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The influence of yeast format and pitching rate on Scotch malt whisky fermentation kinetics and congeners

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Abstract

Yeast format and pitching rate are variables which can be easily manipulated in a distillery environment but are seldom altered. Fermentations using dried and liquid yeast were studied at laboratory scale and compared by application of a 4-parameter logistic model to measurements of the decline in apparent extract during fermentation. Congener analysis of new make distillate allowed comparison between compounds of interest produced during fermentation. The liquid yeast format demonstrated a significant reduction ($p<0.05$) in lag time, which was 68% shorter than dried yeast. Despite this, longer overall fermentation times were observed due to a slower exponential phase as compared to the dried yeast format. Alteration of the yeast pitching rate using dried and liquid formats, demonstrated that high rates resulted in reduced lag times. The maximum fermentation rates (V_{max}) were assessed from the fermentation models and no consistent trend could be identified. Dried yeast was observed to achieve its maximum fermentation rate when underpitched. Substantially higher V_{max} values were obtained with overpitched liquid yeast fermentations, resulting in faster fermentations, compared to underpitched liquid yeast fermentations. The liquid yeast format created more esters compared to dried yeast. The concentration of ethyl esters generally trended downwards for fermentations pitched with less yeast which fully attenuated. By combining the results of these studies, distillers can make informed decisions to optimise spirit character, quality, and distillery production.

Keywords:
fermentation, whisky, new make spirit, yeast, pitching rate, format

Introduction

The production of Scotch malt whisky can be summarised in four steps from grain to product: mashing, fermentation, distillation and maturation. During wort production, carbohydrates are released from malted barley through the action of endogenous enzymes (Sim and Berry, 1996) degrading starch into fermentable sugars that are metabolised by yeast into ethanol and flavour molecules (congeners). At the end of fermentation, the wash has an ethanol content of 7-9% v/v, dependent on the original extract/gravity which is often in the range of 15-20°P (Russell and Stewart, 2014). In a typical malt whisky distillery, the wash is distilled twice in copper pot stills, producing a distillate of 65-72% ABV. This freshly distilled, new make spirit is matured in wooden casks for at least three years before it can be legally called 'whisky' (in Scotland). The flavour profile of the new make spirit is a critical crucial control point for spirit character, quality in the production process and the final product.

During the filling of the washback (fermenter), yeast is pitched either in-line, by mixing yeast with the wort before transfer to the washback or by direct addition to wort in the washback. The former process is used when a liquid or rehydrated (dried) yeast is used, whereas the latter is 'direct pitching' and used with dried or caked yeast (Table 1). After pitching, the yeast cells undergo lag phase (Figure 1A) after which the cells start to divide, and the population increases (exponential phase). Cell growth ceases when nutrients are depleted (depending on the substrate used), and the stationary phase is characterised by no increase in cell number. During yeast growth, the metabolism of sugar results in the decline in the specific gravity of the fermenting wort. When plotted, the data exhibits an inverted sigmoidal curve caused by the formation of ethanol and evolution of carbon dioxide (MacIntosh et al, 2016). The shape of this curve is comparable to that of yeast cell growth (Figure 1B) (Reid, 2021). The curve follows the same regression pattern as observed during brewery fermentations (Speers et al, 2003). This is despite the action of limit dextrinase degrading dextrins during distillery fermentations due to wort not being boiled (Daute et al, 2021; Vriesekoop et al, 2010). These sigmoidal curves or fermentation 'models' have been investigated in the literature, providing a fermentation 'tool' for the prediction of premature yeast flocculation (Armstrong et al, 2018; ASBC, 2012; Lake et al, 2008), fermentation rates (Reid et al, 2021) and novel fermentation processes (Reid et al, 2020).

Yeast health and metabolism will influence the decline in density during fermentation as well as the increase in cell biomass. The shapes of these curves will also be determined by the environment the yeast experiences during fermentation. This includes the availability of free amino nitrogen (FAN), which is important for cell metabolism (Black et al, 2020) and the condition of the cells prior to pitching (Browning et al, 1994; Haukeli and Lie, 1971).

Few distilling yeasts are used commercially, favouring strains that produce high yields of ethanol and are tolerant to stress factors during fermentation (Gibson et al, 2007). Such yeasts consume all fermentable carbohydrates in wort (including maltotriose), maximising yield and preventing fouling in the still (Watson, 1981). These distilling strains are distinct from yeasts used in brewing and oenology (Gonçalves et al, 2016; Krogerus et al, 2017).

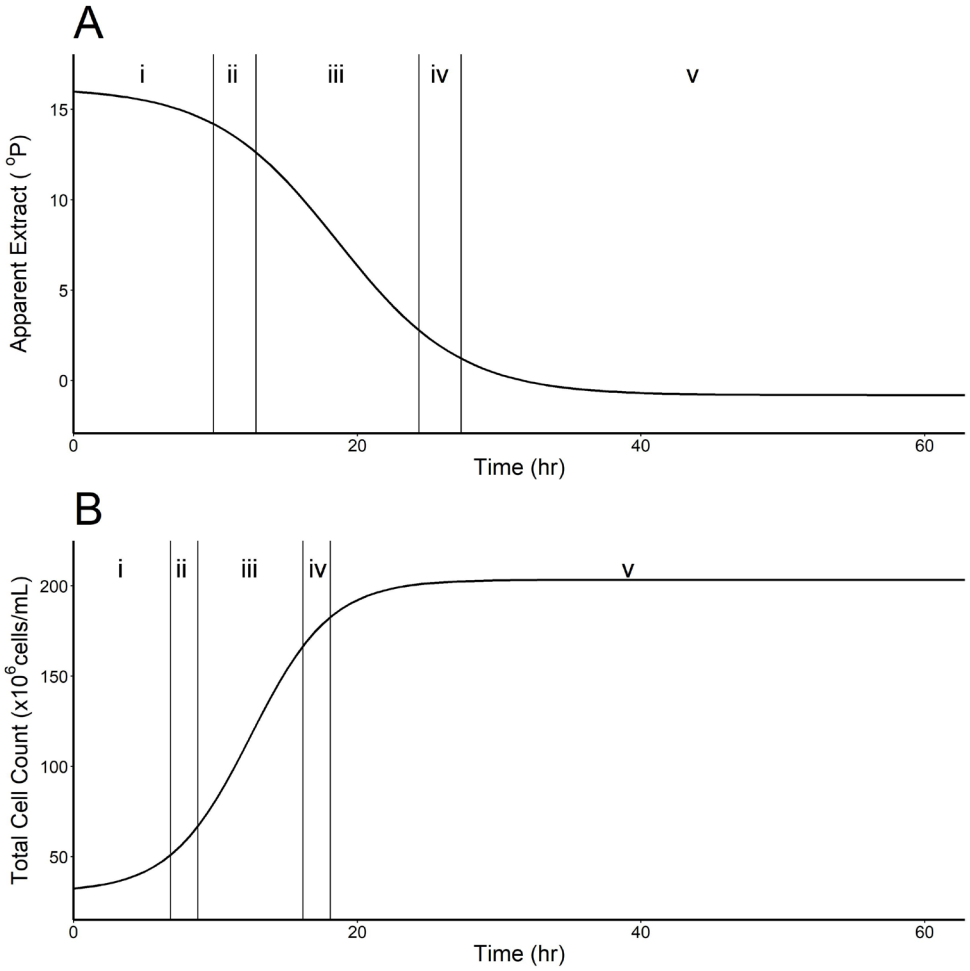
Table 1.

