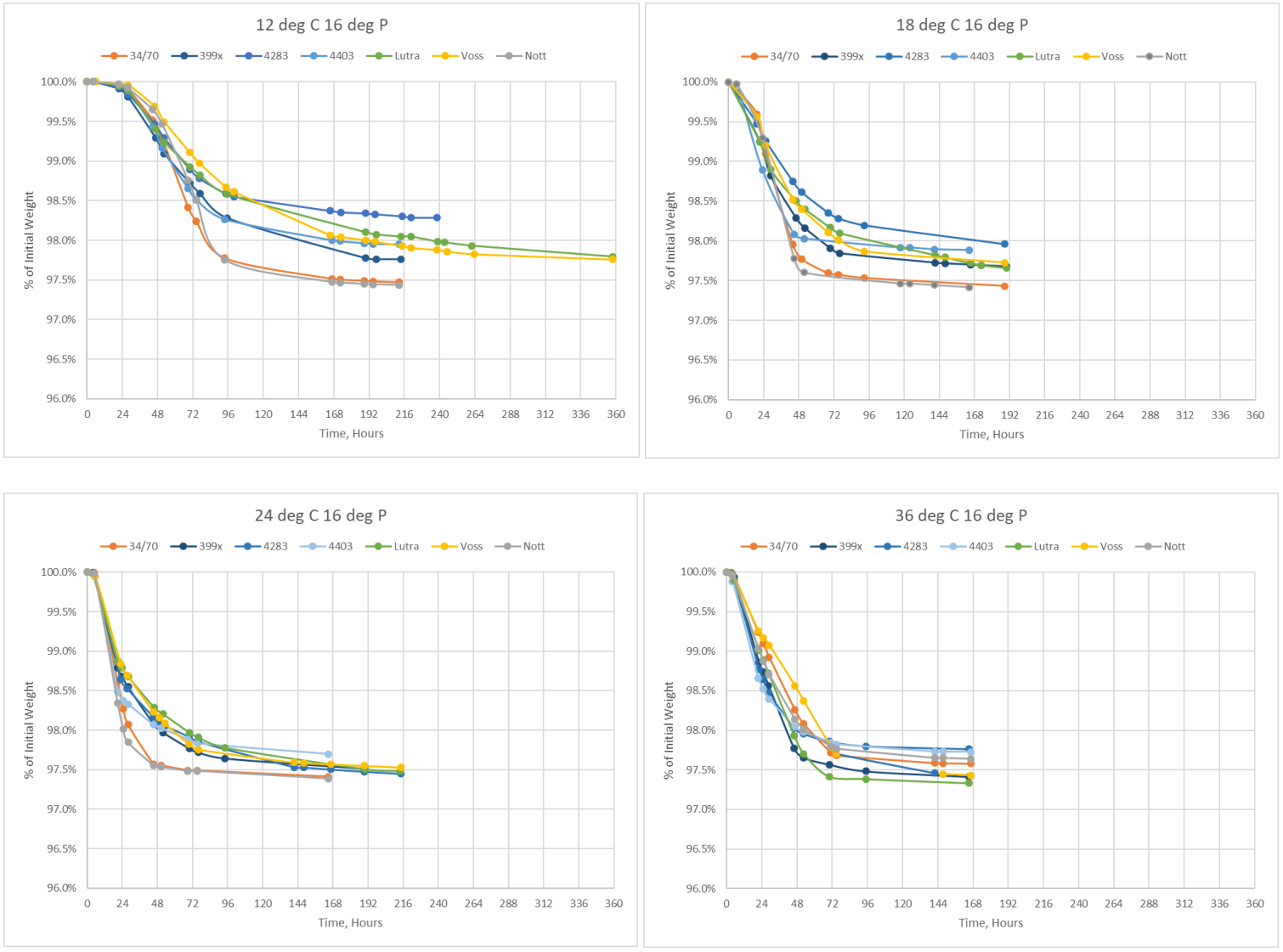




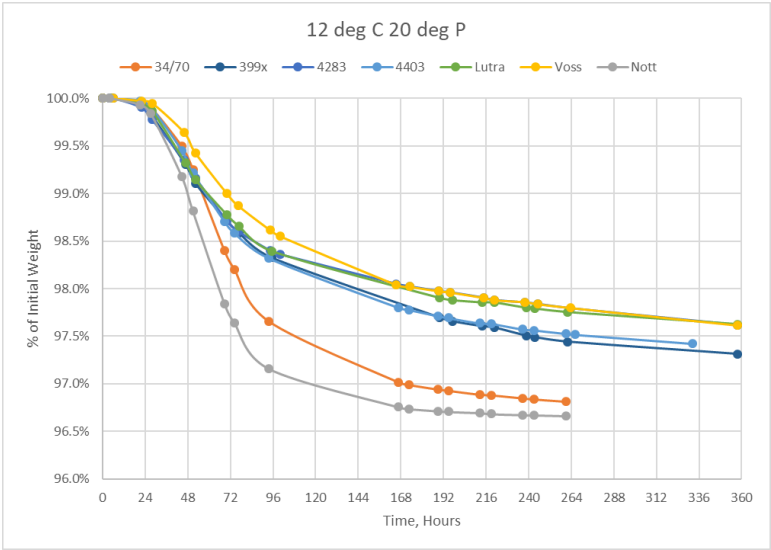


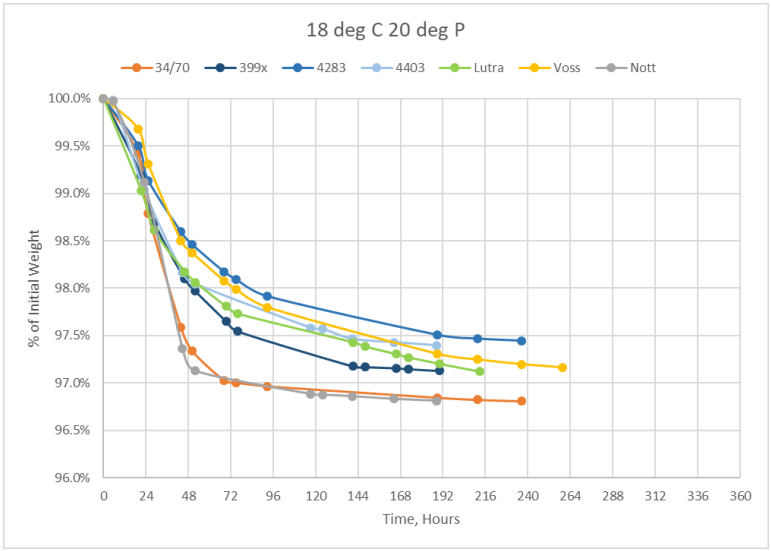


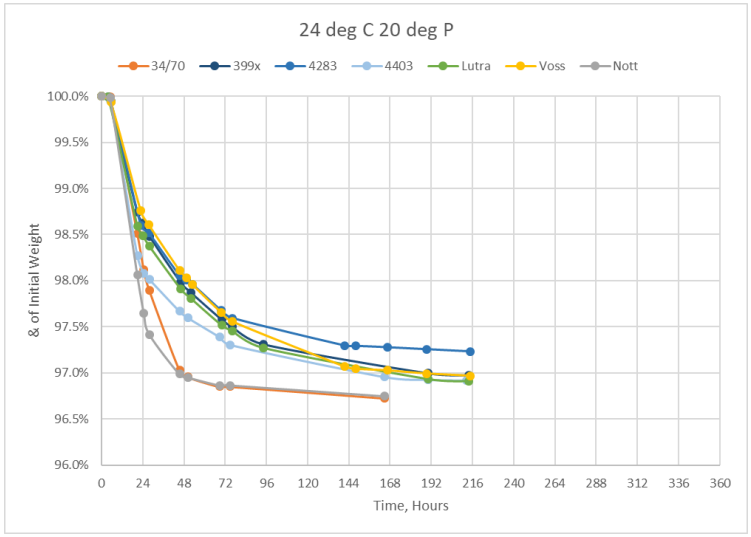
Appendix 2. Fermentation profiles of yeasts strains at 16°P. Fermentation temperatures applied were 12, 18, 24 and 36°C.



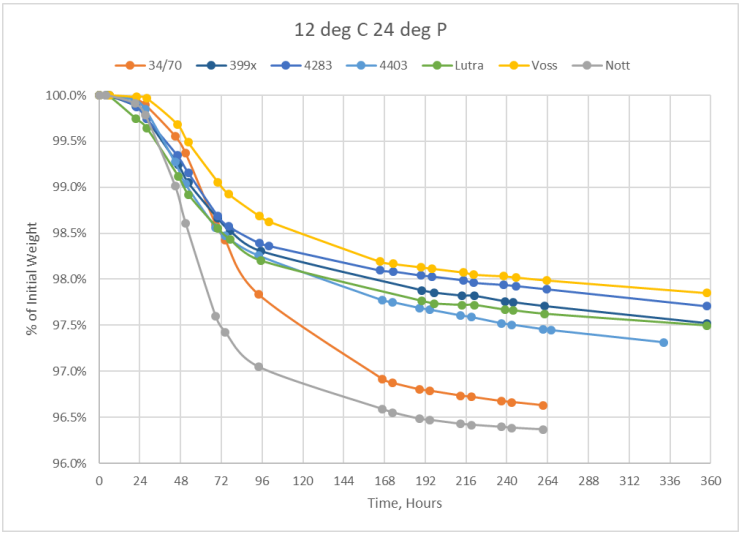
Appendix 3. Fermentation profiles of yeasts strains at 20°P. Fermentation temperatures applied were 12, 18 and 24°C.

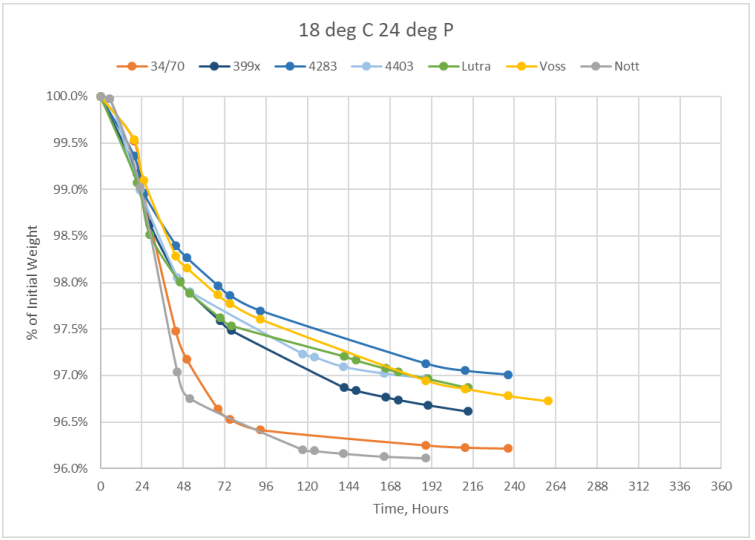


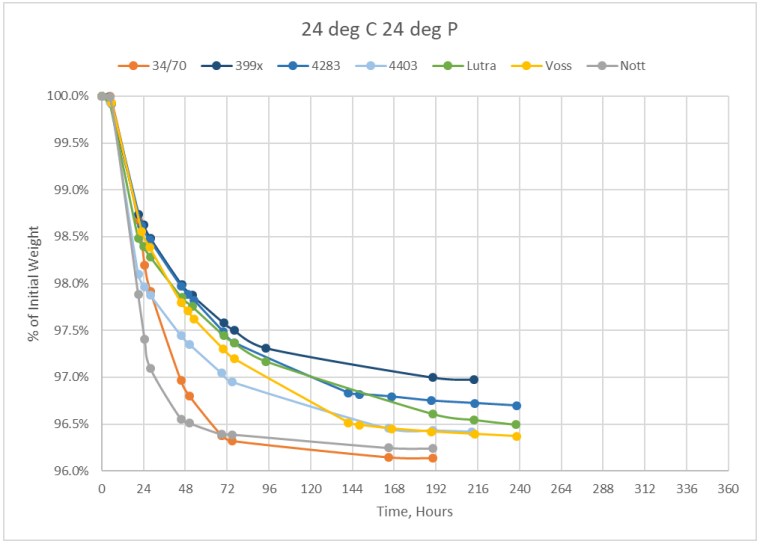




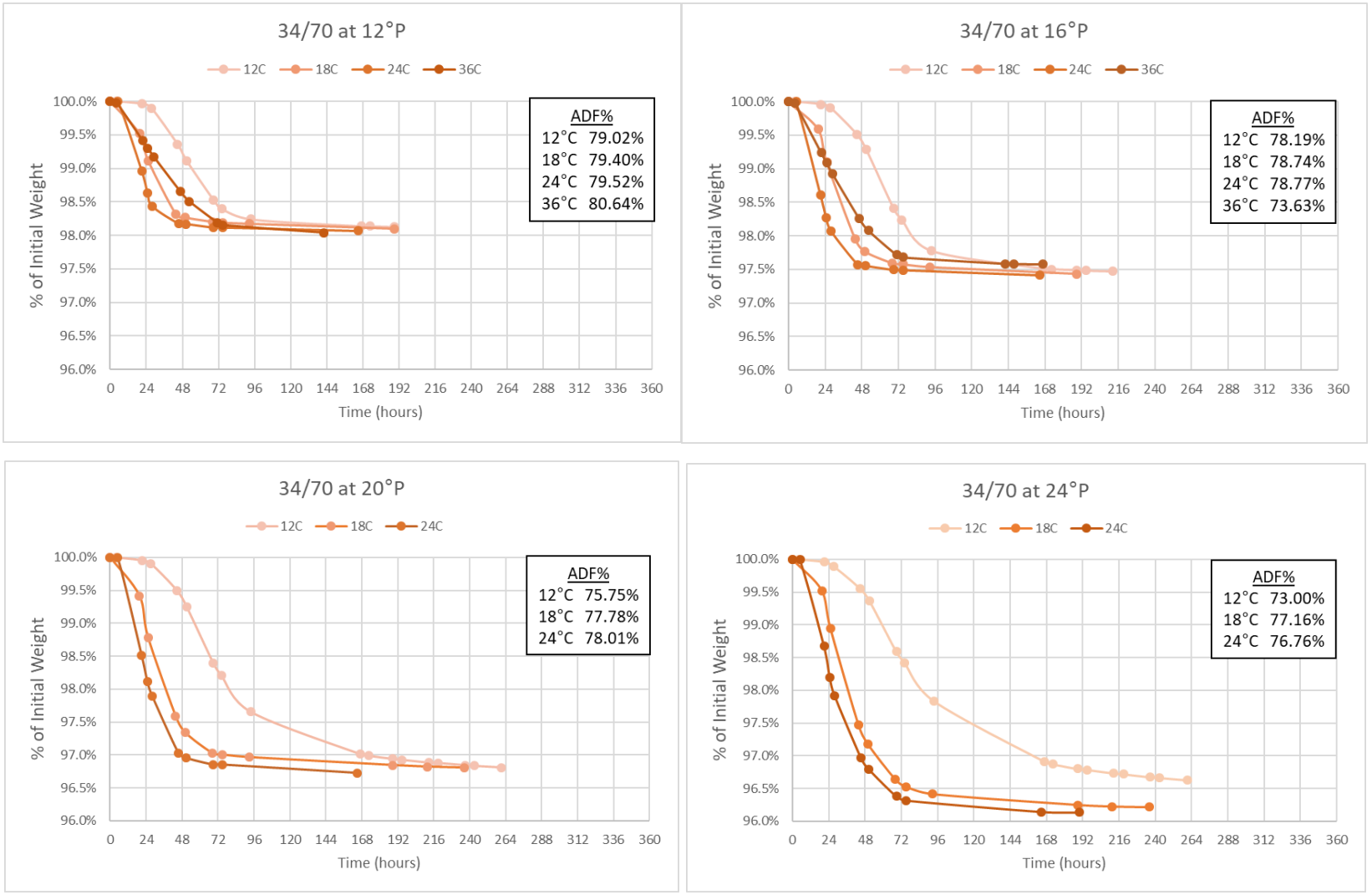
Appendix 4. Fermentation profiles of yeasts strains at 24°P. Fermentation temperatures applied were 12, 18 and 24°C.



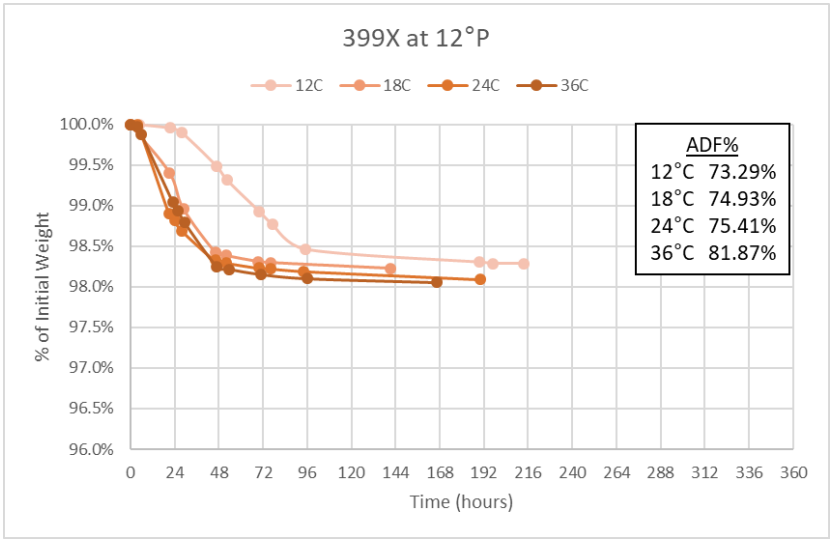


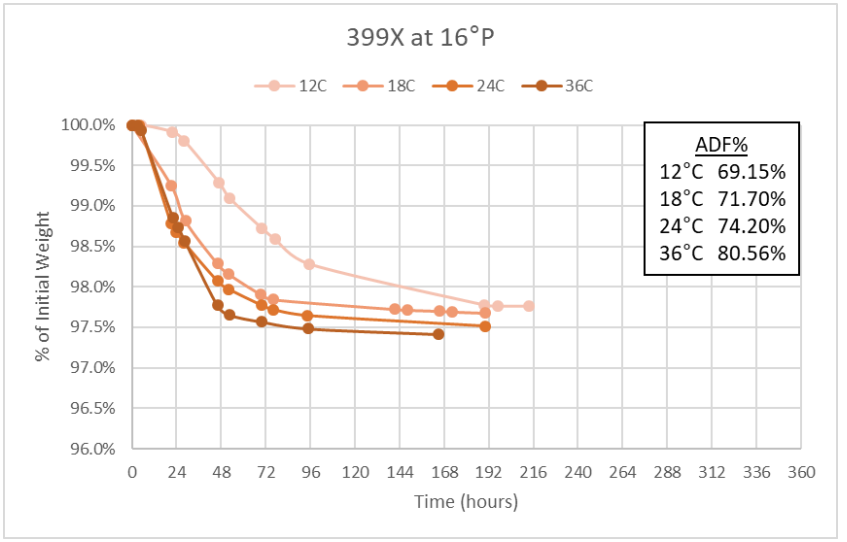


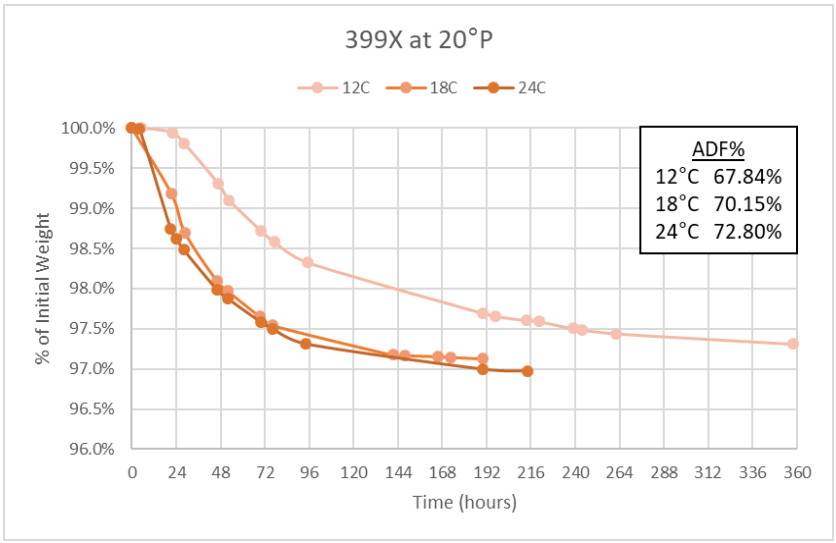
Appendix 5. Fermentation profiles of yeast strain W34/70 at 12, 16, 20 and 24°P. Fermentation temperatures applied include 12, 18, 24 and 36°C. ADF values are included as a reference point only.

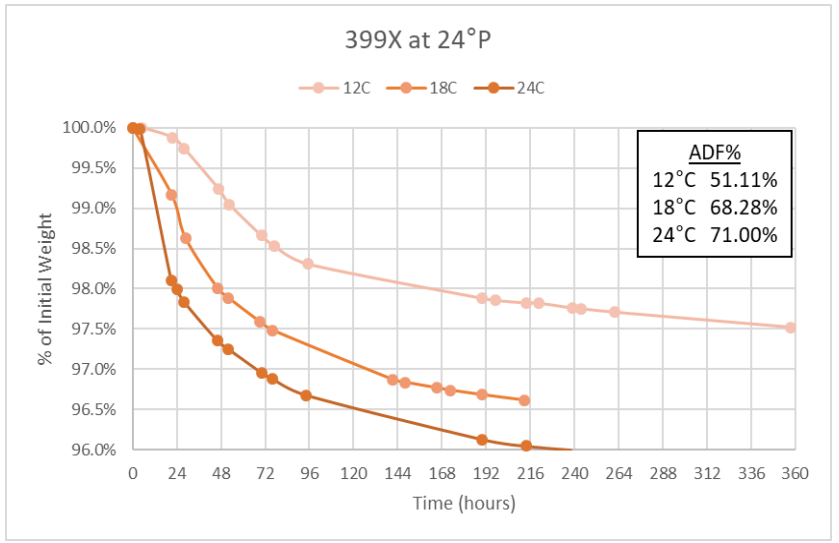


Appendix 6. Fermentation profiles of yeast strain 399X at 12, 16, 20 and 24°P. Fermentation temperatures applied include 12, 18, 24 and 36°C. ADF values are included as a reference point only.

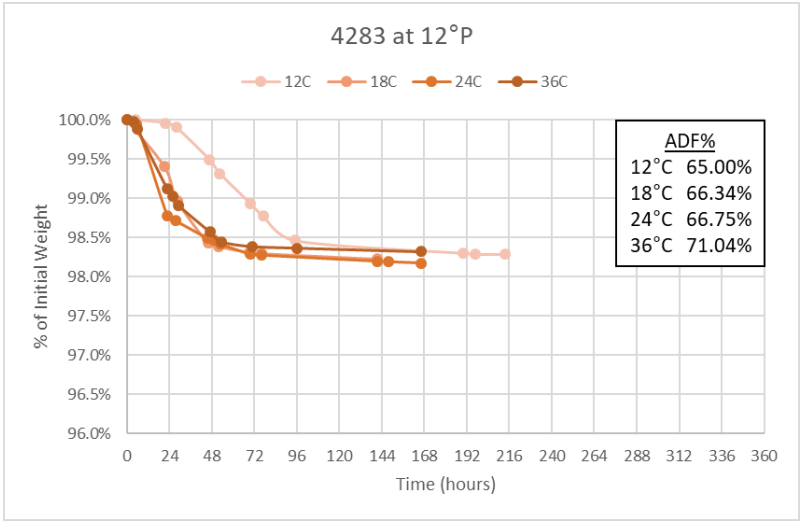


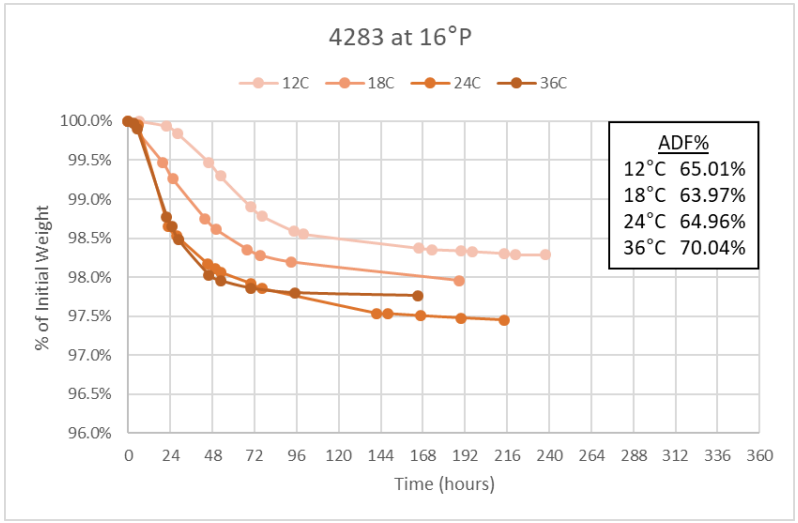


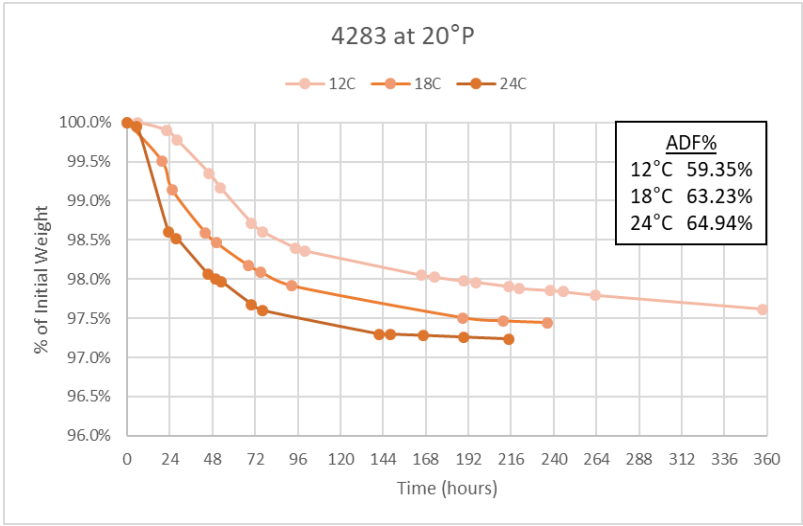


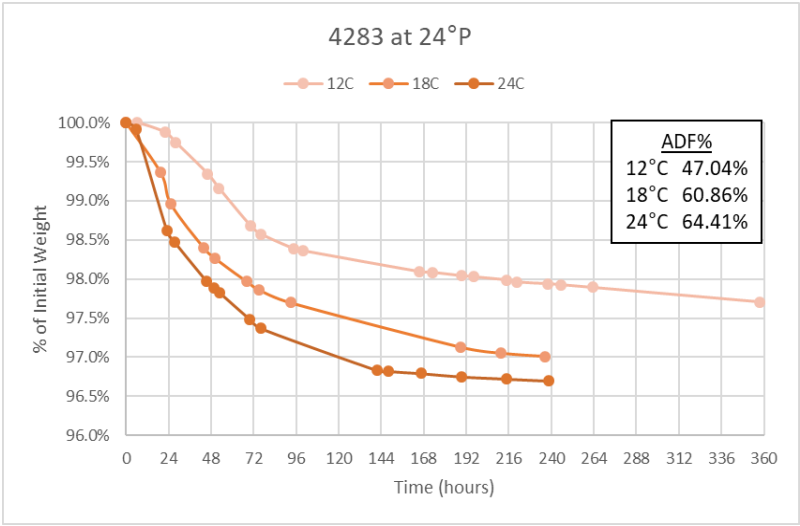


Appendix 7. Fermentation profiles of yeast strain 4283 at 12, 16, 20 and 24°P. Fermentation temperatures applied include 12, 18, 24 and 36°C. ADF values are included as a reference point only.

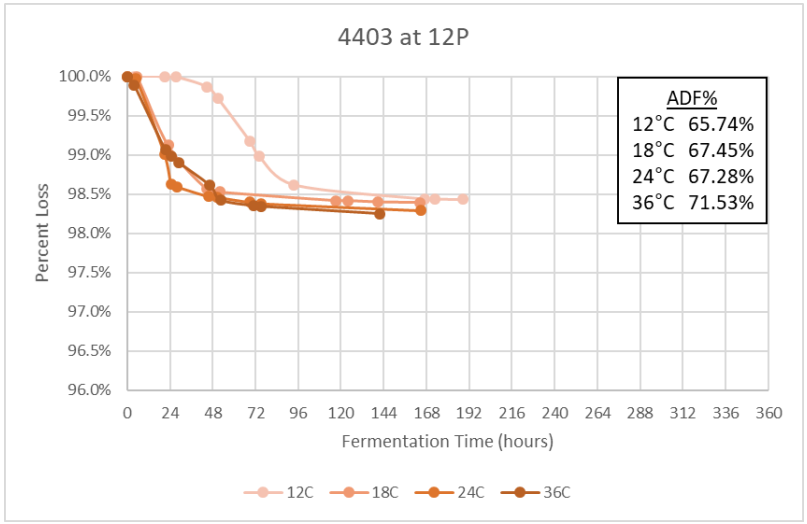


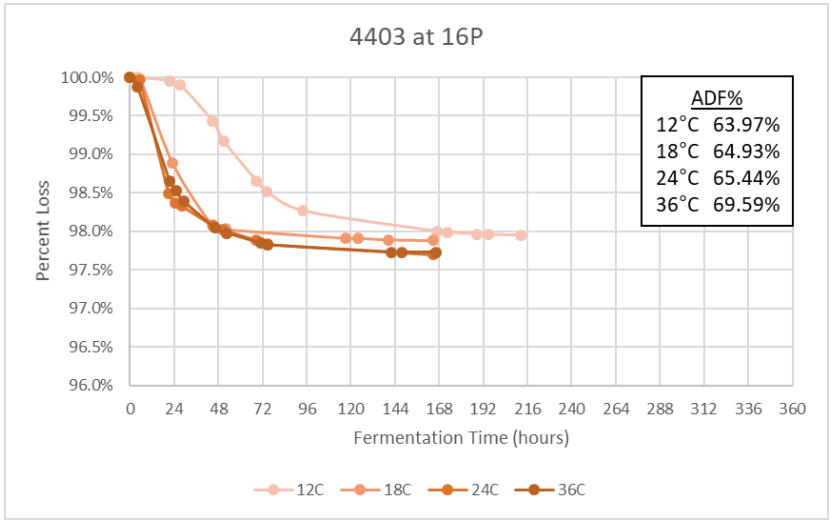


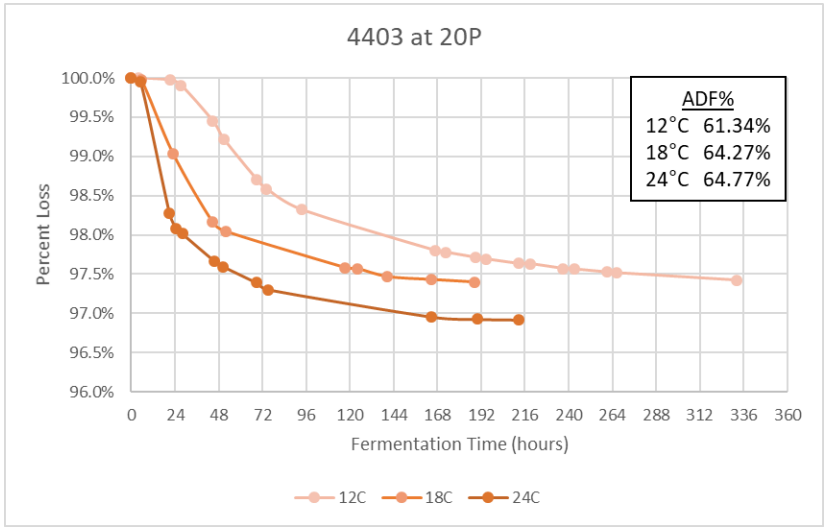


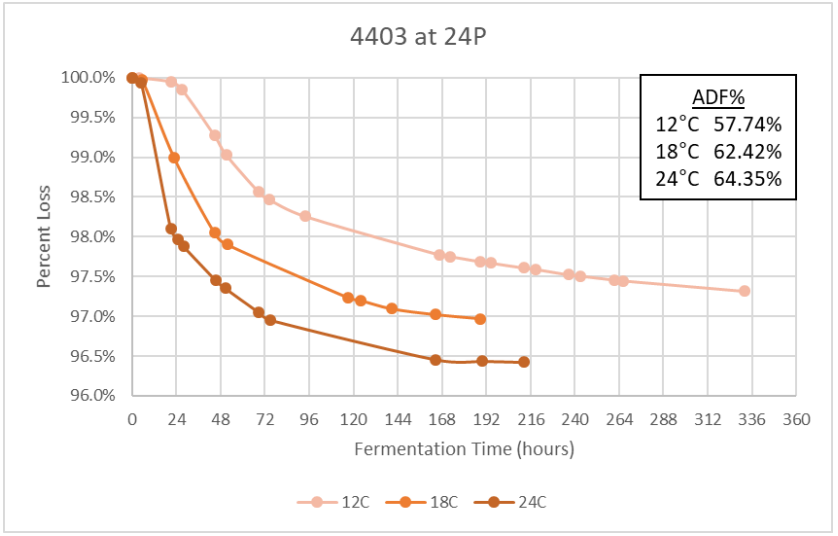


Appendix 8. Fermentation profiles of yeast strain 4403 at 12, 16, 20 and 24°P. Fermentation temperatures applied include 12, 18, 24 and 36°C. ADF values are included as a reference point only.

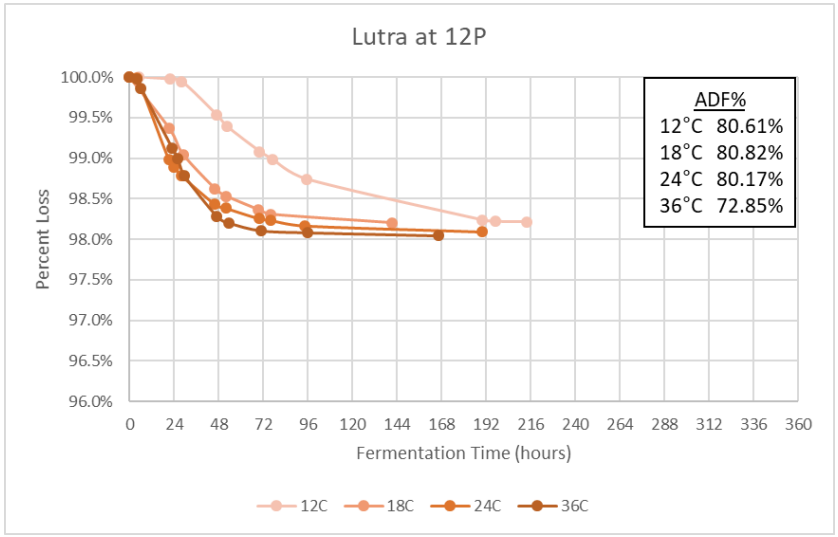


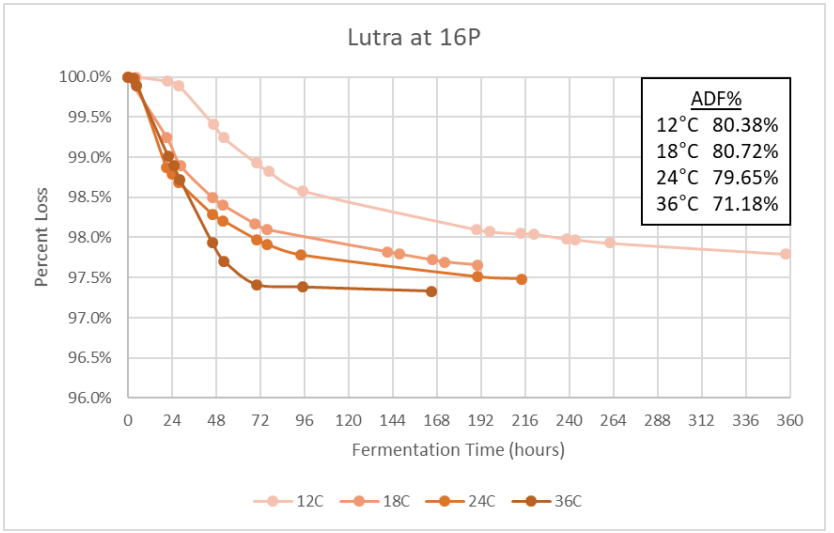


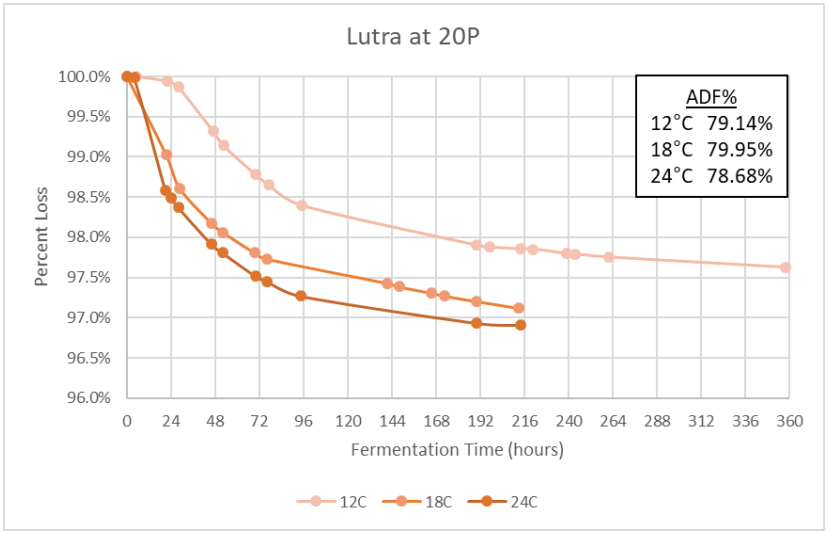


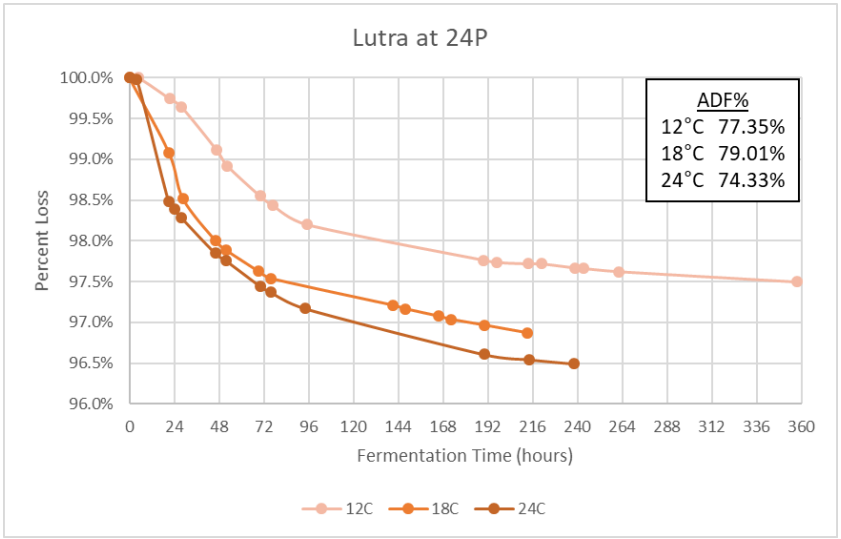


Appendix 9. Fermentation profiles of yeast strain Lutra at 12, 16, 20 and 24°P. Fermentation temperatures applied include 12, 18, 24 and 36°C. ADF values are included as a reference point only.