Commercial yeast formats available to the Scotch whisky industry (Ingledew, 2017)

Format	Storage temperature (°C)	Shelf life	% Dry matter	Yeast cell concentration
Dried	4-20	1 year +	92-96	~ 2.2 x10^{10} cells/g
Compressed	1-4	1 month	24-33	6-13 x10^{9} cells/mL
Liquid	1-4	1-2 weeks	15-20	~ 3.0 x10^{8} cells/mL
Stabilised Liquid	1-4	12-16 weeks	16-19	~ 3.0 x10^{8} cells/mL

Figure 1.

Fermentation models (4-parameter logistic) plotted with respect to (A) cell count and (B) apparent extract. The phases of fermentation are derived from cell growth and are i) lag phase, ii) acceleration phase, iii) exponential phase, iv) deceleration phase, v) stationary phase. As shown, the phases of fermentation with respect to cell growth (A) do not match those of the decline in specific gravity (B). For this reason, the observation of one regression cannot be used to deduce information about the other and vice-versa. Adapted from Reid (2021).



Further, the lack of wort boiling allows for the continued action of limit dextrinase, such that the final specific gravity of the wash is <0.00°P (<1.000) (Figure 1B). This is markedly lower than brewery fermentation where the wort is boiled (Russell and Stewart, 2014).

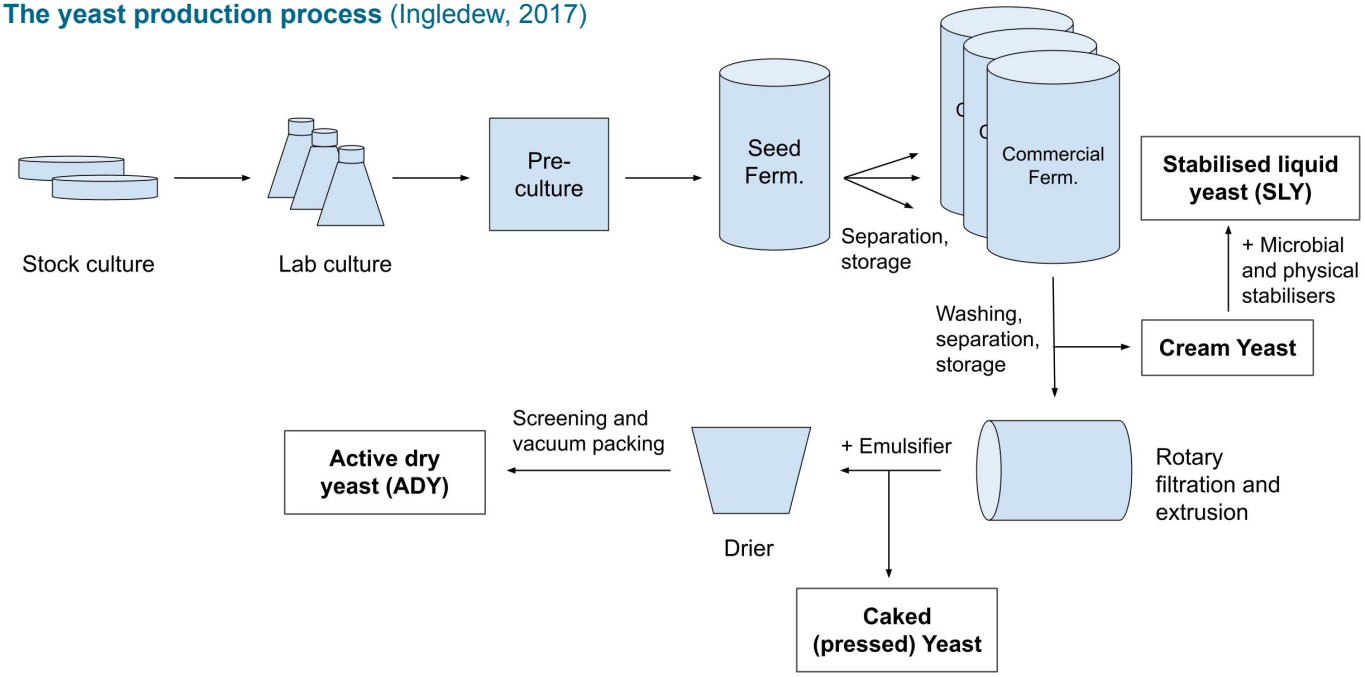
Yeast format

Commercial yeast strains are available in a variety of formats (Table 1). Yeast strains are grown from pure cultures stored either in a glycerol stock (in liquid nitrogen or at – 80°C) or on an agar slant and are propagated as summarised in Figure 2. Following a fed-batch fermentation (Zulauf process), the yeast is centrifuged using a disc-plate centrifuge to create liquid cream yeast. With the addition of emulsifiers and stabilisers, the stabilised liquid yeast is filtered under vacuum and extruded to form caked (compressed) yeast. The yeast is dried with a fluidised bed drier, and the 'active dry yeast' (ADY) is packed under vacuum. Beneficially, ADY has a long shelf-life (Table 1) and can be stored at room temperature without compromising viability. Further, by refrigerating dried yeast, losses in viability can be <4% per annum (Russell, 2003) and, if stored below room temperature (<15°C), fermentations kinetics are optimised (Manson and Slaughter, 1986).

However, as sustainability become increasingly important to the industry, the use of liquid yeast has an advantage due to the reduced energy required to manufacture. The energy intensive, drying process can impose stress, causing genome level modifications in yeast which impact ethanol tolerance (Cheung et al, 2012).

Figure 2.

The yeast production process (Ingledew, 2017)



Dried yeast formats allow distillers to trial new strains. This enables diversity and product differentiation by applying yeasts from different industries including brewing, biofuels and other alcoholic beverages (wine, rum) (Berbert de Amorim Neto et al, 2008, 2009; Ensor et al, 2015; Hill et al, 2018; Muir-Taylor, 2020; Waymark and Hill, 2021).

Liquid yeast is considered to have a short lag phase compared to other forms as adaption stress - which can affect yeast health during rehydration is not present (Jenkins et al, 2010, 2011). This short lag phase is beneficial to distillery fermentations as it gives competing bacteria, such as *Lactobacillus* minimal time to proliferate (Watson, 1981). Other advantages of liquid yeast include the lack of manual handling through automatic dosing systems and improved mixing both yeast and wort homogenous. Liquid yeast may also be stabilised using xanthan gum and glycerol (Richards, 2015). The glycerol acts to maintain yeast vitality during storage and the xanthan gum provides a matrix which stops yeast sedimentation (Degre et al, 2011).

The use of compressed (pressed, caked) yeast is declining in the Scotch whisky market, due to the high degree of manual handling and relatively short shelf-life compared to the dried format. Therefore, compressed yeast was not evaluated in this study.