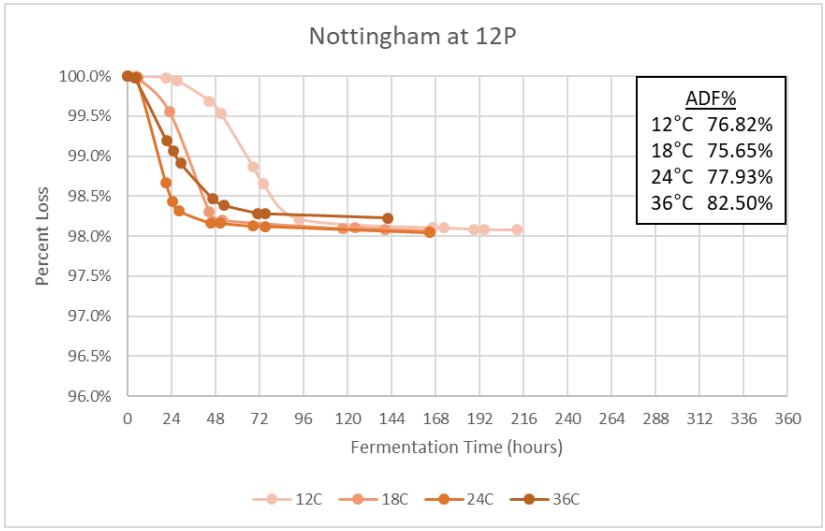


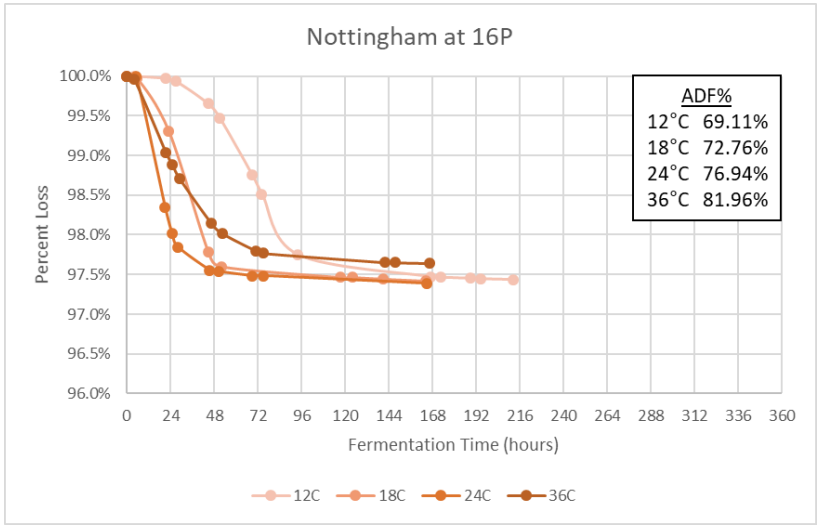


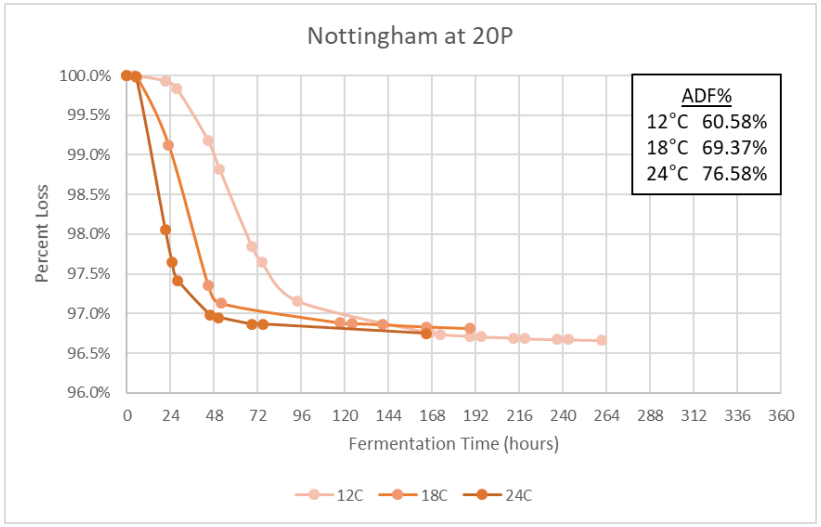


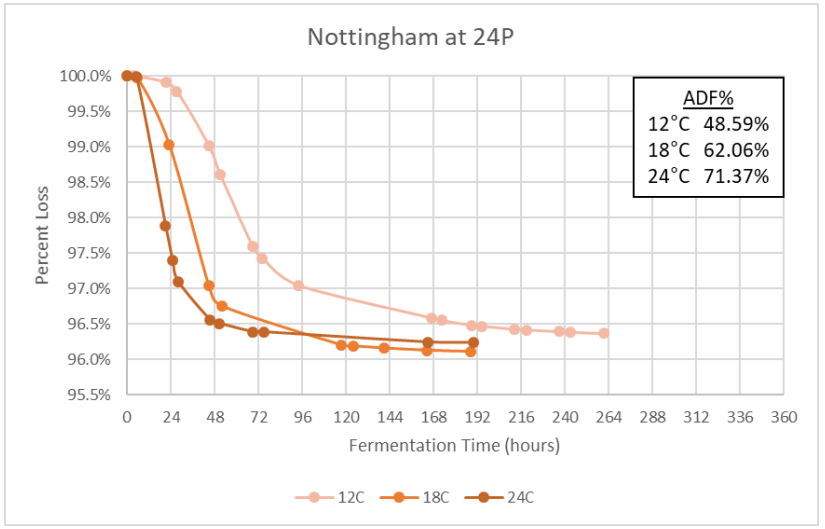


Appendix 10. Fermentation profiles of yeast strain Nottingham at 12, 16, 20 and 24°P. Fermentation temperatures applied include 12, 18, 24 and 36°C. ADF values are included as a reference point only.

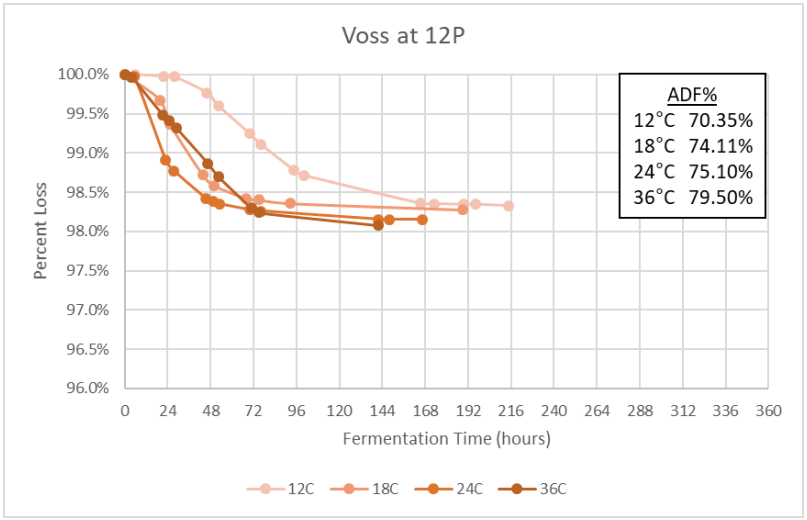


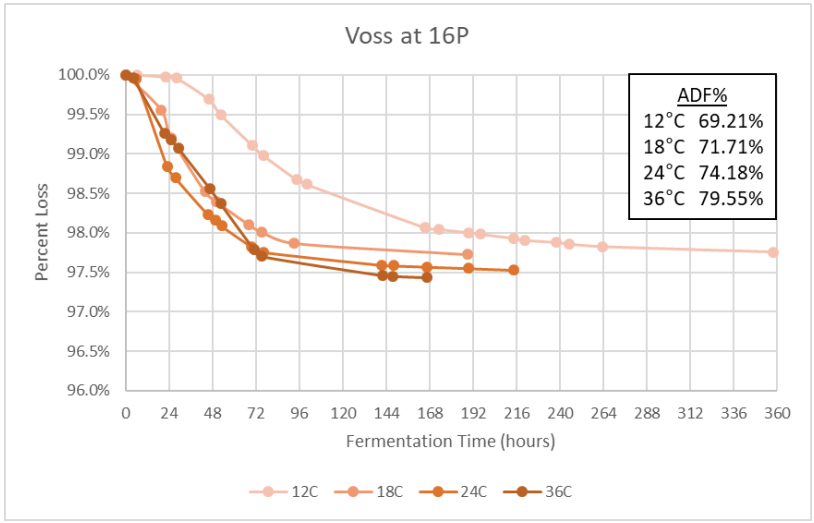


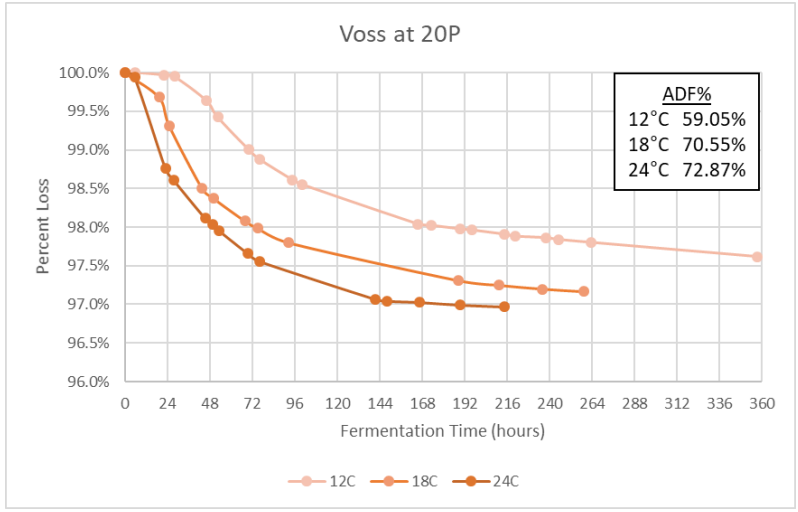


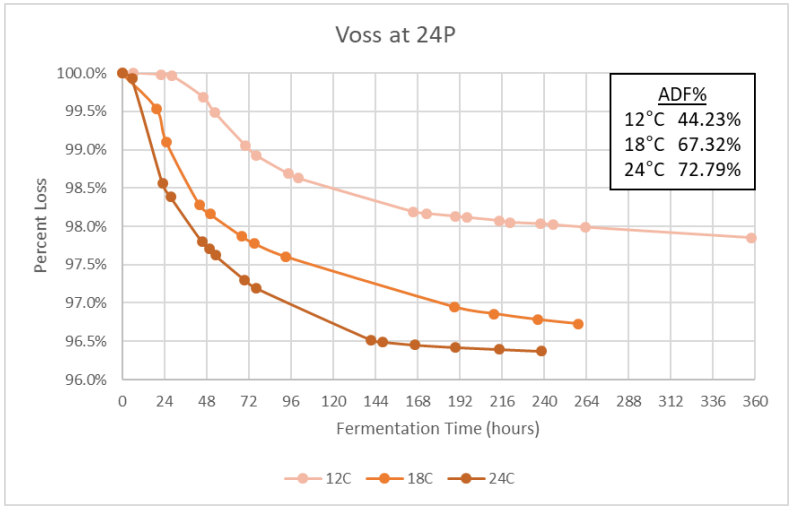


Appendix 11. Fermentation profiles of yeast strain Voss at 12, 16, 20 and 24°P. Fermentation temperatures applied include 12, 18, 24 and 36°C. ADF values are included as a reference point only.









Appendix 12. Headspace Gas Chromatography (GC) data by temperature and wort gravity. All values indicate mg/L and, for comparative purposes, the highest values for each flavour are indicated in red and the lowest in blue.

12.1. Fermentation conditions: 12°C and 12°P

	399X	34/70	4283	4403	Lutra	Nott	Voss
Acetaldehyde	9.675	6.488	13.338	7.595	7.885	8.636	8.848
Ethyl acetate	10.384	11.600	9.097	8.253	10.615	9.313	10.886
Isobutyl acetate	0.000	0.045	0.000	0.012	0.006	0.028	0.012
1-Propanol	18.102	17.302	13.840	13.745	16.139	29.106	14.149
Ethyl butyrate	0.053	0.038	0.034	0.044	0.042	0.060	0.045
Isobutanol	10.914	17.729	8.282	7.355	11.467	21.952	13.254
Isoamyl acetate	0.299	0.878	0.146	0.542	0.183	0.470	0.276
Isoamyl alcohol	102.509	118.501	93.462	103.386	107.790	105.833	110.802
Ethyl hexanoate	0.179	0.138	0.094	0.115	0.098	0.078	0.144
Ethyl octanoate	0.403	0.403	0.285	0.273	0.241	0.269	0.346

12.2. Fermentation conditions: 12°C and 16°P

	399X	34/70	4283	4403	Lutra	Nott	Voss
Acetaldehyde	9.075	12.197	17.685	14.454	8.688	6.693	5.444
Ethyl acetate	20.441	21.117	13.938	16.394	20.652	14.240	21.501
Isobutyl acetate	0.000	0.053	0.000	0.021	0.011	0.038	0.015
1-Propanol	17.897	24.017	16.269	15.843	17.826	41.800	16.292
Ethyl butyrate	0.081	0.065	0.057	0.063	0.073	0.069	0.069
Isobutanol	9.754	21.522	10.165	8.241	11.134	25.656	11.871
Isoamyl acetate	0.396	1.369	0.234	0.766	0.296	0.783	0.368
Isoamyl alcohol	104.318	137.013	102.046	103.647	110.296	144.478	120.983
Ethyl hexanoate	0.228	0.192	0.141	0.159	0.124	0.087	0.188
Ethyl octanoate	0.481	0.561	0.329	0.350	0.286	0.335	0.442

12.3. Fermentation conditions: 12°C and 24°P

	399X	34/70	4283	4403	Lutra	Nott	Voss
Acetaldehyde	9.862	13.401	8.481	12.005	13.489	15.478	11.334
Ethyl acetate	25.777	34.603	21.434	30.867	26.839	41.484	29.413
Isobutyl acetate	0.012	0.047	0.033	0.036	0.021	0.100	0.017
1-Propanol	20.527	22.929	21.648	20.926	22.996	65.412	19.693
Ethyl butyrate	0.115	0.083	0.103	0.118	0.097	0.111	0.091
Isobutanol	12.543	17.679	14.940	13.787	14.652	35.649	16.262
Isoamyl acetate	0.353	1.297	0.207	0.930	0.305	2.389	0.252
Isoamyl alcohol	85.407	128.240	97.932	101.752	80.403	166.259	90.064
Ethyl hexanoate	0.274	0.138	0.136	0.172	0.093	0.097	0.194
Ethyl octanoate	0.467	0.375	0.245	0.355	0.239	0.204	0.334

12.4. Fermentation conditions: 18°C and 12°P

	399X	34/70	4283	4403	Lutra	Nott	Voss
Acetaldehyde	3.635	8.185	8.870	13.325	10.459	4.953	3.674
Ethyl acetate	13.199	15.380	15.358	11.357	14.431	10.913	18.288
Isobutyl acetate	0.036	0.039	0.023	0.025	0.037	0.054	0.042
1-Propanol	21.824	17.675	19.764	18.472	19.650	27.206	19.913
Ethyl butyrate	0.052	0.033	0.047	0.036	0.066	0.047	0.051
Isobutanol	15.864	18.802	10.217	12.621	13.951	31.458	17.475
Isoamyl acetate	0.734	0.808	0.653	0.739	1.065	0.643	0.779
Isoamyl alcohol	144.524	127.773	117.624	124.726	168.214	132.078	134.001
Ethyl hexanoate	0.227	0.182	0.124	0.107	0.253	0.099	0.239
Ethyl octanoate	0.467	0.377	0.264	0.338	0.617	0.317	0.339

12.5. Fermentation conditions: 18°C and 16°P

	399X	34/70	4283	4403	Lutra	Nott	Voss
Acetaldehyde	11.274	14.028	13.998	6.692	5.918	4.400	5.897
Ethyl acetate	27.992	28.345	26.153	19.754	31.367	19.738	31.971
Isobutyl acetate	0.061	0.056	0.038	0.031	0.064	0.082	0.055
1-Propanol	24.477	26.650	19.861	26.621	26.391	38.520	20.892
Ethyl butyrate	0.095	0.054	0.080	0.059	0.131	0.058	0.082
Isobutanol	21.506	23.497	12.763	14.019	21.064	40.253	20.562
Isoamyl acetate	1.212	1.382	0.947	1.125	1.690	1.361	1.131
Isoamyl alcohol	144.576	146.607	118.786	147.924	149.239	184.961	143.282
Ethyl hexanoate	0.432	0.252	0.252	0.195	0.374	0.135	0.359
Ethyl octanoate	0.672	0.496	0.362	0.574	0.691	0.449	0.519

12.6. Fermentation conditions: 18°C and 20°P

	399X	34/70	4283	4403	Lutra	Nott	Voss
Acetaldehyde	5.314	13.149	11.832	9.173	5.922	10.774	12.520
Ethyl acetate	36.574	41.439	34.396	33.760	44.590	50.300	42.620
Isobutyl acetate	0.043	0.053	0.033	0.048	0.067	0.181	0.048
1-Propanol	26.336	35.993	25.921	22.895	30.773	67.706	24.284
Ethyl butyrate	0.111	0.065	0.091	0.099	0.151	0.124	0.117
Isobutanol	21.243	31.378	15.786	14.110	27.672	55.848	18.808
Isoamyl acetate	1.291	1.611	0.831	1.450	1.959	3.160	1.191
Isoamyl alcohol	155.485	157.937	129.295	121.422	157.754	230.983	133.412
Ethyl hexanoate	0.381	0.262	0.236	0.259	0.407	0.175	0.391
Ethyl octanoate	0.607	0.497	0.351	0.548	0.720	0.414	0.377

12.7. Fermentation conditions: 18°C and 24°P

	399X	34/70	4283	4403	Lutra	Nott	Voss
Acetaldehyde	8.919	9.538	8.109	9.445	7.352	13.048	9.869
Ethyl acetate	52.261	69.485	43.733	43.955	46.705	75.301	64.050
Isobutyl acetate	0.055	0.064	0.035	0.054	0.057	0.246	0.064
1-Propanol	33.342	32.480	32.820	30.271	30.044	90.187	31.481
Ethyl butyrate	0.157	0.088	0.120	0.118	0.158	0.143	0.165
Isobutanol	22.614	24.362	20.213	18.661	21.138	62.998	23.293
Isoamyl acetate	1.539	2.276	0.982	1.501	1.928	4.976	1.652
Isoamyl alcohol	140.422	153.568	133.203	117.377	139.916	231.702	147.320
Ethyl hexanoate	0.441	0.221	0.211	0.246	0.351	0.153	0.496
Ethyl octanoate	0.483	0.363	0.230	0.443	0.564	0.358	0.606

12.8. Fermentation conditions: 24°C and 12°P

	399X	34/70	4283	4403	Lutra	Nott	Voss
Acetaldehyde	3.060	14.083	4.043	2.552	7.420	5.483	6.465
Ethyl acetate	14.544	15.235	13.619	12.514	16.905	14.007	19.122
Isobutyl acetate	0.047	0.036	0.044	0.038	0.041	0.061	0.056
1-Propanol	16.800	16.276	16.824	17.042	21.405	26.467	17.413
Ethyl butyrate	0.053	0.023	0.040	0.033	0.061	0.034	0.052
Isobutanol	21.278	16.333	18.979	17.470	23.687	40.600	23.844
Isoamyl acetate	0.644	0.710	0.666	0.738	0.721	0.602	0.718
Isoamyl alcohol	119.253	123.343	110.064	118.035	134.422	140.385	120.943
Ethyl hexanoate	0.205	0.123	0.106	0.097	0.185	0.066	0.221
Ethyl octanoate	0.387	0.235	0.300	0.300	0.298	0.199	0.289

12.9. Fermentation conditions: 24°C and 16°P

	399X	34/70	4283	4403	Lutra	Nott	Voss
Acetaldehyde	6.507	16.877	3.119	4.094	9.430	7.660	9.531
Ethyl acetate	26.457	34.174	23.192	19.836	31.348	24.520	31.629
Isobutyl acetate	0.061	0.052	0.041	0.045	0.051	0.107	0.061
1-Propanol	21.126	23.834	19.100	16.706	27.329	37.405	22.025
Ethyl butyrate	0.094	0.050	0.073	0.055	0.113	0.045	0.084
Isobutanol	25.112	19.318	17.047	14.209	29.569	50.033	29.237
Isoamyl acetate	0.967	1.258	0.784	0.870	0.985	1.115	0.925
Isoamyl alcohol	126.882	135.630	108.623	93.751	128.979	179.293	132.750
Ethyl hexanoate	0.348	0.193	0.199	0.149	0.258	0.089	0.295
Ethyl octanoate	0.504	0.362	0.467	0.357	0.305	0.247	0.347

12.10. Fermentation conditions: 24°C and 20°P

	399X	34/70	4283	4403	Lutra	Nott	Voss
Acetaldehyde	5.971	11.758	3.294	3.966	8.269	12.133	12.759
Ethyl acetate	30.493	38.659	25.513	28.267	34.261	45.464	42.534
Isobutyl acetate	0.041	0.032	0.037	0.062	0.042	0.182	0.057
1-Propanol	24.173	22.114	23.384	17.135	30.975	55.237	26.108
Ethyl butyrate	0.110	0.055	0.076	0.128	0.129	0.079	0.113
Isobutanol	24.898	15.455	19.120	15.414	30.549	61.404	29.678
Isoamyl acetate	0.885	1.228	0.861	0.992	1.003	2.017	1.056
Isoamyl alcohol	121.946	123.559	118.650	80.524	137.882	200.122	132.321
Ethyl hexanoate	0.353	0.155	0.201	0.209	0.265	0.110	0.376
Ethyl octanoate	0.455	0.277	0.450	0.384	0.301	0.247	0.366

12.11. Fermentation conditions: 24°C and 24°P

	399X	34/70	4283	4403	Lutra	Nott	Voss
Acetaldehyde	7.742	9.768	5.320	6.113	8.169	10.949	11.844
Ethyl acetate	45.905	51.583	30.572	49.233	48.426	44.888	57.564
Isobutyl acetate	0.058	0.034	0.037	0.067	0.051	0.179	0.063
1-Propanol	27.241	28.199	26.967	29.608	34.543	63.966	34.504
Ethyl butyrate	0.181	0.094	0.085	0.123	0.211	0.081	0.152
Isobutanol	25.276	14.380	21.494	21.989	28.979	60.094	31.869
Isoamyl acetate	1.068	1.462	0.760	1.638	1.208	2.293	1.247
Isoamyl alcohol	107.267	117.499	105.303	121.259	125.140	206.387	131.104
Ethyl hexanoate	0.422	0.143	0.169	0.235	0.275	0.093	0.311
Ethyl octanoate	0.484	0.225	0.285	0.392	0.303	0.169	0.306

12.12. Fermentation conditions: 36°C and 12°P

	399X	34/70	4283	4403	Lutra	Nott	Voss
Acetaldehyde	14.083	4.349	15.813	16.774	18.657	6.282	3.961
Ethyl acetate	12.103	5.399	7.320	9.007	9.190	7.972	6.323
Isobutyl acetate	0.010	0.000	0.011	0.013	0.013	0.016	0.005
1-Propanol	10.026	8.436	9.030	10.578	11.347	16.978	7.335
Ethyl butyrate	0.017	0.000	0.009	0.013	0.016	0.017	0.009
Isobutanol	20.133	7.930	15.660	15.106	20.047	20.550	17.350
Isoamyl acetate	0.174	0.086	0.127	0.183	0.186	0.163	0.073
Isoamyl alcohol	84.102	53.914	84.844	68.933	95.051	81.107	83.931
Ethyl hexanoate	0.064	0.025	0.013	0.023	0.036	0.036	0.035
Ethyl octanoate	0.106	0.072	0.052	0.058	0.094	0.090	0.065

12.13. Fermentation conditions: 36°C and 16°P

	399X	34/70	4283	4403	Lutra	Nott	Voss
Acetaldehyde	11.196	6.204	9.446	18.002	12.206	4.831	7.042
Ethyl acetate	15.240	6.832	10.356	21.176	13.755	8.600	16.123
Isobutyl acetate	0.000	0.000	0.009	0.034	0.021	0.012	0.018
1-Propanol	12.138	8.688	11.628	15.767	13.501	17.363	11.314
Ethyl butyrate	0.019	0.000	0.011	0.035	0.024	0.009	0.031
Isobutanol	22.501	8.106	18.613	18.304	27.996	17.197	23.385
Isoamyl acetate	0.208	0.095	0.191	0.397	0.277	0.141	0.171
Isoamyl alcohol	108.318	53.280	104.554	72.318	116.288	72.846	87.582
Ethyl hexanoate	0.059	0.021	0.021	0.034	0.043	0.035	0.061
Ethyl octanoate	0.128	0.081	0.063	0.061	0.113	0.073	0.098

Appendix 13: Publications submitted and in preparation

Isbell, T & Powell, C. (2025). Best practices for kveik yeast management and fermentation. The Brewer & Distiller. Submitted.

Isbell, T., Li, Y. and Powell, C. D. (In Preparation). Flavour production in kveik yeast strains. Journal of the CIBD. To be submitted 2025.

Appendix 14: List of presentations

1. Brewers Congress – Todd Isbell Invited Speaker **(800+ attendees)**

2. CIBD Scottish Section Symposium - Todd Isbell Invited Speaker **(150+ attendees)**

3. University of Nottingham 2024 Brewing Apprentice Conference & Graduation Event - Todd Isbell Keynote Speaker **(250+ attendees)**

4. Teaching and Training:
    a. HIT Training Level 4 Lecture Series - Todd Isbell (multiple deliveries **100+ attendees)**
    b. University of Nottingham - Todd Isbell / Chris Powell Lectures to a mixture of UG and PG students on Brewing, Microbiology, Food Science and Biotechnology degree courses **(50+ attendees)**

5. University of Nottingham Bioscience Seminar Series - Todd Isbell Invited Speaker **(50+ attendees)**

Appendix 15: Example of presentation content

Presentation content can be found in the slides reproduced on the following pages.

ICBS International Centre for Brewing Science

The University of Nottingham
UNITED KINGDOM • CHINA • MALAYSIA

Invited Speaker
The Brewers Congress, 2004

Todd Isbell, Yishen Li & Chris Powell

Brewers' Research & Education Fund Brewers' Company

1

ICBS International Centre for Brewing Science

The University of Nottingham
UNITED KINGDOM • CHINA • MALAYSIA

Traditional Norwegian Kveik: Novel Yeast Strains and Beer Fermentation







Todd Isbell,
Yishen Li & Chris Powell

Brewers' Research & Education Fund Brewers' Company

2

What is Kveik?

The University of Nottingham
UNITED KINGDOM • CHINA • MALAYSIA

- Kveik are a subspecies of *Saccharomyces cerevisiae* (Ale) and originate from isolated farms or towns in rural Norway.
- These strains were often used indiscriminately for both brewing and the baking of bread.







- Kveik is not a beer style, but refers to any type of beer made with these particular yeast cultures.



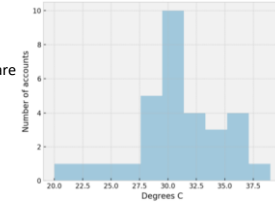
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What is Kveik?

The University of Nottingham
UNITED KINGDOM • CHINA • MALAYSIA

- In Norwegian, the word kveik means 'to bring a spark to' or 'bring life to.'
- Some kveik cultures comprise single strains
  - Others are mixtures, and some contain bacteria, such as *LAB* species.
- Kveik are POF- meaning they do not create phenolics, nor are they a *diastaticus* subspecies (super attenuator).
- These yeasts exhibit flocculation and are fully accustomed to consuming malt sugars
- They are famous for their ability to ferment well at high temperatures.

Pitch temperatures reported by owners of collected kveik strains (Garshol, 2021).



4

What is Kveik?

The University of Nottingham
UNITED KINGDOM • CHINA • MALAYSIA

- Traditional kveik beers can be made with 'alternative' ingredients, such as juniper.
- Contemporary hopped worts can also be used!





Unusual Yeast Handling



Kveikstokk



Gjærkrans

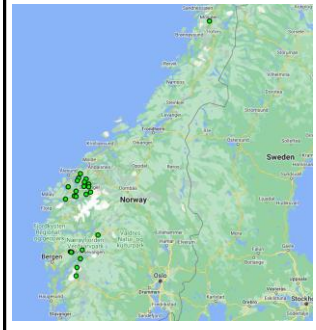


Dessication for long-term storage

5

Sources of Kveik Strains

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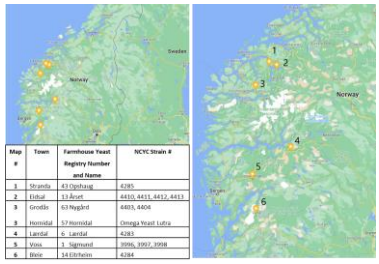


https://www.garshol.priv.no/download/farmhouse/kveik.html#kv51

6

Strains Used in This Study

Kveik



Map #	Town	Farmhouse Yeast Registry Number and Name	NCYC Strain #
1	Stranda	43 Opshaug	4285
2	Eidsal	13 Åraet	4410, 4411, 4412, 4413
3	Grodås	63 Nygård	4403, 4404
3	Hornidal	57 Hornidal	Omega Yeast Lutra
4	Lærdal	6 Lærdal	4283
5	Voss	1 Sigmund	3996, 3997, 3998
6	Ekre	14 Eikheim	4284

Conventional

Yeast Identifier	Type
Nottingham	Ale
NCYC 1332	Ale
M2	Ale
CBS 1174	Lager (Saaz)
CBS 1260	Lager (Frohberg)
W34/70	Lager (Frohberg)

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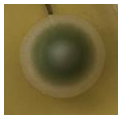
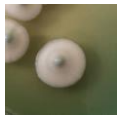
Aims & Objectives

- Develop a simple method for identifying isolates within kveik cultures
- Analyse the attributes of kveik yeast in comparison to conventional strains
  - Stress tolerance
  - Fermentation performance
- Determine the relative contribution of yeast strains from mixed kveik cultures
  - Population dynamics
  - Flavour production
- Investigate yeast handling properties of kveik strains
  - Tolerance to storage
  - Acid washing

8

How to Differentiate Kveik Strains

- Typically, genetic tools are used to identify and characterise yeast strains.
- This approach is not always suitable for mixed cultures and culture-based methods remain the preferred approach.
- How can we differentiate kveik strains ?
- Which methods can we use to analyse mixed cultures of cells ?
- Colony morphology analysis

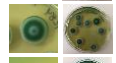


9

Kveik Strain Colony Morphology

Single Strain Cultures

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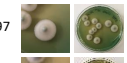

2-Strain Culture

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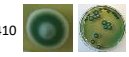
4404


3-Strain Culture

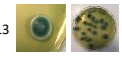
3996

3997

3998


4-Strain Culture

4410

4411

4412

4413


10

ICBS International Centre for Brewing Science

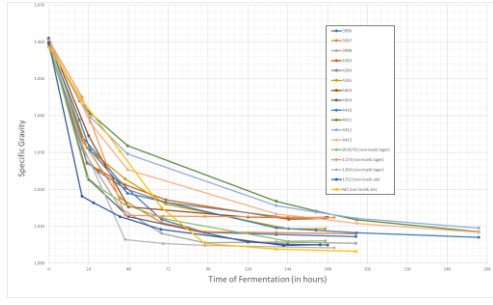
What Are The Fermentation Properties of Kveik Strains ?




11

Sugar Utilisation (gravity)

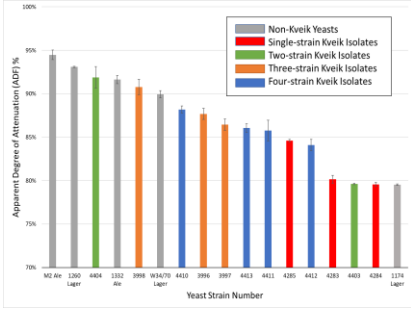
Wort gravity (1.060)
Pitch rate (1 million cells/mL/°P)
All fermentations 20°C



12

Apparent Degree of Fermentation

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13

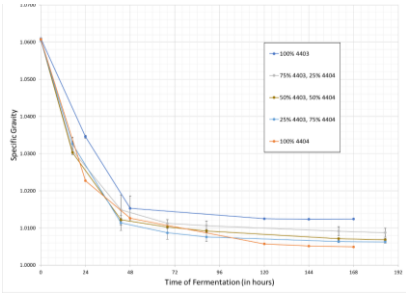
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14

Fermentation Performance



Example from 2-strain Culture

4403

4404

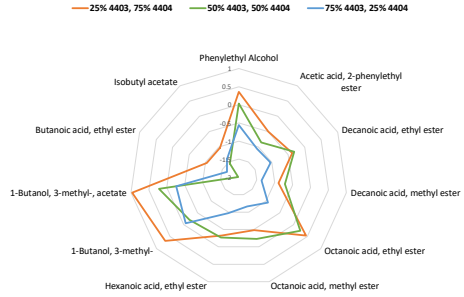


Isbell & Powell (Article In Preparation)

Note: Also performed using a 3-strain culture (data not shown here)

15

Flavour Production

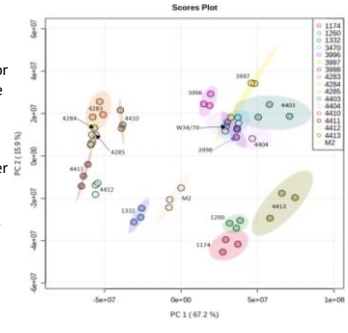


Isbell & Powell (Article In Preparation)

16

Flavour Mapping – APCI-MS

- This method provides a characteristic 'fingerprint' for each strain based on volatile compound production.
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- Strains show distinct flavour profiles, even within mixed isolate cultures.



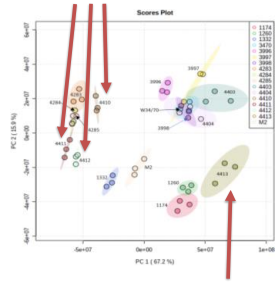
Isbell & Powell (Article In Preparation)

17

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Isolates 4410, 4411, 4412



Isolate 4413

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18

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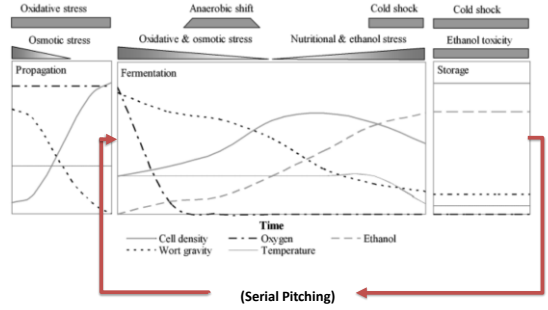
What Are the physiological capacities of Kveik strains? How do they handle Stress?





19

Fermentation Related Stressors



Gibson et al., 2007

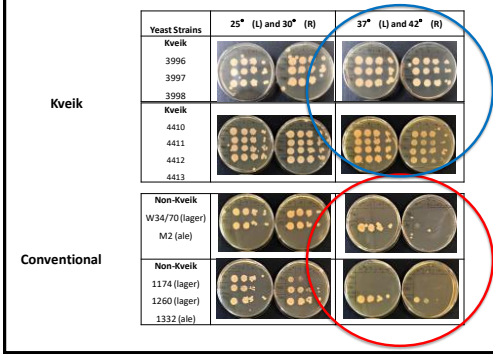
20

Kveik Response to Stress

- Tolerance to alcohol
  - Capacity to grow in the presence of ethanol
- Tolerance to high temperature
  - Ability to proliferate at high temperatures
- Tolerance to osmotic stress
  - Ability to adapt to changing osmotic conditions and to survive in conditions of high osmolality
- Tolerance to oxidative stress
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21

Response of Yeast to Thermal Stress



22

Stress tolerance: Summary

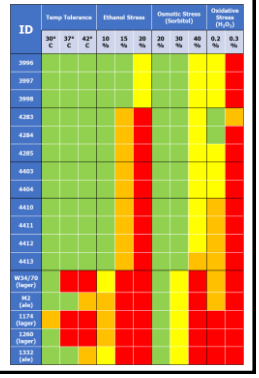
- Temperature (30°C, 37°C, 42°C)
- Ethanol (10%, 15%, 20%
- Osmotic (20%, 30%, 40%)
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- Tolerance is indicated by colour
  - Red = low
  - Green = High

Kveik

- Kveik strains are universally robust

Conventional



Isbell & Powell (Article In Preparation)

23

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Can Kveik Strains be Handled in the Same Way as Conventional Brewing Yeasts?





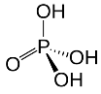
24

Response to cold storage & acid washing

- **Cold Storage**
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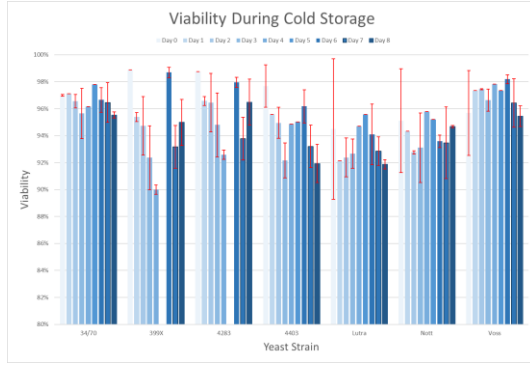


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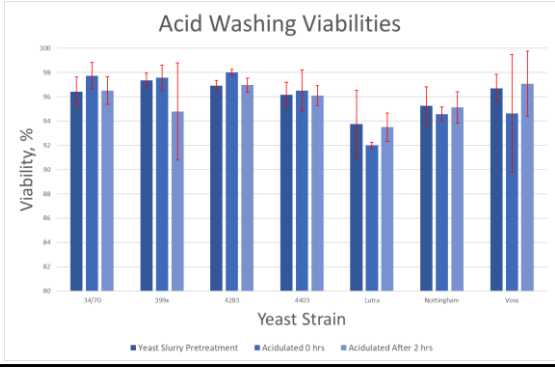
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Response to Cold Storage



26

Response of Yeast to Acid Washing



27

Current & Future Work

- **What is the capacity of kveik strains to perform at high gravity ?**
  - High (16-18° Plato), very high (18-22° Plato) and ultra high (>22° Plato) gravity worts.
  - Fermentation performance & flavour production analysed.
- **How does temperature affect the flavour profile of beer produced using kveik strains ?**
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  - Is it possible to produce a lager-type product at elevated temperatures using kveik?
- **Explore performance, flavour and aroma profiles of kveik isolates when fermenting non-malt based substrates.**
  - Wine, mead, cider, seltzers, RTDs, Biofuels.

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Summary

- Kveik isolates in mixed cultures can be identified based on colony morphology.
- Isolates within multi-strain cultures have individual characteristics.
  - Fermentation profiles and flavour production.
  - The use of mixed cultures can introduce complexity, but also offers unique performance.
- Kveik strains exhibit positive response to real-life brewing stressors.
  - Kveik strains can ferment wort to create beers in less time than many contemporary yeasts.
  - More tolerant to most industrially-relevant stress factors than conventional strains.
- Kveik yeasts show similar handling properties to standard brewing yeasts.
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- The potential of kveik strains across the fermentation industry remains to be fully appreciated.