Pitching rate

In a Scotch malt whisky distillery, the yeast pitching rate is defined by malt tonnage or fermentation volume. As a result, several assumptions are made about the fermentation conditions that the yeast will be exposed to. This includes the original gravity, nutrient content of mash liquor and other stressors such as metabolites from competing bacteria. It also assumes that the yeast used is consistent in both dried and liquid formats during storage. The effect of pitching rate on whisky fermentation was investigated by Ramsay and Berry (1984) and showed that at high pitching rates, fatty acids and their corresponding esters were reduced with an increase in total higher alcohols. It has also been demonstrated that yeast will ferment faster and be more resilient to high gravity conditions when the pitching rate is increased (Erten et al, 2007; Walker et al, 2008).

Studies in the field of brewing have demonstrated that fermentations will be faster when yeast pitching rate is increased with more biomass formed (Casey and Ingledew, 1983). It has also been demonstrated that increasing the pitching rate changes the formation of flavour compounds, decreasing desirable esters such as isoamyl acetate and changing the ratio of branched higher alcohols that are formed (Verbelen et al, 2009).

The yeast pitching rate used in Scotch malt whisky distilleries are often significantly greater than for other distilled spirits categories, commonly pitching 30-40 x 10^6 cells/mL (liquid) or 0.9-1g DW/L (dry weight) of dried yeast, independent of original extract. The liquid pitching rate exceeds the brewing 'rule of thumb' of 1x10^6 cells/mL /°P, however dried pitching rates are comparable. Other distilled spirits producers in America or Canada utilise yeast pitching rates in the range of 4-8x10^6 cells/mL (liquid) or 0.25-0.5g DW/L (dried, typically 0.35 g DW/L) (Collicutt, 2009; Russell and Stewart, 2014). The elevated yeast pitching rates in whisky fermentations are to overcome high levels of bacteria associated with malted barley and to achieve fast fermentation kinetics.

This study explores how yeast format and pitching rate impacts the fermentation model with respect to the decline in extract and yeast growth. These models are then be related to the production of flavour congeners produced during these fermentations.

Materials and Methods

Wort production

Wort (200L per batch) was produced at the International Centre for Brewing and Distilling research brewery using Concerto distillers malted barley (Simpsons Malt, Berwick-upon-Tweed, GBR). Malt (55kg) was milled using a two-roll mill (Fraser Agricultural, Inverurie, GBR) and the grist was mashed with water (150L). The strike temperature of the water was adjusted so that the resulting mash temperature of the water was 64°C, which was maintained for 1 hr with constant agitation. The wort was separated in a lauter tun, and sparged (81°C) until the specific gravity in the reception vessel was 16.1°P (1.066). This wort was then frozen is 20L batches and thawed prior to further use.

Yeast handling

The yeast was supplied by Lallemand Biofuels and Distilled Spirits (Lallemand Inc, Montreal, CAN) and was an industrial strain used in the production of both malt and grain whisky. The strain was provided in liquid and dried form.

Liquid yeast was stored on agar slants of YPD (10% w/v yeast extract, 20% w/v peptone, 20% w/v glucose and 15% w/v nutrient agar (Fisher-Scientific, Loughborough, GBR). Fresh slants were prepared from stocks (frozen at -80°C) every 4 weeks. For all experiments, yeast was freshly propagated prior to pitching. To propagate, yeast was transferred from YPD slant to water (5mL) and kept for 24hr at room temperature. The suspended yeast was transferred to 150mL of liquid YPD and grown with shaking (300rpm) at 27.5°C for 24hr. Following growth, the supernatant was discarded, and the cells counted by haemocytometer (ASBC, 2016). Stepwise propagation continued a further four times, with a starting volume of 250mL, 500mL, 1.0L and 1.5L of YPD media to which 25mL, 50mL, 100mL and 150mL of yeast culture from the previous stage was added. The propagation process was conducted in triplicate, with each replicate used to pitch one fermentation. Post propagation, yeast concentration was 1.39-1.50 x10^{10}cells/mL, with viability > 98.5-99.0%. Yeast was pitched by live cell count (see Yeast pitching rate below).

Yeast cell counts

Total and methylene blue stained cells were recorded using the haemocytometer, counting between 100-200 cells (ASBC, 2016, 2010). Measurements were performed in triplicate, enabling the determination of cell number and viability.

Yeast pitching rate

Dried yeast was stored in vacuum sealed bags and refrigerated for no longer than a year. Dried yeast was pitched directly into fermenter at a rate of 0.5g/L (under-pitched), 1g/L (control pitch), 2g/L (over-pitched). Liquid yeast was pitched at six pitching rates: 0.04, 0.2, 0.4, 2, 20 (control) and 200 x 10^6 viable cells/mL. The control pitching rate was identified from industrial studies using the same yeast strain (Reid et al, 2020).

Fermentation

Fermentations (20L) were performed in 33L brewing buckets (Young's Home Brew Ltd, Bilston, GBR). Buckets were fitted with a lid and airlock in a temperature-controlled environment (20°C) for

75 hr (dried yeast) and up to 100hr (liquid yeast) fermentations. Apparent extract (Cutaia et al, 2009) was determined by filtering wash (50mL) through grade 1 filter paper (Whatman, Maidstone, GBR) with measurement using an Anton-Paar DMA 35 (Anton-Paar, St Albans, GBR). Yeast counts were performed using the ASBC Yeast-4 method (ASBC, 2016). Final extracts and ethanol concentrations in wash were obtained by filtering wash (25mL) as above with measurement using an Anton-Paar DMA 4500 fitted with an Alcolyzer ME module using the `wash' program.

Distillation

The wash (20L) was first distilled using a steam operated, hybrid glass and copper pot still (Reid, 2021; Reid et al, 2021). The alcohol concentration of the distillate was monitored using a Anton-Paar DMA35 until the % ABV (alcohol by volume) dropped to 1% v/v, at which point the distillation was stopped.

Following wash distillation, 3L of the low wines were added to a 5L copper Alembic style still (Al-Ambiq, Gandra, PRT) fitted with a worm tub style condenser. This was heated using an isomantle (Electrothermal, Staffordshire, GBR). Foreshots (50mL) were collected and the ABV was monitored during the spirit cut and was allowed to drop by 12% v/v from the end of the foreshots cut. When the 12% w/v drop was completed, the distillation was stopped.

Higher alcohols

The analysis of higher alcohols was conducted using gas chromatography flame ionisation detection (GC-FID) operating in liquid injection mode. An Agilent 7820 A (Agilent Technologies, California, USA) was fitted with an Agilent DB-WAX 30m x 0.25mm x 0.25µm column. The oven program started at 35°C with a 6 min hold followed by a temperature ramp to 60°C at a rate of 5°C/min. After a 2 min hold, the temperature was raised to 210°C at a rate of 20°C/min and held for 1 min. Finally, the temperature was raised to 250°C at 70°C/min and held for 1 min. The detector was maintained at 270°C throughout analysis.

Sample (1 mL) was added to a vial together with 100µL of pentan-1-ol internal standard (final concentration 500 mg/L). The liquid injector autosampler withdrew 1µL of sample and injected this onto the column. Quantification was of higher alcohols and esters relevant to malt whisky spirit and included acetaldehyde, ethyl acetate, acetal, methanol, n¬-propanol, isobutanol, isoamyl acetate, n-butanol, isoamyl alcohol and furfural.