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Acknowledgements & Resources

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- Lars Marius Garshol's Blog: https://www.garshol.priv.no/
- Farmhouse Yeast Registry: https://www.garshol.priv.no/download/farmhouse/kveik.html
- Viking Age Brew, by Mika Laitinen
- Brewing Nordic Website, by Mika Laitinen: https://www.brewingnordic.com/

Brewers' Research & Education Fund Brewers' Company @Brewers' Hall

30

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The University of Nottingham
UNITED KINGDOM • CHINA • MALAYSIA

Invited Speaker
Chartered Institute of Brewers and Distillers – Scottish Section

Todd Isbell, Yishen Li & Chris Powell

Brewers' Research & Education Fund Brewers' Company of Brewers' Hall

1

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The University of Nottingham
UNITED KINGDOM • CHINA • MALAYSIA

Traditional Norwegian Kveik: Novel Yeast Strains and Beer Fermentation







Todd Isbell,
Yishen Li & Chris Powell

Brewers' Research & Education Fund Brewers' Company of Brewers' Hall

2

What is Kveik?

The University of Nottingham
UNITED KINGDOM • CHINA • MALAYSIA

- Kveik are a subspecies of *Saccharomyces cerevisiae* (Ale) and originate from isolated farms or towns in rural Norway.
- 'Word-of-mouth' heritage
  - Little written records



- Kveik is not a beer style, but refers to any type of beer made with these particular yeast cultures.



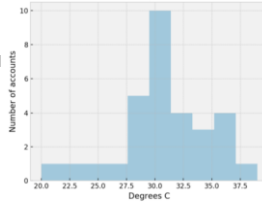
3

What is Kveik?

The University of Nottingham
UNITED KINGDOM • CHINA • MALAYSIA

- Some kveik cultures comprise single strains
  - Others are mixtures, and some contain bacteria, such as *LAB* species.
- Kveik are POF- meaning they do not create phenolics, nor are they a *diastaticus* subspecies (super attenuator).
- These yeasts exhibit flocculation and are fully accustomed to consuming malt sugars
- They are famous for their ability to ferment well at high temperatures.

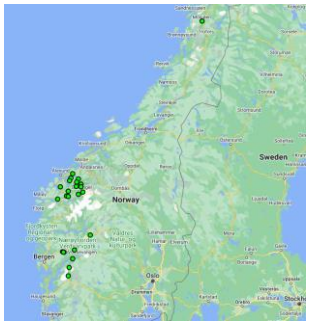
Pitch temperatures reported by owners of collected kveik strains (Garshol, 2021).

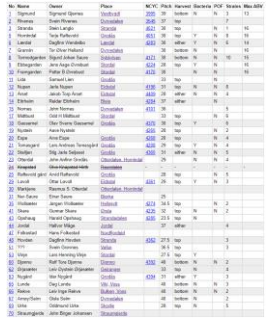


4

Sources of Kveik Strains

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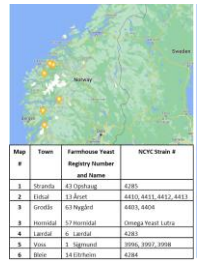
https://www.garshol.priv.no/download/farmhouse/kveik.html#kv51

5

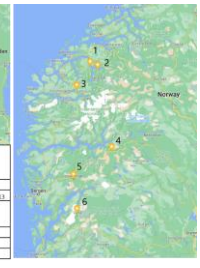
Strains Used in This Study

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Kveik



Map #	Town	Farmhouse Yeast Registry Number and Name	NCYC Strain #
1	Stranda	43 Djohvag	4285
2	Eidsal	53 Åsvet	4410, 4411, 4412, 4413
3	Grodås	63 Nygård	4403, 4404
3	Hornidal	57 Hornidal	Omega Yeast Lutra
4	Lærdal	6 Lærdal	4283
5	Voss	1 Sigmund	3996, 3997, 3998
6	Weie	14 Eikrheim	4284



Conventional

Yeast Identifier	Type
Nottingham	Ale
NCYC 1332	Ale
M2	Ale
CBS 1174	Lager (Saaz)
CBS 1260	Lager (Frohberg)
W34/70	Lager (Frohberg)

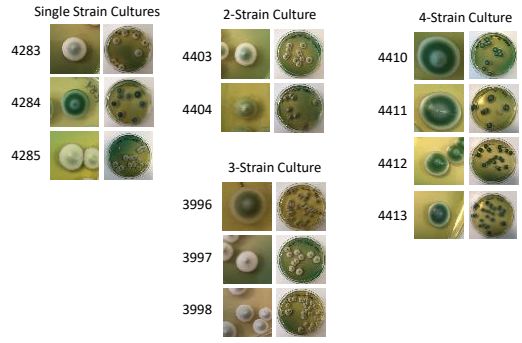
6

Aims & Objectives

- Develop a simple method for identifying isolates within kveik cultures
  - Genetic tools are not always suitable for mixed cultures
- Analyse the attributes of kveik yeast in comparison to conventional strains
  - Stress tolerance
  - Fermentation performance
- Determine the relative contribution of yeast strains from mixed kveik cultures
  - Population dynamics
  - Flavour production
- Investigate yeast handling properties of kveik strains
  - Tolerance to storage
  - Acid washing

7

Strain Identification - Colony Morphology



8

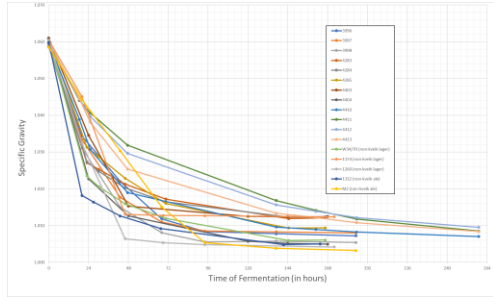
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9

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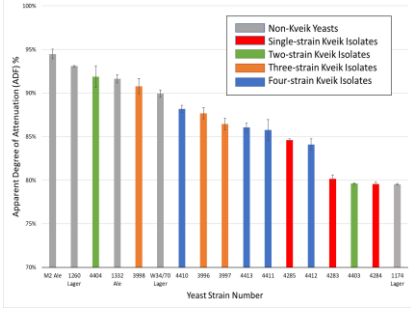
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Pitch rate (1 million cells/mL/°P)
All fermentations 20°C



10

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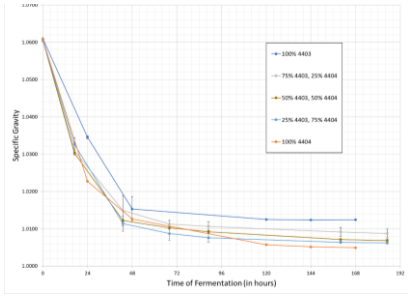
11

What is the Relative Contribution of Individual Isolates Within a Kveik Culture ?



12

Isolates within Mixed Culture Fermentation Performance



Example from 2-strain Culture

4403

4404



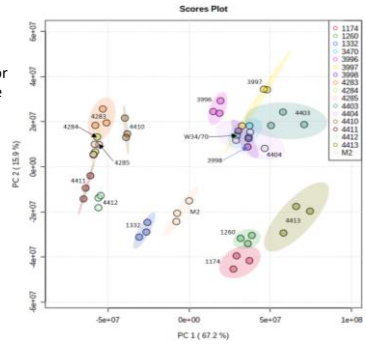
Isbell & Powell (Article In Preparation)

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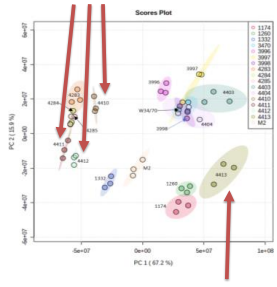
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Isolate 4413

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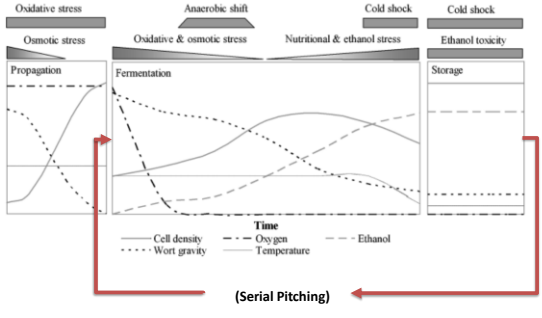
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Gibson et al., 2007

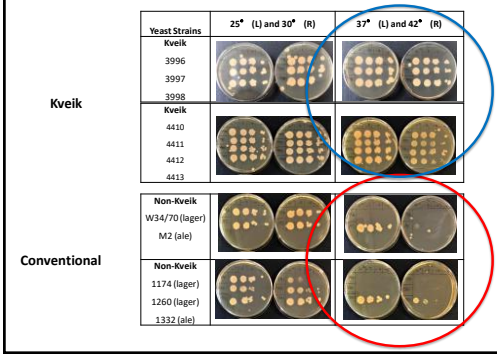
17

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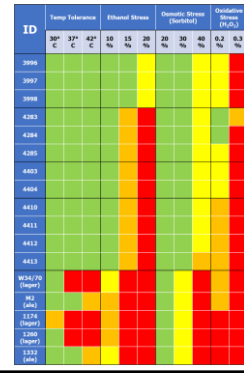
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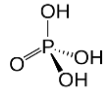
21

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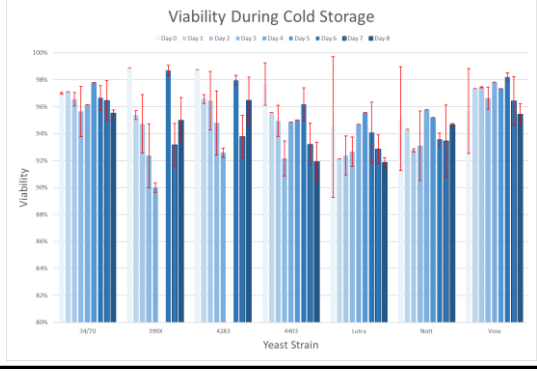


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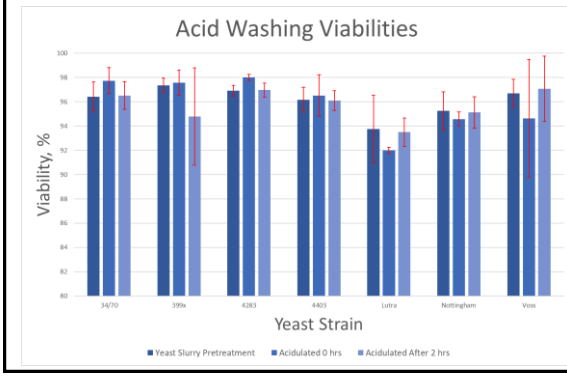
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Response to Cold Storage



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Response of Yeast to Acid Washing



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Current & Future Work

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- Viking Age Brew, by Mika Laitinen
- Brewing Nordic Website, by Mika Laitinen: https://www.brewingnordic.com/

Brewers' Research & Education Fund Brewers' Company @Brewers' Hall

27



28

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Keynote Speaker
2024 Brewing Apprenticeship Conference
University of Nottingham

Todd Isbell, Yishen Li & Chris Powell

Brewers' Research & Education Fund Brewers' Company

1

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UNITED KINGDOM • CHINA • MALAYSIA

Traditional Norwegian Kveik: Novel Yeast Strains and Beer Fermentation







Todd Isbell
Chris Powell & Yishen Li

Brewers' Research & Education Fund Brewers' Company

2

What is kveik?

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- Kveik are a subspecies of *Saccharomyces cerevisiae* (Ale) and originate from isolated farms or towns in rural Norway.
- These strains were often used indiscriminately for both brewing and the baking of bread.







- Kveik is not a beer style, but refers to any type of beer made with these particular yeast cultures.



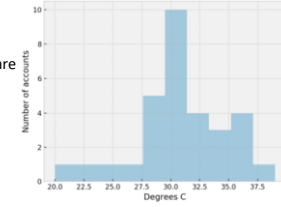
3

What is kveik?

The University of Nottingham
UNITED KINGDOM • CHINA • MALAYSIA

- In Norwegian, the word kveik means 'to bring a spark to' or 'bring life to.'
- Some kveik cultures comprise single strains
  - Others are mixtures, and some contain bacteria, such as *LAB* species.
- Kveik are POF- meaning they do not create phenolics, nor are they a *diastaticus* subspecies (super attenuator).
- These yeasts exhibit flocculation and are fully accustomed to consuming malt sugars
- They are famous for their ability to ferment well at high temperatures.

Pitch temperatures reported by owners of collected kveik strains (Garshol, 2021).



4

What is kveik?

The University of Nottingham
UNITED KINGDOM • CHINA • MALAYSIA

- Traditional kveik beers can be made with 'alternative' ingredients, such as juniper.
- Contemporary hopped wort is often used.







Kveikstokk



Gjærkrans

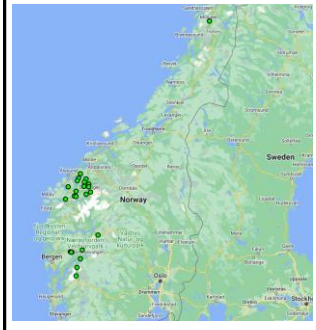


Dessication for long-term storage

5

Sources of kveik strains

The University of Nottingham
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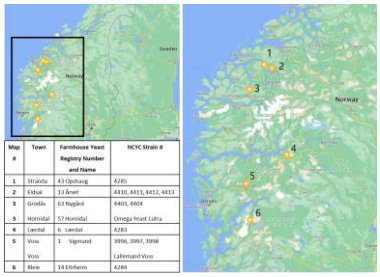


https://www.garshol.priv.no/download/farmhouse/kveik.html#kv51

6

Strains used in this study

Kveik



Map #	Town	Farmhouse Yeast Registry Number and Name	NCYC Strain #
1	Strandа	43 Opshaug	4285
2	Eidsvl	13 Arvet	4410, 4411, 4412, 4413
3	Grodås	63 Nygård	4403, 4404
3	Homstøl	57 Homstøl	Omega Yeast Lutra
4	Lærdal	6 Lærdal	4283
5	Voss	1 Sigmund	3996, 3997, 3998
	Voss		Lallemand Voss
6	Bøle	14 Elthelm	4284

Conventional

Yeast Identifier	Type
Nottingham	Ale
NCYC 1332	Ale
M2	Ale
CBS 1174	Lager (Saaz)
CBS 1260	Lager (Frohberg)
W34/70	Lager (Frohberg)

7

Aims & Objectives

- Analyse the attributes of kveik in comparison to conventional strains
  - Stress tolerance
  - Fermentation performance
- Determine the relative contribution of yeast strains from mixed kveik cultures
  - Population dynamics
  - Flavour production
- Investigate yeast handling properties of kveik strains
  - Tolerance to storage
  - Acid washing

8

What Are The Fermentation Properties of Kveik Strains ?

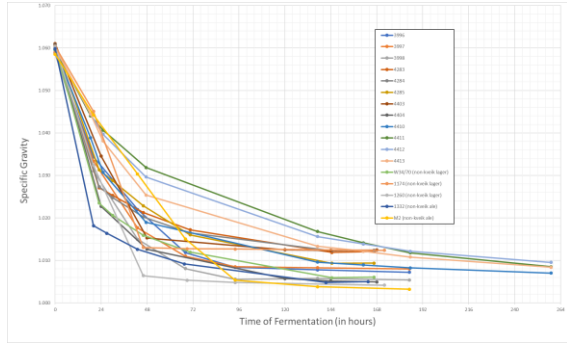




9

Sugar Utilisation (gravity)

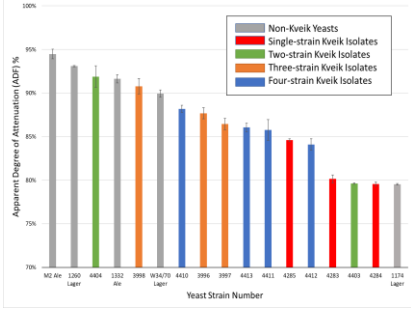
Wort gravity (1.060) Pitch rate (1 million cells/mL/°P) All fermentations 20°C



10

Apparent degree of fermentation

Wort gravity (1.060)
Pitch rate (1 million cells/mL/°P)
All fermentations 20°C



11

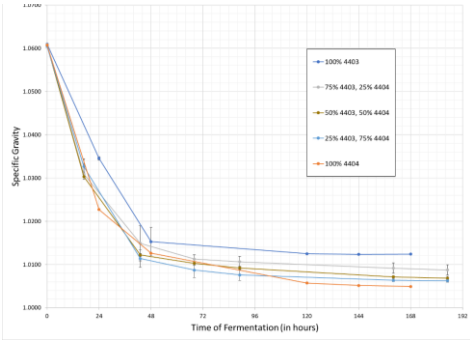
What is the Relative Contribution of Individual Isolates Within a Kveik Culture ?





12

Fermentation Performance



Isbell & Powell (Article In Preparation)

Note: Also performed using a 3-strain culture (data not shown here)

13

ICBS International Centre for Brewing Science

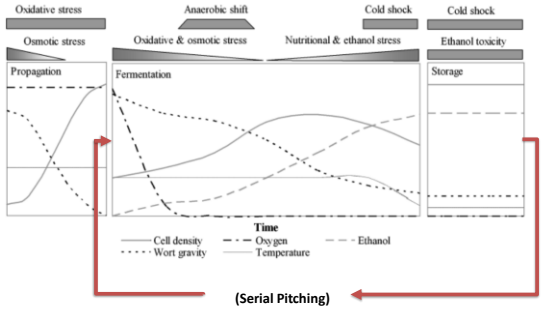
What Are the physiological capacities of Kveik strains? How do they handle Stress?





14

Fermentation relates stressors



Gibson et al., 2007

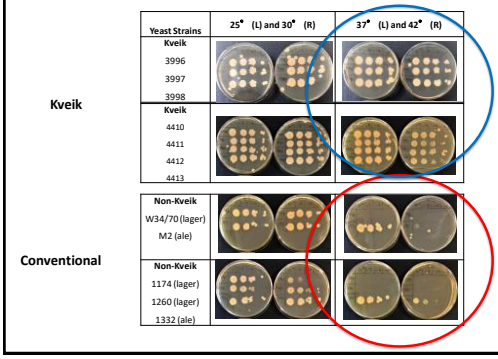
15

Kveik Response to Stress

- Tolerance to alcohol
  - Capacity to grow in the presence of ethanol
- Tolerance to high temperature
  - Ability to proliferate at high temperatures
- Tolerance to osmotic stress
  - Ability to adapt to changing osmotic conditions and to survive in conditions of high osmolality
- Tolerance to oxidative stress
  - Ability to breakdown or resist free radicals produced during fermentation
- **How do kveik strains compare to conventional brewing yeasts ?**

16

Response of yeast to thermal stress



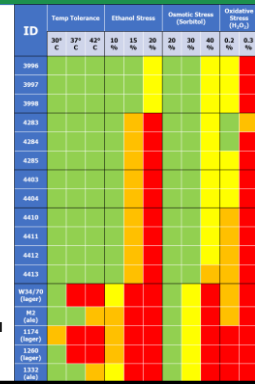
17

Stress tolerance: Summary

- Temperature (30°C, 37°C, 42°C)
- Ethanol (10%, 15%, 20%
- Osmotic (20%, 30%, 40%)
- Oxidative (0.2%, 0.3%)

- Tolerance is indicated by colour
  - Red = low
  - Green = High

- Kveik strains are universally robust



Isbell & Powell (Article In Preparation)

18

Current & Future Work

- **What is the capacity of kveik strains to perform at high gravity ?**
  - Standard (12°P), High (16°P), very high (20°P), and ultra high (24°P) gravity worts.
  - Fermentation performance & flavour production analysed.
- **How does temperature affect the flavour profile of beer produced using kveik strains ?**
  - What are the effects of low/high fermentation temperature on flavour production in kveik?
  - What is the maximum temperature that can be applied to rapidly create acceptable ale-type beers?
  - Is it possible to produce a lager-type product at elevated temperatures using kveik?
- **Explore performance, flavour and aroma profiles of kveik isolates when fermenting non-malt based substrates.**
  - Wine, mead, cider, seltzers, RTDs, Biofuels.

19

Summary

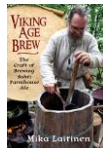
- Isolates within multi-strain cultures have individual characteristics.
  - Fermentation profiles and flavour production.
  - The use of mixed cultures can introduce complexity, but also offers unique performance.
- Kveik strains exhibit positive response to real-life brewing stressors.
  - Kveik strains can ferment wort to create beers in less time than many contemporary yeasts.
  - More tolerant to most industrially-relevant stress factors than conventional strains.
- Kveik yeasts show similar handling properties to standard brewing yeasts.
  - Resistant to acid washing.
  - Robust during cold storage.
- The potential of kveik strains across the fermentation industry remains to be fully appreciated.

20

Acknowledgements & Resources

Brewers Research & Education Fund

University of Nottingham Flavour Laboratory
University of Nottingham Fermentation Suite
University of Nottingham Research Pilot Brewery





- Historical Brewing Techniques: The Lost Art of Farmhouse Brewing by Lars Marius Garshol
- Lars Marius Garshol's Blog: https://www.garshol.priv.no/
- Farmhouse Yeast Registry: https://www.garshol.priv.no/download/farmhouse/kveik.html
- Viking Age Brew, by Mika Laitinen
- Brewing Nordic Website, by Mika Laitinen: https://www.brewingnordic.com/

Brewers' Research & Education Fund Brewers' Company

21



22

Teaching and Training

1. University of Nottingham Brewing Apprenticeship Scheme in partnership with Hospitality Industry Training (HIT)
2. University of Nottingham Degree Courses delivery to UG and PG students on Brewing, Microbiology, Food Science and Biotechnology degree courses

Todd Isbell, Yishen Li & Chris Powell

Brewers' Research & Education Fund Brewers' Company

1

Traditional Norwegian Kveik: Novel Yeast Strains and Beer Fermentation





Todd M. Isbell

Based on thesis submitted to the University of Nottingham for the degree of Master of Research from Nov 2021 to Sept 2022 titled: Assessment of Stress Tolerance and Fermentation Performance in Traditional Norwegian Landrace (Kveik) Yeast.

2

What is Beer?

Beer is an alcoholic beverage where the sugars are supplied by a grain. It consists of: malted barley, hops, water, and yeast







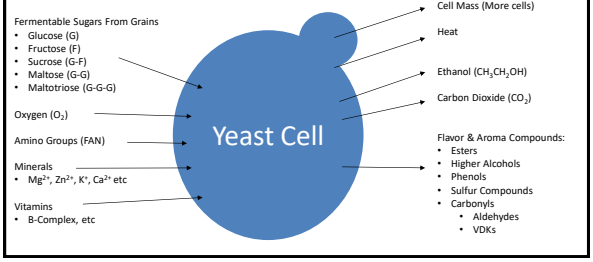




3

What is Beer Fermentation?

Fermentation of beer is the process where yeast consumes the sugars and nutrients extracted from grains and produces CO_2, alcohol, and a multitude of flavour and aroma compounds



4

There are two main types of Brewing Yeast:

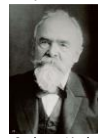
Ales	Lagers
Saccharomyces cerevisiae	*Saccharomyces pastorianus*

Differences Between Ale and Lager Yeast	
Ales	**Lagers**
Can grow up to 37˚C	Cannot grow above 34˚C
Typically used in fermentations at 18˚C - 22˚C	Typically used in fermentations at 8˚C - 15˚C
Faster fermentation at several days	Slower fermentations at 1 -3 weeks
Higher ester and higher alcohol production	Lower ester and higher alcohol production
Lower sulphur production	Higher sulphur production
Cannot ferment melibiose	Able to ferment melibiose

5

Throughout history, beers were fermented by a multitude of yeasts and bacteria


Louis Pasteur


Carl von Linde


Emil Hansen


Harry Brearley

- 1864: Pasteurisation
- 1873: Refrigeration
- 1883: Pure culture isolation
- 1913: Stainless Steel

Now brewers can produce beers created from a single culture with less bacterial infections to produce 'clean' ales and lagers with longer shelflife.

The vast majority of beers consumed in the world today are created using these methods

6

What is Kveik?

- Kveik are a unique subspecies of *Saccharomyces cerevisiae* which originate from isolated farms or towns in rural Norway.
  - Yeast cultures traditionally passed down between generations within a family a farming region for decades (perhaps centuries).
  - Little written historical information available as most and techniques were passed down generationally by word-of-mouth.
- These strains were often used indiscriminately for both brewing and the baking of bread.







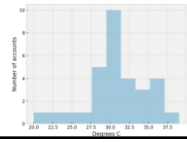
- Kveik is not a beer style, but refers to any type of beer style made with those particular yeast cultures.



7

What is Kveik?

- In Norwegian, the word kveik means 'to bring a spark to' or 'bring life to.'
- Some Kveik possess single cultures, but others are comprised of multiple distinct strains (Tormodgarden has 10 known isolates). Some do contain bacteria, such as *LAB* species.
- Kveik are POF- meaning they do not create phenolics, nor are they a *diastaticus* subspecies (super attenuator).
- These yeasts exhibit flocculation and are fully accustomed to consuming malt sugars
- They are famous for fermenting at very high temperatures relative to standard commercial ale strains without creating off flavours. Temperatures often exceed 30°C and many approach 40°C. They are known to create fruity and tropical flavours and aromas.



Pitch temperatures reported by the owners of collected kveiks (Garshol, 2021).

8

What is Kveik?

While hops are often used for flavour, aroma, and for its antimicrobial properties, often Kveik beers are made using teas of juniper or juniper as the base filter in their mash tuns.





Unique Yeast Handling



Kveikstokk



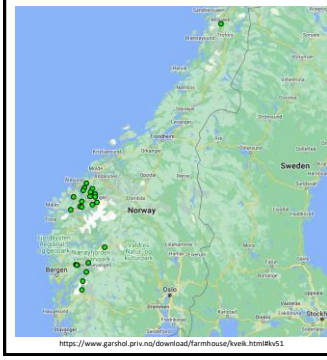
Gjærkrans



Dessication for long-term storage

9

Known Kveik Sources



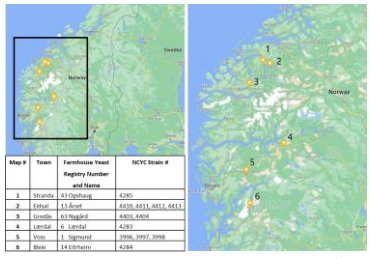
https://www.garshol.priv.no/download/farmhouse/kveik.html#kv51

Kveik often acquire their names from the surname of the family or the location of the farm or town.

No.	Name	Owner	Place	NCYC	Pitch	Hanover	Bacteria	POF	Strains	Max ABV
1	Sigmund	Sigmund Gjernes	Hjelmeland	3385	30	bottom	N	N	1	10
2	Rosnes	Svein Rosnes	Dyrvekjeller	3640	37	top			1	
3	Granvik	Stein Lengvik	Granvik	4521	30	top		N	1	16
4	Hornnes	Tarjei Rafthevold	Grindflo	4552	30	top	Y	N	0	16
5	Lauvtun	Dagfinn Mandelkow	Lauvtun	4283	30	either	Y	N	0	14
6	Granvin	Tor Olav Hetland	Dyrvekjeller		30	bottom	N	N	0	16
7	Tormodgarden	Sigurd Johan Skaare	Stallkroken	4171	30	bottom	N	N	10	16
8	Ellingsnæs	Jens Aage Christiansen	Ellingsnæs	4229	30	top	Y	N	0	16
9	Farmgarden	Petter B Grindstuen	Grindflo	4173	30		N	N	0	16
10	Lida	Samuel Lida	Grindflo		33	top		N	0	
11	Kvasir	John Kvasir	Kvasir	4196	33	top	N	N	0	
12	Aved	Jakob Tarp Aved	Kvasir	4283	30	either	N	N	0	3
13	Eikheim	Reidar Eikheim	Reko	4284	37	either		N	0	
14	Nonnes	John Nonnes	Dyrvekjeller	4191	96				0	5
15	Wolfkvil	Odd H Wolfkvil	Grindflo		33	top			N	5
16	Gausemel	Olav Gravna Gausemel	Grindflo	4375	90	top	Y		N	6
17	Kyrkjeb	Aave Kyrkjeb	Grindflo	4284	28	top		N	0	4
18	Espe	Arne Espe	Grindflo	4286	29	top		N	N	4
19	Tomasgard	Lars Andreas Tomasgard	Grindflo	4285	29	top	Y	N	0	4
20	Nerbø	Stig Jarle Nerbøvik	Grindflo	4380	31	either	N	N	N	5
21	Vikerlid	John Arthur Grindlo	Vikerlidens, Hornindal		29		N	N	0	5
22	Voss	Olve Haugland Hille	Vossakilden							
23	Hothmyr	Hothmyr gard	Grindflo		33	top			N	5
24	Lund	Stig Lund	Lund	4282	29	top	Y	N	0	5
25	Mathisen	Rasmus S. Vikerlid	Vikerlidens, Hornindal							
26	Ten Oever	Olve Grindlo	Maska		25					
27	Hothmyr	Jørgen Hothmyr	Hothmyr	4279	34.5	top		N	0	2
28	Øksne	Gunnar Øksne	Grindflo	4278	30	top	N	N	0	4
29	Gjerkeng	Harald Gjerkeng	Gråmålarstuen	4285	27.5	top	N			
30	Jordal	Halfdan Mage	Jordal		37	either				4
31	Folkestad	Hans Folkestad	Nordfjordeid							
32	Nordtun	Dagfinn Nordtun	Granvik	4282	27.5	top			0	
33	???	Svein Grannes	Valley		30.5	top			0	
34	Voje	Lars Henning Voje	Grindflo		27.5	top	Y		0	
35	Djønno	Rolf Tore Djønno	Djønno	4282	40	bottom	N	N	0	2
36	Grytøyrstøl	Lars Gystein Grytøyrstøl	Grytøyrstøl		35	top	N		0	4
37	Nygård	Ole Nygård	Grindflo	4284	31	either	Y		0	5
38	Lunde	Stig Lunde	Vik Vare		40	bottom	N	N	0	5
39	Bakke	Lars Inge Bakke	Bakkeli, Voss		40	bottom	N	N	0	2
40	Arvey Gaia	Gaia Gaino	Dyrvekjeller		40	bottom	N		0	2
41	Ulma	Oddmund Ulma	Ulmaflo		30	top	N		0	5
42	Skrungarde	John Birger Johansen	Skrungarde							

10

Kveik Used in the Study



Map #	Town	Farmhouse Yeast Registry Number and Name	NCYC Strain #
1	Stranda	43 Opshaug	4285
2	Eidsøl	13 Aved	4430, 4431, 4612, 4413
3	Grodås	63 Nygård	4403, 4404
4	Lærdal	6 Lærdal	4283
5	Voss	1 Sigmund	3996, 3997, 3998
6	Reko	14 Eikheim	4284

Contemporary Brewing Yeasts in Study

Yeast Identifier	Origination of Strain
NCYC 1332	National Collection of Yeast Cultures, *Saccharomyces cerevisiae*
M2	Oxford Brookes University Brewing Yeast Culture Collection, *Saccharomyces cerevisiae*
CBS 1174	Westerdijk Fungal Biodiversity Institute, *Saccharomyces pastorianus*
CBS 1260	Westerdijk Fungal Biodiversity Institute, *Saccharomyces pastorianus*
W34/70	Hefebank Weihenstephan, *Saccharomyces pastorianus*

11

Goals of the Study

- Investigate distinctiveness between isolates within multiple strain cultures
- To compare the responses of kveik isolates to various stressors that are present during normal beer fermentations:
  - Oxidative Stress
  - Osmotic Stress
  - Alcoholic Stress
  - Thermal Stress
- Compare fermentation performance between isolates (rate of fermentation and degree of attenuation) both within multiple strain cultures as well as compared to contemporary brewing yeast strains, both ales and lager.
- Compare flavour production of kveik isolates within their respective cultures

12

What is the colony morphology of kveik strains?

Can we use this as a method for differentiation?

13

Kveik Morphology Using WLN Media

3-Strain Culture

3996

3997

3998



2-Strain Culture

4403

4404

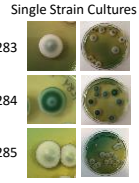


Single Strain Cultures

4283

4284

4285



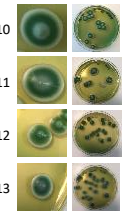
4-Strain Culture

4410

4411

4412

4413



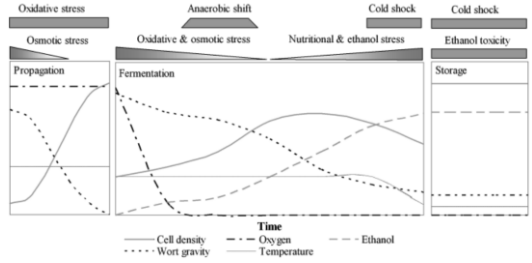
14

Key stress factors that yeast are subjected to during fermentation

15

Various Stressors Experienced by Yeast

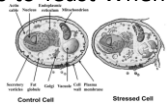
Oxidative, Osmotic, Alcoholic, and Thermal stresses were examined in the study



Stress factors associated with brewery yeast handling (Gibson et al., 2007)

16

What Happens to Yeast When it is Stressed?

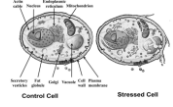


Artistic impression of the effect of stress factors on yeast organelles (Stewart, 2020).

- **Oxidative Stress**
  - Reactive Oxygen Species (ROS) including molecular oxygen (O_2), but also superoxide (O_2^-), peroxide anions (O_2^{2-}), hydrogen peroxide (H_2O_2), hydroxyl radicals ($OH\bullet$), and perhydroxyl radicals ($OOH\bullet$).
  - These degrade lipids causing membrane fluidity and damage cellular proteins and DNA.
  - This can induce petites in mitochondria and create mutations.
- **Osmotic Stress**
  - Cell transitions from nutrient deficiency to a nutrient rich environment at the onset of fermentation
  - Concentration gradient across the cell membrane. This gradient can cause water to leak out of the cell as well as solutes into the cell.
  - This slows cell growth, can stall fermentation, and decrease viability.

17

What Happens to Yeast When it is Stressed?



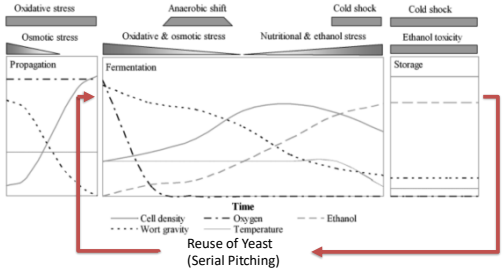
Artistic impression of the effect of stress factors on yeast organelles (Stewart, 2020).

- **Alcohol Stress**
  - Ethanol is a key product created by yeast during fermentation
  - When excessive, ethanol harms membranes and denatures nutrient transport proteins within the cell
  - Degrades cell fluidity
  - Decrease cell viability, including during storage.
  - Can also instigate the production of mitochondrial petites.
- **Thermal Stress**
  - Proteins can be denatured and will directly affect cellular health.
  - Higher temperatures also cause off flavours in beer, particularly higher esters and higher alcohols.
  - When excessive, high temperatures cause cell death.
  - High temperatures exacerbate all other stressors.

18

Serial Repitching of Yeast (Cyclic Stressing)

All negative stressor outcomes are magnified due to serial repitching.



Reuse of Yeast
(Serial Pitching)

19

Yeast Stress Response

Saccharomyces species possess a stress response element (STRE) system that proves to resolve the negative effects of a wide variety of environmental stresses factors

- **Oxidative Stress**
  - Non-enzymatic: Trehalose and Glutathione
    - Trehalose assists in protecting the plasma membrane and enzymes as well as enabling the repair of proteins.
    - Glutathione scavenges radical molecules and reduces oxidants.
  - Enzymatic sources include peroxidases and reductases, as well as catalase which break down ROS species to less harmful compounds
- **Osmotic Stress**
  - Yeast accumulates osmoprotectants such as glycerol, which stabilize cellular components including membranes and proteins.
  - Trehalose, which also assists in oxidative, alcohol, and thermal stability, acts to protect the cell membrane.

20

Yeast Stress Response

Saccharomyces species possess a stress response element (STRE) system that proves to resolve the negative effects of a wide variety of environmental stresses factors

- **Ethanol Stress**
  - At the onset of fermentation, yeast utilize oxygen to develop unsaturated fatty acids and sterols in the cell membrane. This assures efficient membrane transport and assists the cell in combating multiple stresses, including ethanol.
  - Trehalose also plays a role in ethanol tolerance due to its protective effects on the cell membrane.
- **Thermal Stress**
  - The genetic reaction to thermal stress is called Heat Shock Response (HSR).
    - When increasing temperature damage proteins, STRE stimulates the production of Heat Shock Proteins (HSP).
    - These act as 'chaperones' to replace the proteins and enzymes lost to heat-induced denaturation.
  - Cold shock also induces a response by yeast, which accumulates trehalose, glycerol, andHSPs in order to stabilize the cell. This is especially relevant to brewery yeast cultures, since they are stored cold to prevent cellular activity during serial repitching (Cryo Tolerance).

21

How do Kveik strains compare to traditional brewing strains when subjected to multiple stressors?

Analysed using a spot plate based assay using YPD as a base medium

22

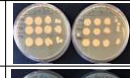
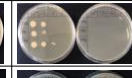
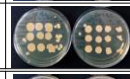
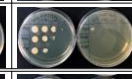
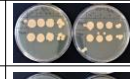
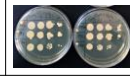
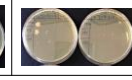
Oxidative Stress Tolerance

Analysis of growth when yeasts were serially diluted on YPD supplemented with Hydrogen Peroxide

23

Oxidative Stress

YPD plates with hydrogen peroxide

Yeast Strains	0% (L) and 0.01% (R) Hydrogen Peroxide	0.02% (L) and 0.03% (R) Hydrogen Peroxide
Kveik 3996 3997 3998		
Kveik 4283 4284 4285		
Kveik 4403 4404		
Non-Kveik W34/70 (lager) M2 (ale) 1174 (lager)		

24

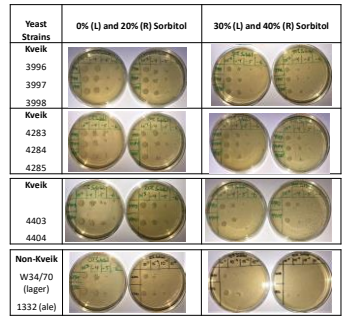
Osmotic Stress Tolerance

Analysis of cell growth when yeasts were serially diluted on YPD supplemented with Sorbitol

25

Osmotic Stress

YPD plates with sorbitol



26

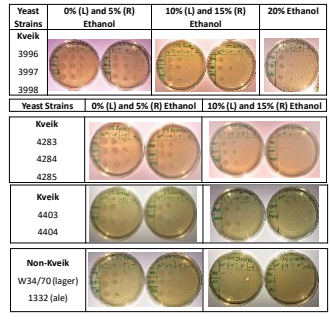
Alcohol Stress Test

Analysis of cell growth when yeasts were serially diluted on YPD supplemented with Ethanol

27

Alcoholic Stress

YPD plates with ethanol



28

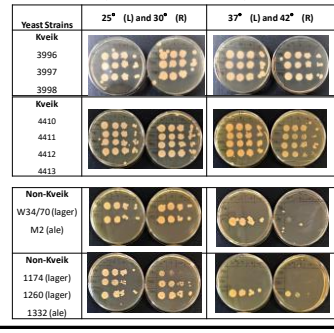
Thermal Stress Testing

Analysis of cell growth when yeasts were serially diluted on YPD at a range of predetermined temperatures

29

Thermal Stress

YPD plates at various temperatures



30

Stress Tolerance Summary

Tolerance to stress. Each 'cell' represents tolerance from green (high) through to red (low)

From poster presented at 2023 ASBC Conference titled: Kveik Yeast Cultures: Population Dynamics and Fermentation Characteristics.