Esters

Esters in the new make spirit were measured using headspace solid phase microextraction gas chromatography mass spectrometry (HS-SPME-GC/MS). Analysis was conducted using a Shimadzu QP2010 Ultra GC/MS using an AOC 500 autosampler (Shimadzu, Tokyo, JPN). Analytes were extracted using PDMS/DVB fibre (65µm, Supleco, Pennsylvania, USA). Sample (1mL) was diluted with distilled water (5mL) and mixed with methyl heptanoate internal standard (10µL, 25mg/L) in a headspace vial. The vial was then incubated at 40°C for 5 min followed by exposure to the fibre (allowing for adsorption) for a further 5 min. The fibre was then injected to the inlet of the GC for 1min at 200°C.

Analytes were separated on an Agilent DB-WAX 30m x 0.25mm x 0.25µm column (Agilent, California, USA). The oven program began at 40°C for 3 min and was ramped to 100°C at a rate of 10°C/min and then immediately raised to 160°C/min at a constant rate of 4°C/min. Finally, the temperature was raised to 220°C at a rate of 10°C/min followed by a 10 min hold. The mass spectrometer operated in both SIM and SCAN modes. SIM ions used were m/z 45, 55, 74, 88, 101, 104 and 129. SCAN mode range from m/z 42 – 400. The quantified congeners were those found in malt new make spirit and included ethyl caproate, ethyl lactate, ethyl caprylate, phenylethyl acetate, ethyl caprate, ethyl laurate and ethyl palmitate. All calibrations levels were recorded in triplicate at a range of 0.1 – 10 mg/L and all curves had $r^2 > 0.99$. All statistical analyses were conducted in Microsoft Excel 2019 (Microsoft, Washington, USA) and R (V4.0.4, The R-Foundation, Vienna, AUS) using the RStudio interface (V1.4, Rstudio, Boston, MA, USA).

Fermentations were modelled using the 4-parameter logistic function (Equation 1) (ASBC, 2012; Reid, 2021; Reid et al, 2021).

$$P_i = P_e + \frac{P_i - P_e}{1 + e^{-B(t-M)}} \quad \text{(Eqn.1)}$$

Where:
$P_{(t)}$ = the apparent extract (AE, specific gravity) at time *t*,
P_i = the initial asymptotic extract (OE),
P_e = the final asymptotic extract (FE),
B is a function of the gradient (M) of the curve at the inflection point,
M is the inflection (midway point of the asymptote) with respect to time.

Triplicate data points were combined to determine one model per fermentation condition. From the fermentation modelling, associated parameters were determined including lag time, maximum fermentation rate (V_{max}) and total fermentation time. Maximum fermentation rates were determined through assessment of the first differential of the model at the inflection point, M.

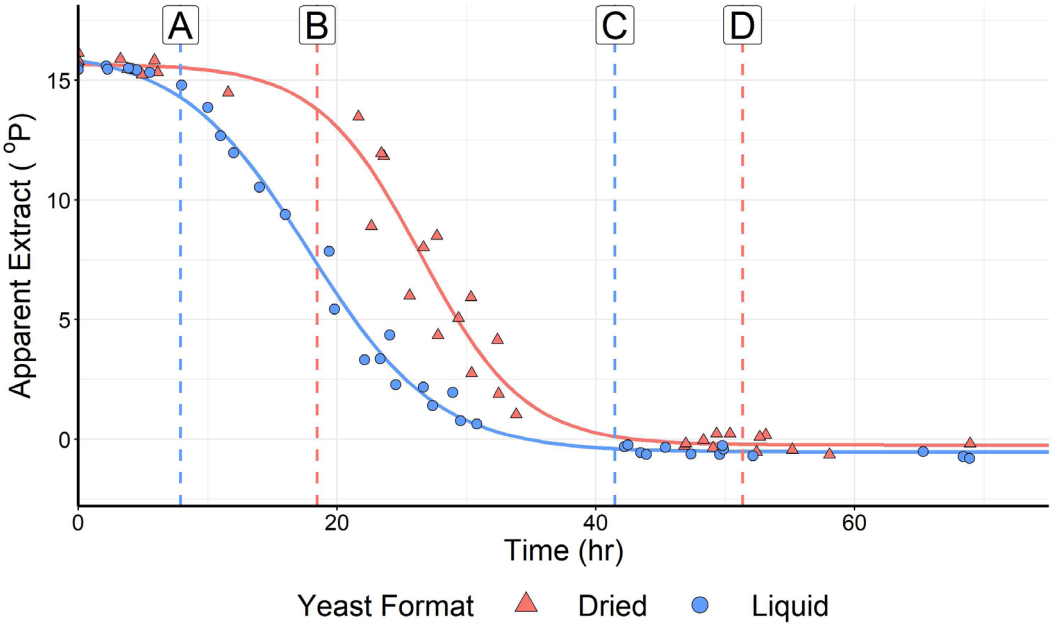
The lag time was determined by determining the intersection of the line y = P_i and the straight line that intersects the inflection point at the gradient (V_{max}) of the model at that point. Fermentation end point was determined when the gradient of the model was <0.1°P/h.

Significance levels were calculated at the 95% confidence interval unless otherwise stated. The four-parameter model was also adjusted to model cell growth during fermentation. The adapted model contained the variables C_i, which described the initial asymptotic cell concentration (comparable to the pitching rate), C_e which described the final asymptotic cell concentration (upper yeast cell concentration). The model also described the maximum growth rate, μ_{max} and generation time. The time total number of generations (n), or population doubling was determined from the cell growth model using Equation 2.

$$n = \frac{\ln\left(\frac{C_e}{C_i}\right)}{\ln(2)} \quad \text{(Eqn.2)}$$

Figure 3.

Apparent extract of fermentations with dried and liquid format of yeast. The decline in apparent extract is modelled using the 4-parameter logistic model. Dash lines represent the lag and end of fermentation times of liquid yeast (A, C) and dry yeast (B, D), calculated by analysis of model parameters and assessing the differential of the produced model. Each data point represents one observation and the model the average of triplicate fermentations.



Results and discussion

The effect of yeast format on fermentation and congener production

Comparison of the yeast format (Figure 3) showed that fermentation kinetics were fastest when using the liquid (cream) yeast format. This appears to result from changes in the lag phase of fermentation, which was shorter with liquid yeast compared to dried yeast. The difference of ca. 8-10 hours was notable at both the midpoint and end of fermentation, with both showing an increase of 8 hours when using the dried yeast. This delay was determined to be significant ($p<0.05$). However, the calculated values for V_{max} demonstrated that fermentations had similar maximum rates.

No significant difference ($p>0.05$) was found for the original and final asymptotic value (P_i and P_e respectively, eqn.1) for these fermentations, with model parameters showing parity. The final wash alcohol concentration was lower on average using dried yeast, which is reflected in the output model parameters ($P_i - P_e$), however, these differences were not found to be significant ($p>0.05$).