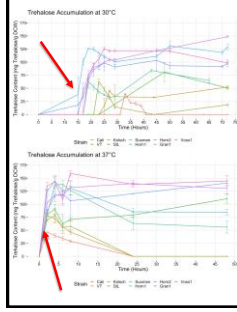
Isbell & Powell
(Article in preparation)



31

Why does kveik exhibit such responses to stressors?

The answer may be Trehalose: a disaccharide which stabilises the cell membrane by strengthening the lipid bilayer.



A study (Foster et al., 2022) comparing heat and trehalose:

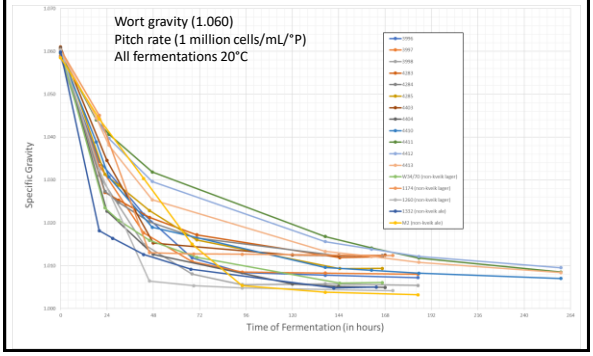
- At 30°C (top diagram) none of the strains make trehalose until ~ 15 hours after growth begins.
- At 37°C (bottom diagram) suddenly all the strains start making trehalose right away. So clearly the kveiks (and other strains) use trehalose to protect themselves against heat. And the kveiks make much more of it than the other strains.
- The kveik strains also retained trehalose, perhaps in anticipation of potential desiccation and long-term storage.

32

Do Kveik strains show the same fermentation properties as contemporary yeasts ?

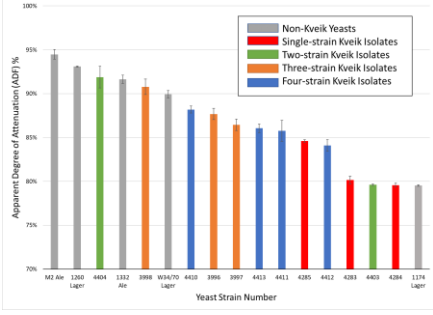
33

Fermentation Performance – All Yeasts in Study



34

Apparent Degree of Fermentation (ADF) of yeast strains by percentage, sorted from highest to lowest.



35

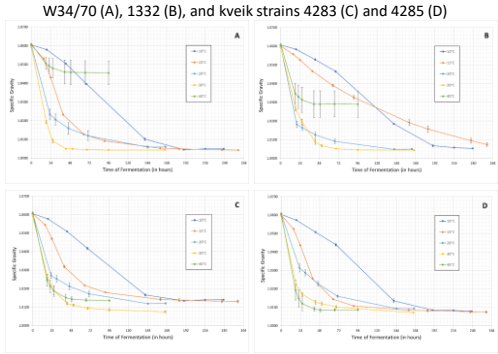
How do Kveik strains perform at elevated temperatures in comparison to contemporary yeasts?

36

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Relationship Between Temperature and Fermentation Performance
W34/70 (A), 1332 (B), and kveik strains 4283 (C) and 4285 (D)



37

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What is the relative contribution of individual yeast isolates within a Kveik culture ?

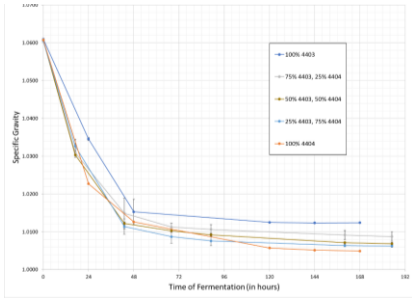
Isolates derived from 2- and 3-strain cultures were fermented using pre-determined percentages

38

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Fermentation Performance with 2-Strain Culture Isolates



2-Strain Culture

4403

4404



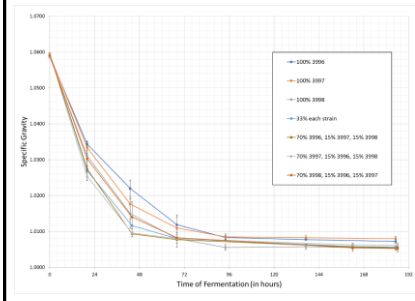
2-Strain Culture using 4403, 4404

39

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Fermentation Performance with 3-Strain Culture Isolates



3-Strain Culture

3996

3997

3998



3-Strain Culture using 3996, 3997, 3998

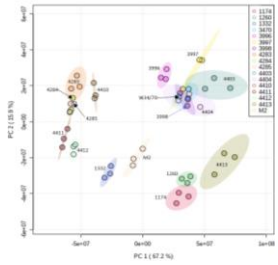
40

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Flavour Production of the Isolates

Principal component projection of flavour compounds produced by kveik and traditional non-kveik isolates utilising Atmospheric Pressure Chemical Ionization Mass Spectrometry (APCI-MS)



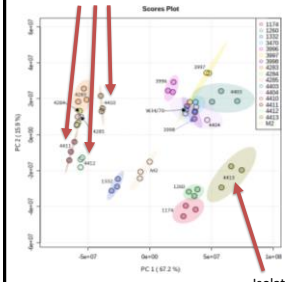
- Shows distinct flavour profiles, even within the mixed isolate cultures – this demonstrates that brewers can choose yeast strains for particular attributes according to the beer style they are creating.
- Although this method does not denote specific compounds, it provides a characteristic 'fingerprint' representation of volatile compounds produced by each yeast during fermentation

41

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4-strain culture isolates:
4410, 4411, 4412

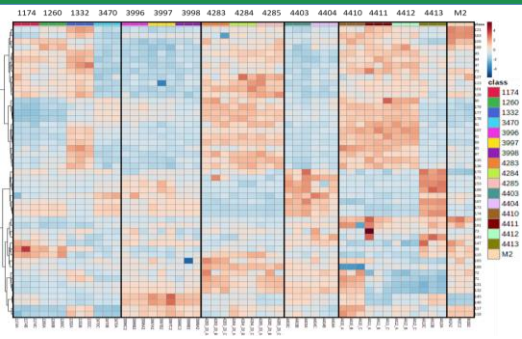


Isolate 4413

Notice the 4413 'outlier'

- Potentially has a different heritage than the other strains and entered the culture at an unknown time in the past.
- A 'contaminant' yeast, since brewers would borrow yeast from local farms if fermentations didn't proceed as planned?
- A mutation of an isolate within the culture?
- This 'anomalous' yeast allowed desirable flavour impact on the beers, or yielded improved fermentation efficiency, such that the culture was not discarded and was reused, eventually developing into its own unique blend.

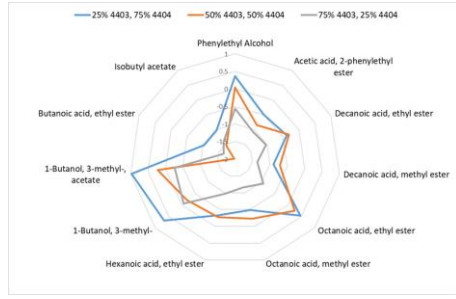
42



Distribution of volatile compounds for each fermentation, as determined by APCI-MS. Each yeast strain is coded by colour, and in each instance, data indicates those obtained from triplicate. The Y-axis displays the respective molecular weight of volatiles, determined algorithmically for generation of this heat map (by molecular weight minus 1).

43

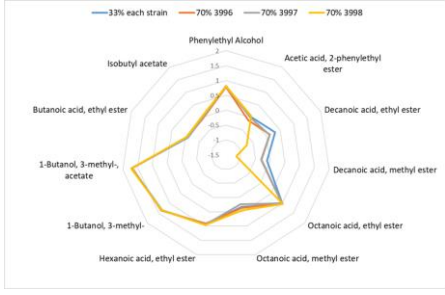
Spider Chart showing the blends of Kveik strains 4403 and 4404



Presented in log scale for ease of viewing

44

Spider Chart showing the blends of Kveik strains 3996, 3997, and 3998



Presented in log scale for ease of viewing

45

Conclusions

- Kveik strains exhibit positive response to real-life brewing stressors
- Kveik strains can ferment wort to create beers in less time than many contemporary yeasts
- Isolates within multi-strain cultures have shown to individuality by exhibiting differing fermentation profiles as well as differing flavour and aroma production

Future Endeavours

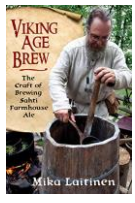
- Brewery throughput can increase without purchasing more tanks as individual beers can be created in less time.
- Capacity for rapid high gravity brewing fermentations can assist not only the brewing industry, but others as well (bioenergy).
- Examine the flavour profiles of kveik strains when fermented at 'normal' ale temperatures. Anecdotal opinions believe that these fermentations provide lager-like beers.
- Further explore flavour and aroma profiles of kveik isolates when fermenting non-malt substrates (wine, mead, cider, seltzers, RTDs)

46

Great Reading

- Historical Brewing Techniques: The Lost Art of Farmhouse Brewing by Lars Marius Garshol
- Lars Marius Garshol's Blog: https://www.garshol.priv.no/
- Farmhouse Yeast Registry: https://www.garshol.priv.no/download/farmhouse/kveik.html
- Viking Age Brew, by Mika Laitinen
- Brewing Nordic Website, by Mika Laitinen: https://www.brewingnordic.com/
- Milk the Funk Website: https://www.milkthefunk.com/wiki/Main_Page







47

Any Questions?



48

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University of Nottingham Invited Seminar Series

Todd Isbell, Yishen Li & Chris Powell

Brewers' Research & Education Fund Brewers' Company

1

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Traditional Norwegian Kveik: Novel Yeast Strains and Beer Fermentation





Todd M. Isbell

Based on thesis submitted to the University of Nottingham for the degree of Master of Research from Nov 2021 to Sept 2022 titled: Assessment of Stress Tolerance and Fermentation Performance in Traditional Norwegian Landrace (Kveik) Yeast.

2

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What is Beer?

Beer is an alcoholic beverage where the sugars are supplied by a grain. It consists of: malted barley, hops, water, and yeast









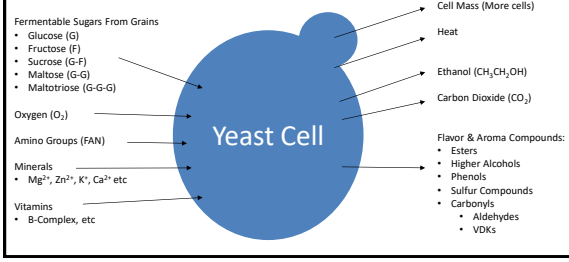


3

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What is Beer Fermentation?

Fermentation of beer is the process where yeast consumes the sugars and nutrients extracted from grains and produces CO_2, alcohol, and a multitude of flavour and aroma compounds



4

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There are two main types of Brewing Yeast:

Ales	Lagers
Saccharomyces cerevisiae	*Saccharomyces pastorianus*

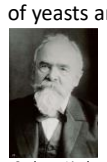
Differences Between Ale and Lager Yeast	
Ales	**Lagers**
Can grow up to 37˚C	Cannot grow above 34˚C
Typically used in fermentations at 18˚C - 22˚C	Typically used in fermentations at 8˚C - 15˚C
Faster fermentation at several days	Slower fermentations at 1 -3 weeks
Higher ester and higher alcohol production	Lower ester and higher alcohol production
Lower sulphur production	Higher sulphur production
Cannot ferment melibiose	Able to ferment melibiose

5

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Throughout history, beers were fermented by a multitude of yeasts and bacteria


Louis Pasteur


Carl von Linde


Emil Hansen


Harry Brearley

- 1864: Pasteurisation
- 1873: Refrigeration
- 1883: Pure culture isolation
- 1913: Stainless Steel

Now brewers can produce beers created from a single culture with less bacterial infections to produce 'clean' ales and lagers with longer shelflife.

The vast majority of beers consumed in the world today are created using these methods

6

What is Kveik?

- Kveik are a unique subspecies of *Saccharomyces cerevisiae* which originate from isolated farms or towns in rural Norway.
  - Yeast cultures traditionally passed down between generations within a family a farming region for decades (perhaps centuries).
  - Little written historical information available as most and techniques were passed down generationally by word-of-mouth.
- These strains were often used indiscriminately for both brewing and the baking of bread.







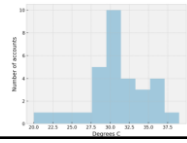
- Kveik is not a beer style, but refers to any type of beer style made with those particular yeast cultures.



7

What is Kveik?

- In Norwegian, the word kveik means 'to bring a spark to' or 'bring life to.'
- Some Kveik possess single cultures, but others are comprised of multiple distinct strains (Tormodgarden has 10 known isolates). Some do contain bacteria, such as *LAB* species.
- Kveik are POF- meaning they do not create phenolics, nor are they a *diastaticus* subspecies (super attenuator).
- These yeasts exhibit flocculation and are fully accustomed to consuming malt sugars
- They are famous for fermenting at very high temperatures relative to standard commercial ale strains without creating off flavours. Temperatures often exceed 30°C and many approach 40°C. They are known to create fruity and tropical flavours and aromas.



Pitch temperatures reported by the owners of collected kveiks (Garshol, 2021).

8

What is Kveik?

While hops are often used for flavour, aroma, and for its antimicrobial properties, often Kveik beers are made using teas of juniper or juniper as the base filter in their mash tuns.





Unique Yeast Handling



Kveikstokk



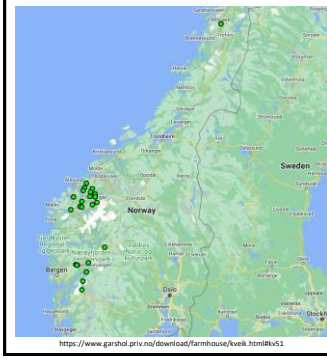
Gjærkrans



Dessication for long-term storage

9

Known Kveik Sources



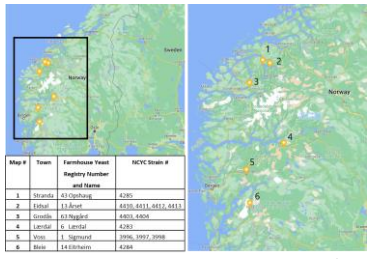
https://www.garshol.priv.no/download/farmhouse/kveik.html#kv51

Kveik often acquire their names from the surname of the family or the location of the farm or town.

No.	Name	Owner	Place	NCYC	Pitch	Hanover	Bacteria	POF	Strains	Max ABV
1	Sigmund	Sigmund Gjernes	Hjelmeland	3385	30	bottom	N	N	1	10
2	Rosnes	Svein Rosnes	Dyrvekjeller	3640	37	top			1	
3	Granvik	Stein Lengvik	Granvik	4521	30	top		N	1	16
4	Hornnes	Tarjei Rafthevold	Grindflo	4552	30	top	Y	N	0	16
5	Lauvtun	Dagfinn Mandelkow	Lauvtun	4283	30	either	Y	N	0	14
6	Granvin	Tor Olav Hetland	Dyrvekjeller		30	bottom	N	N	0	16
7	Tormodgarden	Sigurd Johan Skaare	Stallkroken	4171	30	bottom	N	N	10	16
8	Ellingsnæs	Jens Aage Christiansen	Ellingsnæs	4229	30	top	Y	N	0	16
9	Farmgarden	Petter B Grindstuen	Grindflo	4173	30		N	N	0	16
10	Lida	Samuel Lida	Grindflo		33	top		N	0	
11	Kvasir	John Kvasir	Kvasir	4196	33	top	N	N	0	
12	Aved	Jakob Tarp Aved	Kvasir	4283	30	either	N	N	0	3
13	Eikheim	Reidar Eikheim	Reko	4284	37	either		N	0	
14	Nonnes	John Nonnes	Dyrvekjeller	4191	96				0	5
15	Wolfkvil	Odd H Wolfkvil	Grindflo		33	top			N	5
16	Gausemel	Olav Gravna Gausemel	Grindflo	4375	90	top	Y		N	6
17	Kyrkjeb	Aave Kyrkjeb	Grindflo	4284	28	top		N	0	4
18	Espe	Arne Espe	Grindflo	4286	29	top		N	N	4
19	Tomasgard	Lars Andreas Tomasgard	Grindflo	4285	29	top	Y	N	0	4
20	Nerbø	Stig Jarle Nerbøvik	Grindflo	4380	31	either	N	N	N	5
21	Vikerlid	John Arthur Grindlo	Vikerlidens, Hornindal		29		N	N	0	5
22	Voss	Olve Haugland Hille	Vossakilden							
23	Hothmyr	Hothmyr gard	Grindflo		33	top			N	5
24	Lund	Stig Lund	Lund	4282	29	top	Y	N	0	5
25	Mathisen	Rasmus S. Vikerlid	Vikerlidens, Hornindal							
26	Ten Oever	Olve Grindlo	Maska		25					
27	Hothmyr	Jørgen Hothmyr	Hothmyr	4279	34.5	top		N	0	2
28	Øksne	Gunnar Øksne	Grindflo	4278	30	top	N	N	0	4
29	Gjerkeng	Harald Gjerkeng	Gråmålarstuen	4285	27.5	top	N			
30	Jordal	Halfdan Mage	Jordal		37	either				4
31	Folkestad	Hans Folkestad	Nordfjordeid							
32	Nordtun	Dagfinn Nordtun	Granvik	4282	27.5	top			0	
33	???	Svein Grannes	Valley		30.5	top			0	
34	Voje	Lars Henning Voje	Grindflo		27.5	top	Y		0	
35	Djønno	Rolf Tore Djønno	Djønno	4282	40	bottom	N	N	0	2
36	Grytøyrstøl	Lars Gystein Grytøyrstøl	Grytøyrstøl		35	top	N		0	4
37	Nygård	Ole Nygård	Grindflo	4284	31	either	Y		0	5
38	Lunde	Stig Lunde	Vik Vare		40	bottom	N	N	0	5
39	Bakke	Lars Inge Bakke	Bakkeli, Voss		40	bottom	N	N	0	2
40	Arvey Gaia	Gaia Gaino	Dyrvekjeller		40	bottom	N		0	2
41	Ulma	Oddmund Ulma	Ulmaflo		30	top	N		0	5
42	Skrungarde	John Birger Johansen	Skrungarde							

10

Kveik Used in the Study



Map #	Town	Farmhouse Yeast Registry Number and Name	NCYC Strain #
1	Stranda	43 Opshaug	4285
2	Eidsøl	13 Aved	4430, 4431, 4612, 4413
3	Grodås	63 Nygård	4403, 4404
4	Lærdal	6 Lærdal	4283
5	Voss	1 Sigmund	3996, 3997, 3998
6	Reko	14 Eikheim	4284

Contemporary Brewing Yeasts in Study

Yeast Identifier	Origination of Strain
NCYC 1332	National Collection of Yeast Cultures, *Saccharomyces cerevisiae*
M2	Oxford Brookes University Brewing Yeast Culture Collection, *Saccharomyces cerevisiae*
CBS 1174	Westerdijk Fungal Biodiversity Institute, *Saccharomyces pastorianus*
CBS 1260	Westerdijk Fungal Biodiversity Institute, *Saccharomyces pastorianus*
W34/70	Hefebank Weihenstephan, *Saccharomyces pastorianus*

11

Goals of the Study

- Investigate distinctiveness between isolates within multiple strain cultures
- To compare the responses of kveik isolates to various stressors that are present during normal beer fermentations:
  - Oxidative Stress
  - Osmotic Stress
  - Alcoholic Stress
  - Thermal Stress
- Compare fermentation performance between isolates (rate of fermentation and degree of attenuation) both within multiple strain cultures as well as compared to contemporary brewing yeast strains, both ales and lager.
- Compare flavour production of kveik isolates within their respective cultures

12

What is the colony morphology of kveik strains?

Can we use this as a method for differentiation?