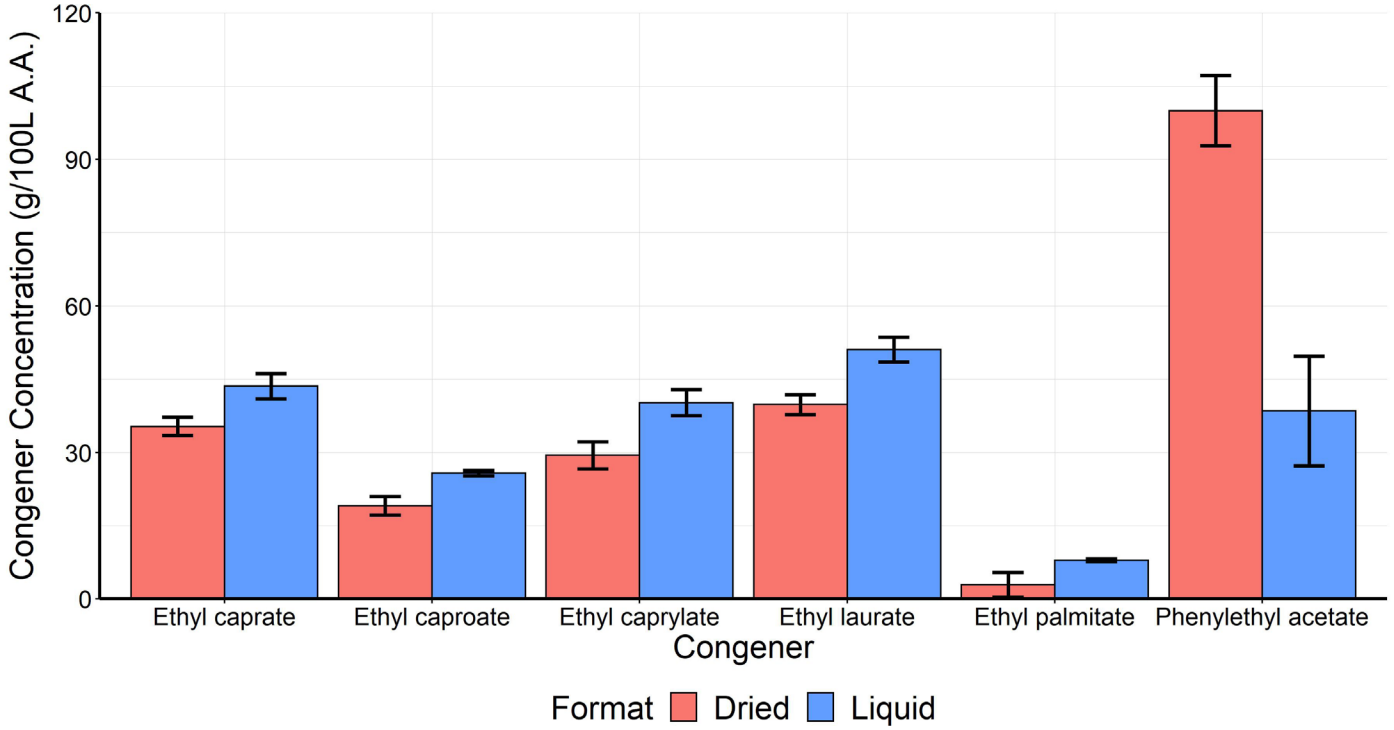
A greater variation using dried yeast in both the final ethanol titres and gravity decline was noted during these fermentations.

Extended fermentation times with dried yeast would be expected as the yeast added to the wort is in a different physiological state, requiring rehydration before the cell cycle starts and fermentation begins. Furthermore, the drying of yeast induces stress to the yeast which can result in reduced viability and ethanol tolerance (Cheung et al, 2012). These issues can be compensated for by rehydrating the yeast prior to use, which also encourages better cell dispersal in the fermentation media (Ferrarini et al, 2007).

New make spirit produced from both fermentations was analysed for higher alcohols and esters by GC-FID and GC/MS (Figure 4). A analysis of the new make spirit indicated an elevated concentration of congeners - excluding acetaldehyde, methanol, furfural and phenylethyl acetate - in the spirit produced with liquid yeast compared to dried yeast. The concentration of volatile esters (except for ethyl acetate and phenylethyl acetate) was significantly ($p<0.05$) higher where the liquid format

Figure 4.

Volatile esters analysis by SPME-GC/MS of new make spirits produced from dried and liquid yeast. Concentration is expressed in g/100L absolute alcohol (A.A.) Error bars represent the standard deviation of the mean of triplicate samples.



was used. Esters are associated with fruity, waxy, or sweet aromas (Daute et al, 2021). Therefore, fermentation with liquid yeast may produce more of these aromas (Burdock, 2010; Reid et al, 2020). The level of acetaldehyde (an intermediate in the production of ethanol) was elevated in new make spirit from dried yeast. This may be a result of incomplete fermentation due to a lack of nutrients, stress from the yeast drying process, increased bacterial competition due to the increased lag time or insufficient time in stationary phase for the conversion to ethanol. The enhanced concentration of phenylethyl acetate from fermentations with dried yeast may be due to an increase in phenylalanine, a yeast autolysis product resulting from the drying process.

The increase in fermentation lag time will also increase the opportunity for competing bacterial microflora to grow, reducing substrate availability for the yeast growth. Fermentation data showed that attenuation was lower during fermentations using liquid yeast compared to dried yeast.

This work was conducted comparing an industrially produced dried yeast and the same propagated liquid yeast. As such, several process parameters during the production of these yeasts will be different. Laboratory propagation of the liquid yeast was without the forced oxygenation used in the industrial process (Figure 2).

Further, fed batch fermentation process was not used. These differences may have resulted in physiological differences, particularly the availability of oxygen during laboratory propagation. It is likely that nutritional differences will also exist between the two propagation methods. Despite these reservations, a comparison of industrially produced liquid and dried yeast formats has been compared at industrial scale, demonstrating similar results for the final spirit (Reid, 2021).

Table 2.

Initial and final yeast cell count. Number of generations from equation 2.

Fermentation	Pitching rate (g/L)	Approx. pitching rate (x10^6 cells/mL)†	Final cell count (x10^6 cells/mL)	Estimated number of generations
Control	1.0	22	143 ± 23	2.7
Overpitched	2.0	44	180 ± 20	2.0
Underpitched	0.5	11	169 ± 14	3.9

† Assumed viable cell concentration in dried yeast of 2.2 x10^{10} cells/g (Table 1).

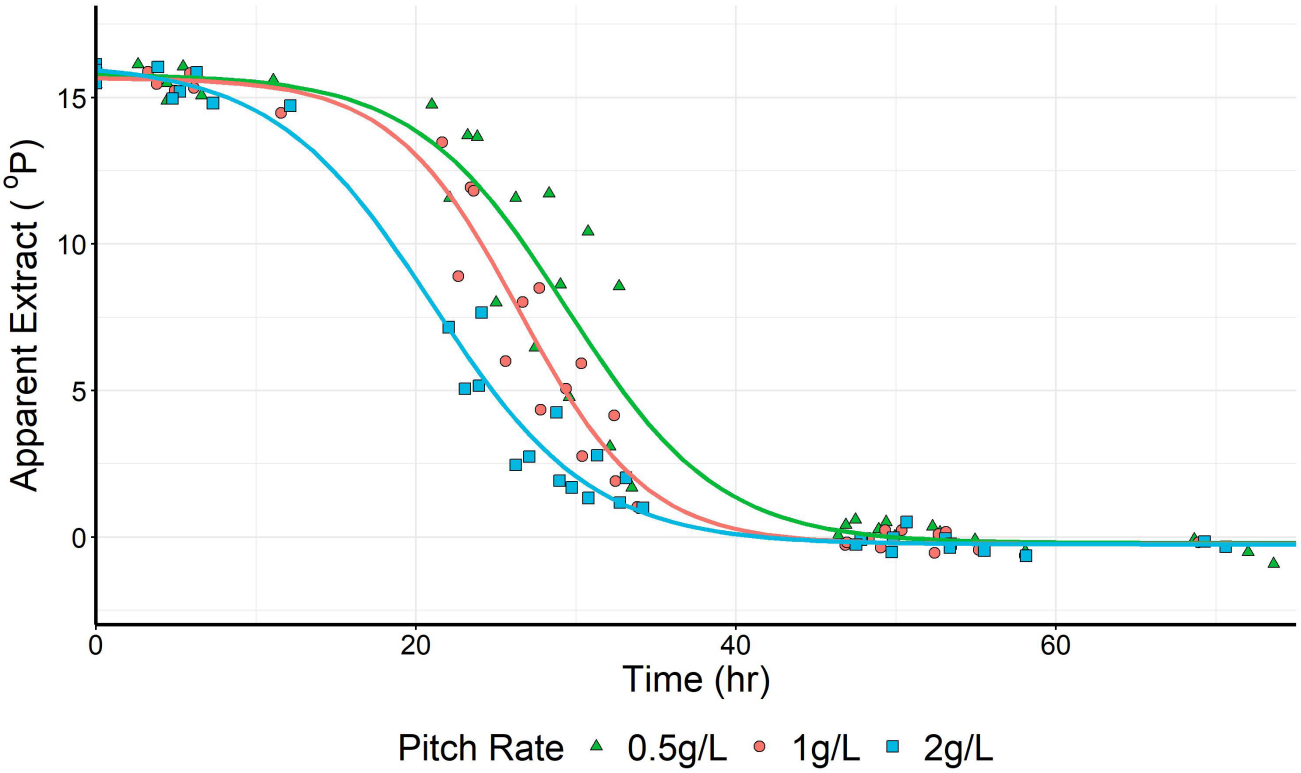
Dried yeast pitching rate

The impact of pitching rate was assessed by varying the quantity of dried yeast added to 20L wort fermentations. Three pitching rates were used, underpitched (0.5g/L), control (1g/L) and overpitched (2g/L), with the fermentation kinetics followed by monitoring the decline in apparent extract (Figure 5). The models showed significantly different ($p<0.05$) profiles when the pitching rate was varied. By increasing the yeast pitching rate, the lag phase was reduced. The longest lag phase was measured with the underpitched yeast and the shortest with the overpitched. The control demonstrated a lag phase like that of the underpitched fermentations.

Changes to the lag phase may reflect changes to the rate yeast adjusts to its new surroundings and to the consumption of nutrients. By increasing the pitching rate, the time before budding begins will be shorter due the larger cell population. Furthermore, a higher pitching rate will out-compete bacteria present in the wort. The differences between the control and underpitched fermentations were small compared to the kinetics of the overpitched fermentation versus the control. Globally, the typical pitching rate of grain based distilling fermentations with dried yeast can be as little as 0.25g/L. Therefore, it is likely that successful fermentation could be conducted with the yeast pitching rate further reduced. It is possible that the lag phase of approximately 20 hours may be limiting for a successful, complete fermentation. Furthermore, the absolute amount of yeast pitched is greater for the overpitched fermentation (40 g) than the difference between the underpitched sample (10 g) and the control (20 g).

Figure 5.

Apparent extract during fermentation with dried yeast where the pitching rate was varied from 0.5-2g/L. The trend in the decline in apparent extract is modelled using the 4-parameter logistic model. Data points are from single fermentations and the model represents the average model for all replicates (n=3).



The maximum consumption rate $|V_{max}|$, was comparable across all fermentations ranging from 0.82–1.0°P/hr, with the highest and lowest values from the under and overpitched fermentations. Nutrient addition, specifically nitrogen, has been demonstrated to increase yeast cell growth and the kinetics and efficiency of fermentation (Stewart, 2017). It is suggested from this work that by increasing the pitching rate, the effect on increasing yeast cell concentration early in fermentation is counteracted by a reduction in yeast cell health due to dilution of wort nutrients across the increased yeast population. Final yeast cell concentration was not significantly different (p<0.05) at the end of fermentation (Table 2), however the estimated number of generations did vary due to differences in pitching rate. These results agree with previous studies that assessed the physiological effects of inoculum size on beer fermentation (Verbelen et al, 2009).

Total fermentation time varied from 45 hours (overpitched) to 51 hours (underpitched), demonstrating that the delay by different lag times is relieved by the end of fermentation. This is reflected by the higher values of V_{max}. These results demonstrate that pitching rate can be increased where a shorter fermentation time (overall) is required. No substantial differences in the lower asymptotic limit of the fermentation models (P_e) were noted (equation 1).

Distillates were analysed by GC-FID and by SPME-GC/MS (Figure 6). The results show that as the yeast pitching rate is increased, the concentration of ethyl esters is reduced. This was found to be significant (p<0.05) when comparing the overpitched new make spirit with the control in the cases of ethyl caprate and ethyl caprylate. These esters are often associated with fruity-waxy characteristics such as apple, pineapple and banana (Burdock, 2010). The opposite trend was observed for phenylethyl acetate (floral, rose) which was significantly lower (p< 0.05) in the underpitched distillate (Burdock, 2010). This trend was also reflected in the analysis of the other acetate esters, ethyl and isoamyl acetate, with isoamyl acetate measured at significantly lower (p<0.05) concentrations in the underpitched new make spirit.

Figure 6.

New make spirit analysis of ethyl esters and phenylethyl acetate from fermentations pitched with varying amounts of dried yeast. Error bars indicate the standard deviation on the mean of replicates (n=3).