13

Kveik Morphology Using WLN Media

3-Strain Culture

3996

3997

3998



2-Strain Culture

4403

4404

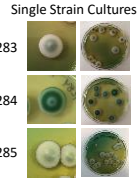


Single Strain Cultures

4283

4284

4285



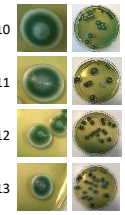
4-Strain Culture

4410

4411

4412

4413



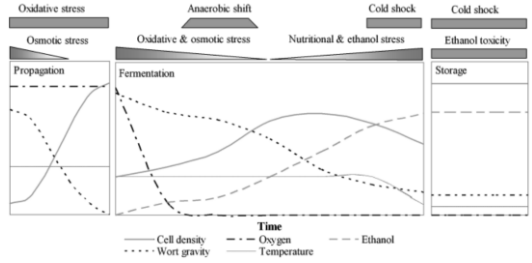
14

Key stress factors that yeast are subjected to during fermentation

15

Various Stressors Experienced by Yeast

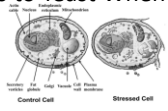
Oxidative, Osmotic, Alcoholic, and Thermal stresses were examined in the study



Stress factors associated with brewery yeast handling (Gibson et al., 2007)

16

What Happens to Yeast When it is Stressed?

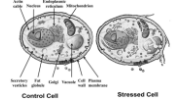


Artistic impression of the effect of stress factors on yeast organelles (Stewart, 2020).

- **Oxidative Stress**
  - Reactive Oxygen Species (ROS) including molecular oxygen (O_2), but also superoxide (O_2^-), peroxide anions (O_2^{2-}), hydrogen peroxide (H_2O_2), hydroxyl radicals ($OH\bullet$), and perhydroxyl radicals ($OOH\bullet$).
  - These degrade lipids causing membrane fluidity and damage cellular proteins and DNA.
  - This can induce petites in mitochondria and create mutations.
- **Osmotic Stress**
  - Cell transitions from nutrient deficiency to a nutrient rich environment at the onset of fermentation
  - Concentration gradient across the cell membrane. This gradient can cause water to leak out of the cell as well as solutes into the cell.
  - This slows cell growth, can stall fermentation, and decrease viability.

17

What Happens to Yeast When it is Stressed?



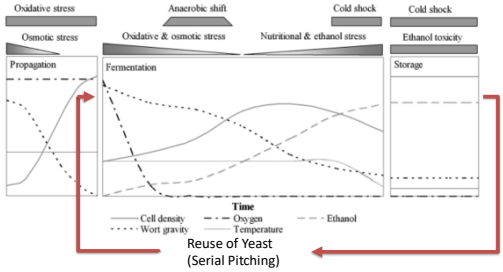
Artistic impression of the effect of stress factors on yeast organelles (Stewart, 2020).

- **Alcohol Stress**
  - Ethanol is a key product created by yeast during fermentation
  - When excessive, ethanol harms membranes and denatures nutrient transport proteins within the cell
  - Degrades cell fluidity
  - Decrease cell viability, including during storage.
  - Can also instigate the production of mitochondrial petites.
- **Thermal Stress**
  - Proteins can be denatured and will directly affect cellular health.
  - Higher temperatures also cause off flavours in beer, particularly higher esters and higher alcohols.
  - When excessive, high temperatures cause cell death.
  - High temperatures exacerbate all other stressors.

18

Serial Repitching of Yeast (Cyclic Stressing)

All negative stressor outcomes are magnified due to serial repitching.



Reuse of Yeast
(Serial Pitching)

19

Yeast Stress Response

Saccharomyces species possess a stress response element (STRE) system that proves to resolve the negative effects of a wide variety of environmental stresses factors

- **Oxidative Stress**
  - Non-enzymatic: Trehalose and Glutathione
    - Trehalose assists in protecting the plasma membrane and enzymes as well as enabling the repair of proteins.
    - Glutathione scavenges radical molecules and reduces oxidants.
  - Enzymatic sources include peroxidases and reductases, as well as catalase which break down ROS species to less harmful compounds
- **Osmotic Stress**
  - Yeast accumulates osmoprotectants such as glycerol, which stabilize cellular components including membranes and proteins.
  - Trehalose, which also assists in oxidative, alcohol, and thermal stability, acts to protect the cell membrane.

20

Yeast Stress Response

Saccharomyces species possess a stress response element (STRE) system that proves to resolve the negative effects of a wide variety of environmental stresses factors

- **Ethanol Stress**
  - At the onset of fermentation, yeast utilize oxygen to develop unsaturated fatty acids and sterols in the cell membrane. This assures efficient membrane transport and assists the cell in combating multiple stresses, including ethanol.
  - Trehalose also plays a role in ethanol tolerance due to its protective effects on the cell membrane.
- **Thermal Stress**
  - The genetic reaction to thermal stress is called Heat Shock Response (HSR).
    - When increasing temperature damage proteins, STRE stimulates the production of Heat Shock Proteins (HSP).
    - These act as 'chaperones' to replace the proteins and enzymes lost to heat-induced denaturation.
  - Cold shock also induces a response by yeast, which accumulates trehalose, glycerol, andHSPs in order to stabilize the cell. This is especially relevant to brewery yeast cultures, since they are stored cold to prevent cellular activity during serial repitching (Cryo Tolerance).

21

How do Kveik strains compare to traditional brewing strains when subjected to multiple stressors?

Analysed using a spot plate based assay using YPD as a base medium

22

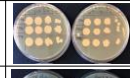
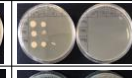
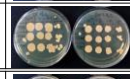
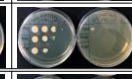
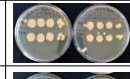
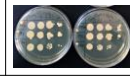
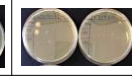
Oxidative Stress Tolerance

Analysis of growth when yeasts were serially diluted on YPD supplemented with Hydrogen Peroxide

23

Oxidative Stress

YPD plates with hydrogen peroxide

Yeast Strains	0% (L) and 0.01% (R) Hydrogen Peroxide	0.02% (L) and 0.03% (R) Hydrogen Peroxide
Kveik 3996 3997 3998		
Kveik 4283 4284 4285		
Kveik 4403 4404		
Non-Kveik W34/70 (lager) M2 (ale) 1174 (lager)		

24

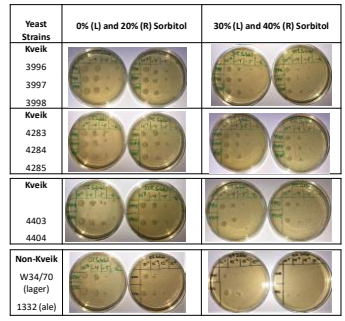
Osmotic Stress Tolerance

Analysis of cell growth when yeasts were serially diluted on YPD supplemented with Sorbitol

25

Osmotic Stress

YPD plates with sorbitol



26

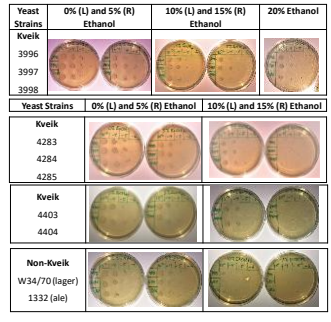
Alcohol Stress Test

Analysis of cell growth when yeasts were serially diluted on YPD supplemented with Ethanol

27

Alcoholic Stress

YPD plates with ethanol



28

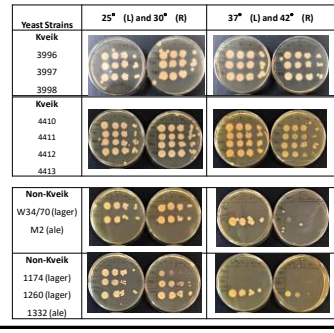
Thermal Stress Testing

Analysis of cell growth when yeasts were serially diluted on YPD at a range of predetermined temperatures

29

Thermal Stress

YPD plates at various temperatures



30

Stress Tolerance Summary

Tolerance to stress. Each 'cell' represents tolerance from green (high) through to red (low)

From poster presented at 2023 ASBC Conference titled: Kveik Yeast Cultures: Population Dynamics and Fermentation Characteristics.

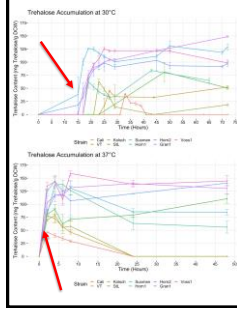
Isbell & Powell
(Article in preparation)



31

Why does kveik exhibit such responses to stressors?

The answer may be Trehalose: a disaccharide which stabilises the cell membrane by strengthening the lipid bilayer.



A study (Foster et al., 2022) comparing heat and trehalose:

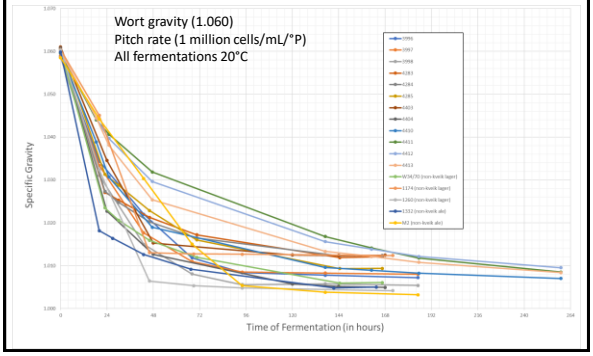
- At 30°C (top diagram) none of the strains make trehalose until ~ 15 hours after growth begins.
- At 37°C (bottom diagram) suddenly all the strains start making trehalose right away. So clearly the kveiks (and other strains) use trehalose to protect themselves against heat. And the kveiks make much more of it than the other strains.
- The kveik strains also retained trehalose, perhaps in anticipation of potential desiccation and long-term storage.

32

Do Kveik strains show the same fermentation properties as contemporary yeasts ?

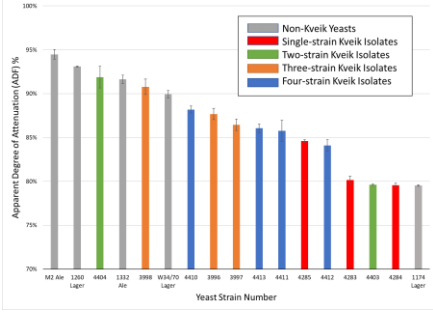
33

Fermentation Performance – All Yeasts in Study



34

Apparent Degree of Fermentation (ADF) of yeast strains by percentage, sorted from highest to lowest.



35

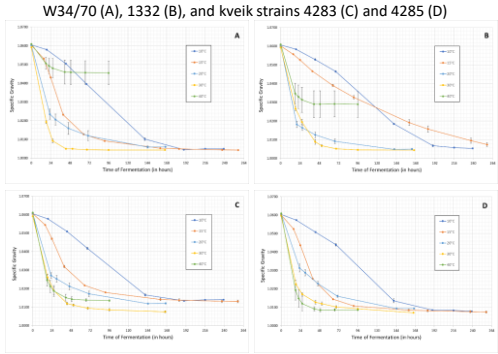
How do Kveik strains perform at elevated temperatures in comparison to contemporary yeasts?

36

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Relationship Between Temperature and Fermentation Performance
W34/70 (A), 1332 (B), and kveik strains 4283 (C) and 4285 (D)



37

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What is the relative contribution of individual yeast isolates within a Kveik culture ?

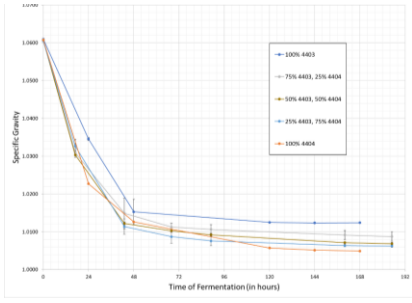
Isolates derived from 2- and 3-strain cultures were fermented using pre-determined percentages

38

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Fermentation Performance with 2-Strain Culture Isolates



2-Strain Culture

4403

4404



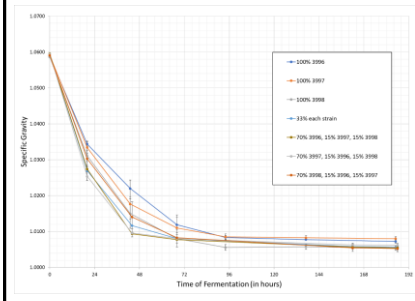
2-Strain Culture using 4403, 4404

39

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Fermentation Performance with 3-Strain Culture Isolates



3-Strain Culture

3996

3997

3998



3-Strain Culture using 3996, 3997, 3998

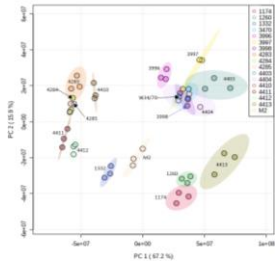
40

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Flavour Production of the Isolates

Principal component projection of flavour compounds produced by kveik and traditional non-kveik isolates utilising Atmospheric Pressure Chemical Ionization Mass Spectrometry (APCI-MS)



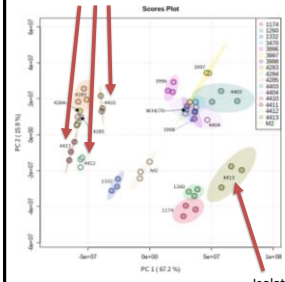
- Shows distinct flavour profiles, even within the mixed isolate cultures – this demonstrates that brewers can choose yeast strains for particular attributes according to the beer style they are creating.
- Although this method does not denote specific compounds, it provides a characteristic 'fingerprint' representation of volatile compounds produced by each yeast during fermentation

41

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4-strain culture isolates:
4410, 4411, 4412

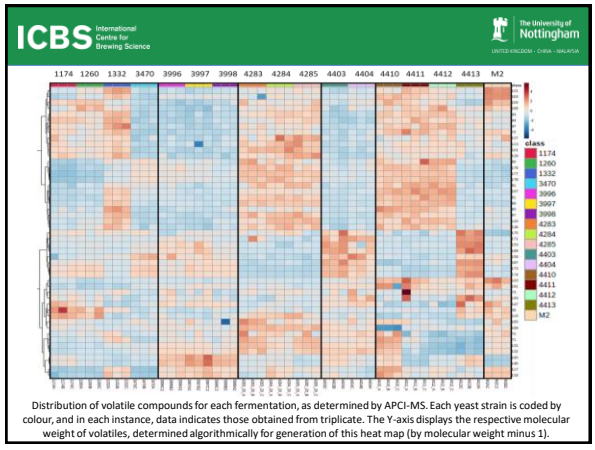


Isolate 4413

Notice the 4413 'outlier'

- Potentially has a different heritage than the other strains and entered the culture at an unknown time in the past.
- A 'contaminant' yeast, since brewers would borrow yeast from local farms if fermentations didn't proceed as planned?
- A mutation of an isolate within the culture?
- This 'anomalous' yeast allowed desirable flavour impact on the beers, or yielded improved fermentation efficiency, such that the culture was not discarded and was reused, eventually developing into its own unique blend.

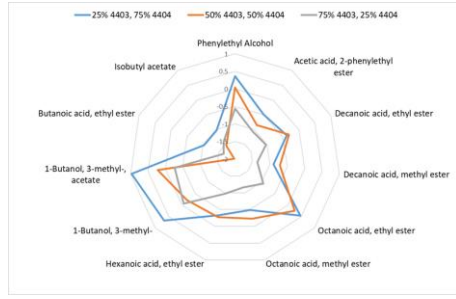
42



Distribution of volatile compounds for each fermentation, as determined by APCI-MS. Each yeast strain is coded by colour, and in each instance, data indicates those obtained from triplicate. The Y-axis displays the respective molecular weight of volatiles, determined algorithmically for generation of this heat map (by molecular weight minus 1).

43

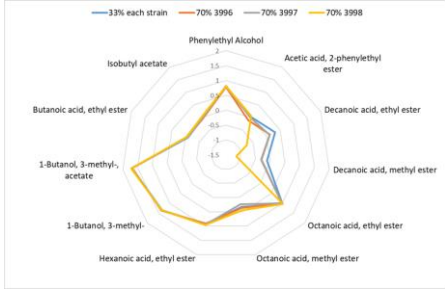
Spider Chart showing the blends of Kveik strains 4403 and 4404



Presented in log scale for ease of viewing

44

Spider Chart showing the blends of Kveik strains 3996, 3997, and 3998



Presented in log scale for ease of viewing

45

Can Kveik Strains be Handled in the Same Way as Conventional Brewing Yeasts?





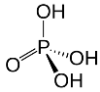
46

Response to cold storage & acid washing

- **Cold Storage**
  - Kveik yeast were fermented in a standard 15°P wort.
  - Slurry was recovered and stored at 4°C for 1 month.
  - Viability assessed using methylene blue staining daily.

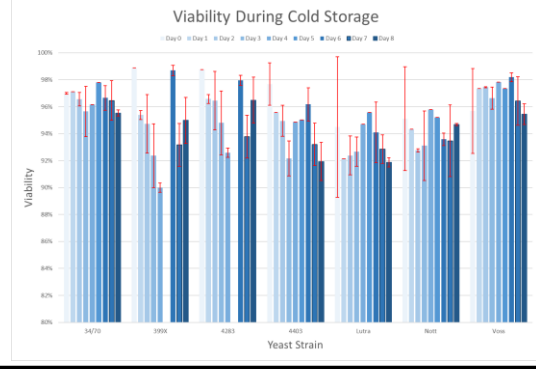


- **Acid Washing**
  - Kveik yeast were fermented in a standard 15°P wort.
  - Post fermentation, yeast slurries were stored at 4°C for one day.
  - Slurries were acidified with phosphoric acid to pH 2.2
  - Slurries were maintained for 2 hours with gentle agitation.
  - Viability was measured after 0 and 2 hours.



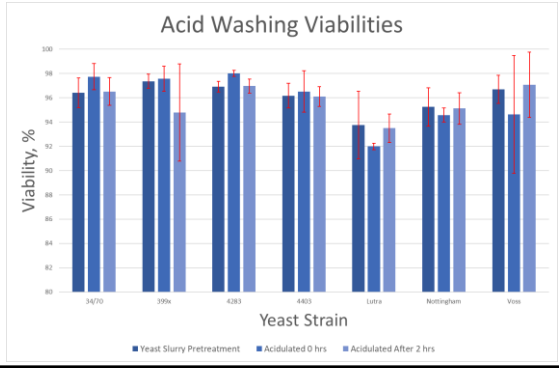
47

Response to Cold Storage



48

Response of Yeast to Acid Washing



49

Conclusions

- Kveik strains exhibit positive response to real-life brewing stressors
- Kveik strains can ferment wort to create beers in less time than many contemporary yeasts
- Isolates within multi-strain cultures have shown to individuality by exhibiting differing fermentation profiles as well as differing flavour and aroma production

Future Endeavours

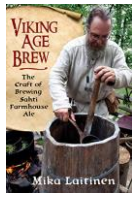
- Brewery throughput can increase without purchasing more tanks as individual beers can be created in less time.
- Capacity for rapid high gravity brewing fermentations can assist not only the brewing industry, but others as well (bioenergy).
- Examine the flavour profiles of kveik strains when fermented at 'normal' ale temperatures. Anecdotal opinions believe that these fermentations provide lager-like beers.
- Further explore flavour and aroma profiles of kveik isolates when fermenting non-malt substrates (wine, mead, cider, seltzers, RTDs)

50

Great Reading

- Historical Brewing Techniques: The Lost Art of Farmhouse Brewing by Lars Marius Garshol
- Lars Marius Garshol's Blog: https://www.garshol.priv.no/
- Farmhouse Yeast Registry: https://www.garshol.priv.no/download/farmhouse/kveik.html
- Viking Age Brew, by Mika Laitinen
- Brewing Nordic Website, by Mika Laitinen: https://www.brewingnordic.com/
- Milk the Funk Website: https://www.milkthefunk.com/wiki/Main_Page





Brettanomyces - Lactobacillus - Pediococcus
Milk the Funk
wiki

51

Any Questions?



52