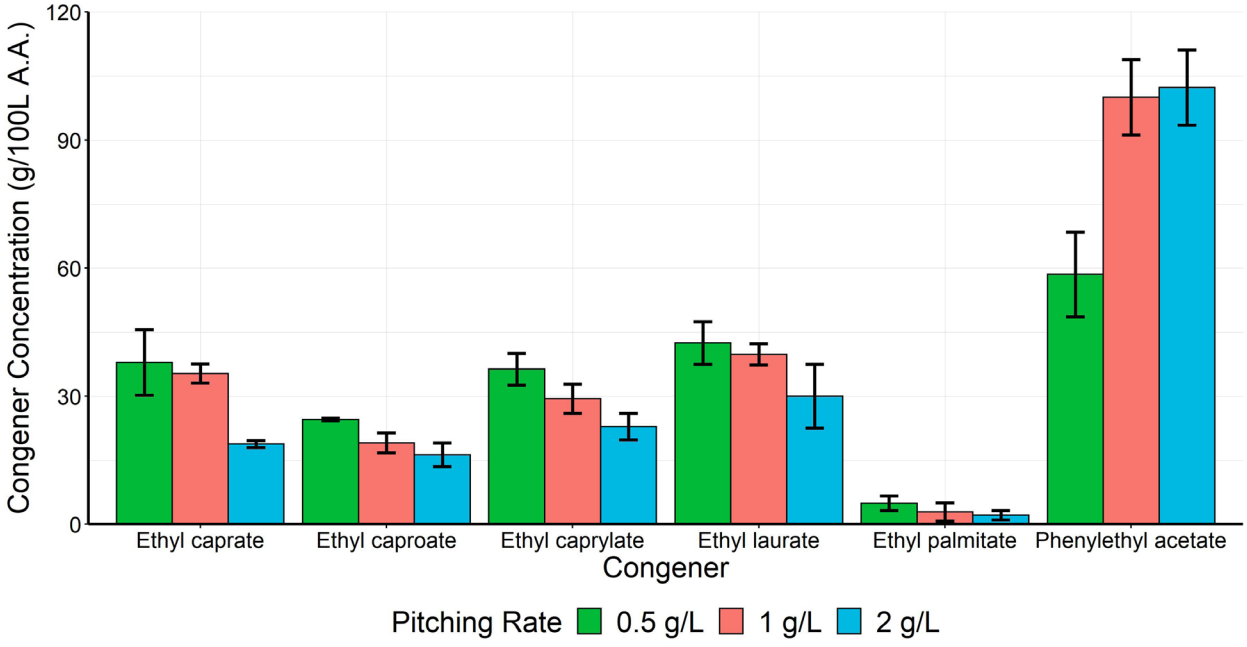
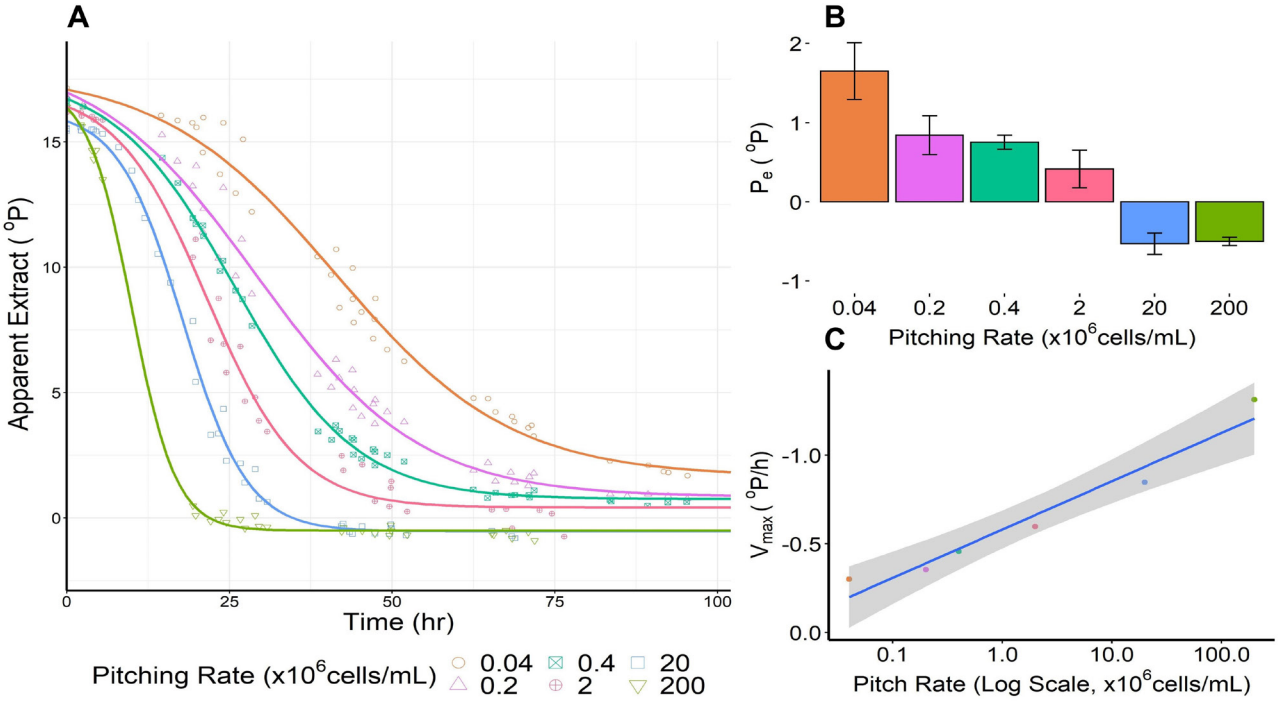


Figure 7.

(A) **Apparent extract of fermentations pitched with varying amounts of liquid yeast**. Each point represents one observation during fermentation. The fitted 4-parameter logistic model represents the average model across triplicate fermentations. (B) **The trend in the lower asymptotic value (P_e of fitted models as pitching rate is increased.** (**C**) **The trend in the maximum consumption rate (V_{max}) as pitching rate is increased fitted with a linear regression.** The shaded area represents the 95% confidence interval on the regression.



Liquid yeast pitching rate

The pitching rates of liquid yeast were varied from 0.04–200 x10^6 cells/mL for fermentation of all malt wort. Measurement of apparent extract throughout fermentation demonstrated that significant differences ($p<0.05$) between the fermentation models existed (Figure 7A). As observed above, the lag time was reduced as pitching rate was increased. This ranged from 1.3 hours (most overpitched) to 14.5 hours (most underpitched), with a calculated lag time during the control fermentations of 6.4hr.

Unlike the results with dried yeast, V_{max} varied substantially with yeast pitching rate (Figure 7C). Values of $|V_{max}|$ ranged from 0.30 to 1.31°P/hr from the most underpitched to the most overpitched. This may be due to several factors. The range of pitching rates in this experiment was broader than that of dried yeast, with the highest pitching rate 5,000 times greater than the lowest pitching rate compared to four times the number of cells with dried yeast (Table 2). It is, therefore, likely within this broader range that differences in V_{max} would be observed. This can be seen when studying the pitching rates 0.2 and 0.4x10^6 cells/mL, whose $|V_{max}|$ values were calculated as 0.35 and 0.45°P/hr. These differences are comparable to those seen for dried yeast at similar differences in pitching rate. Yeast pitched in the liquid format is not required to endure the stresses imposed during the drying process and so V_{max} is likely to be similar to that of a pure culture fermentation. Further, the kinetics are optimal due to sufficient nutrition being provided during the propagation stages. Therefore, fermentations will proceed based on yeast cell concentration rather than nutrient availability. Competition may result in a relative reduction in $|V_{max}|$, which was most observed at low pitching rates.

The final asymptotic value for the models demonstrated that not all fermentations run to completion, with some pitching rates resulting in high final gravities (Figure 7B). Fermentations with a pitching rate lower than 2 x10^6 cells/mL failed to attenuate below 0.5°P. This is likely due to insufficient yeast being produced during fermentation or the stresses imposed on yeast at low pitching rates from both ethanol toxicity and the proliferation of lactic acid bacteria towards the end of fermentation

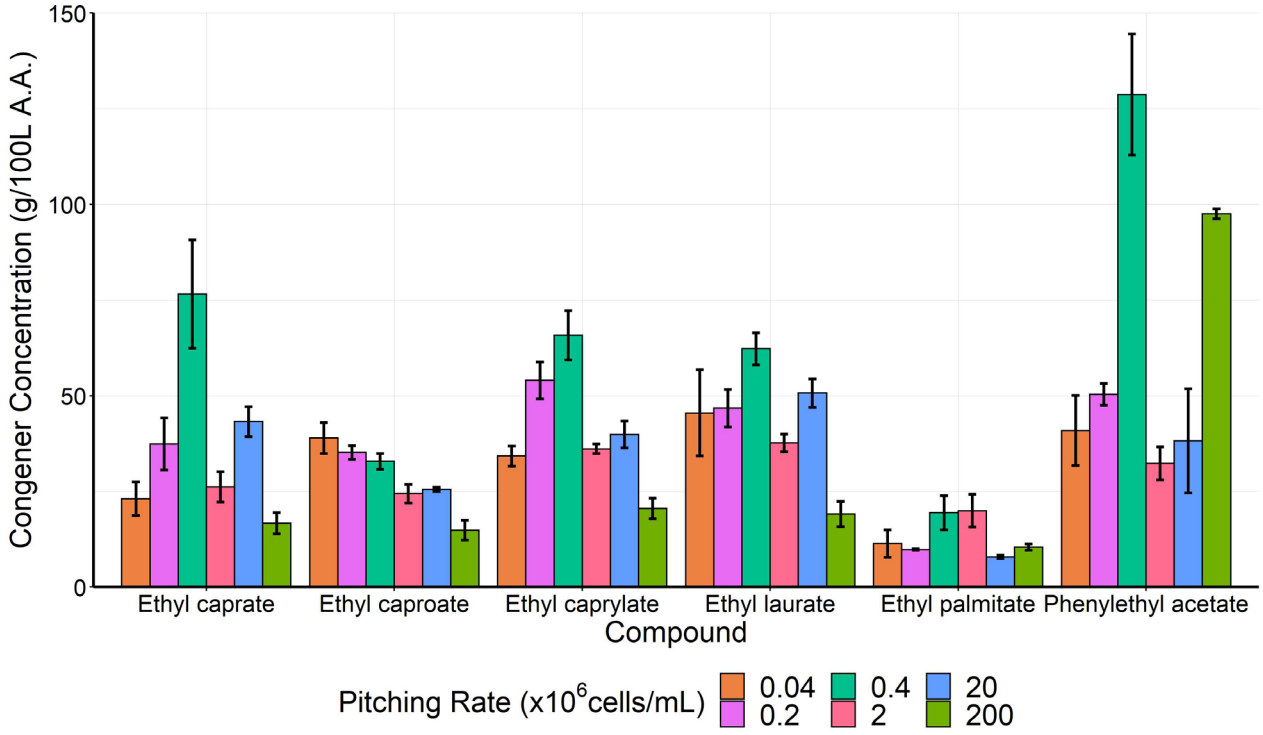
(Ingledew, 2009). This effect may be more substantial at industrial scale as bacterial contamination can be more significant. Microbial contamination at distilleries can be high due to the lack of a wort boiling combined with the increased temperatures that occur through exothermy during fermentation.

The trend in ethyl ester concentration (Figure 8) is less obvious in liquid yeast than dried, with highest values at a pitching rate of 0.4 x10^6 cells/mL. Concentrations of ethyl caprate, ethyl caprylate, ethyl laurate and phenylethyl acetate were significantly higher ($p<0.05$). Ethyl caproate appeared to trend downwards from the lowest pitching rate to the highest. Otherwise, there was a general bell-shaped trend in congener concentration. This reflects the lack of attenuation for fermentations with a very low pitching rate (<0.4 x10^6 cells/mL). As attenuation impacts on the concentration of metabolites (ethanol and congeners) produced by yeast, it is likely that the under attenuation impacts the level of congeners in the new make spirit. Without the impact of reduced attenuation, it can be concluded that reduced pitching rates generally produced a greater concentration of ethyl esters. In the case of phenylethyl acetate, the trend is not clear. The concentration was greatest at a pitching rate of 0.4 x10^6 cells/mL. However, like Figure 6, higher pitching rates also favour the production of this acetate ester.

During the production of new make spirit, wash including the spent yeast and other microflora are transferred to the still. As a result, changes in the pitching rate and final yeast cell concentration would be expected to contribute to the final spirit composition due to volatiles from yeast cell breakdown being transferred or reacting during distillation, together with intracellular ethanol. Furthermore, studies have suggested that esters, capable of adhering to yeast cell wall proteins, are liberated during this process (Saita and Nagata, 1986). An increase in the number of yeast cells may also have a practical impact on distillery through an increase of fouling still heating components and subsequent reduction in heating efficiency (Vigil, 2017).

Figure 8.

New make spirit analysis of ethyl esters and phenylethyl acetate of fermentations pitched with varying amounts of liquid yeast. Error bars indicate the standard deviation on the mean of replicates (n = 3).



Conclusions

The key findings of this study are outlined in Table 3.

This study demonstrates the importance of yeast format and pitching rate on the fermentation kinetics and congener formation in Scotch malt whisky style wort. The choice of yeast format is typically determined by a number of factors including infrastructure, scale, or location of the distillery. With developing technologies such as stabilised liquid yeast, Scotch whisky producers may wish to consider alternative formats based on fermentation rates and flavour formation. By altering the format of the pitching yeast, substantially different fermentation models (apparent extract) were observed. The use of liquid yeast gave a shorter lag phase, resulting in a faster overall fermentation as maximum fermentation rates were not significantly impacted by format. Distillates produced from liquid yeasts were also observed to have higher concentrations of congeners, particularly ethyl esters.

Yeast pitching rates are rarely adjusted in Scotch whisky fermentation, unlike with biofuels where pitching rates may be adjusted to address specific stressors such as contamination (bacteria), high temperature or substrate specific inhibitors (Ingledew, 2009; Odumeru et al, 1992). Indeed, distillery fermentations in Scotland are overpitched, with dried yeast pitching rates more than double those in other parts of world (Collicutt, 2009). Pitching rates are generally high to avoid stuck fermentations, ensuring fast kinetics for distillery throughput and to out-compete the growth of bacteria and wild yeasts. Furthermore, higher pitching rates may also encourage 'late-lactic' or secondary fermentation, contributing to spirit character (Pratt-Marshall, 2002).

Yeast pitching rates were studied using dried yeast between 0.5-2.0g/L. The results show that models of apparent extract decline were significantly different for all three conditions. The most substantial effects were from the overpitched fermentations, where lag times were reduced by 39% in comparison with the control. Changes to the maximum fermentation rate were less dramatic, with the underpitched fermentations exhibiting an increased fermentation rate of 16% compared to the control, although these differences were not significant (p>0.05). The resulting yeast population during the underpitched fermentation was approximately double the number of generations. Although these fermentations have significantly longer lag phases, the efficiency during the exponential phase (indicated by V_{max}) is increased. Distilleries may take advantage of this effect by employing processes such as propagation where yeast could be underpitched.

Table 3.

Effect of yeast format and pitching rate on fermentation rate and ethyl ester concentration in new make spirit.

Yeast format	Pitching rate	Lag time	Fermentation rate	Ethyl ester concentration
Dried	Control	Intermediate	Intermediate	Intermediate
	Low	Long	High	High
	High	Short	Low	Low
Liquid	Control	Intermediate[†]	Intermediate	Intermediate[†]
	Low	Long	Low	High[‡]
	High	Short	High	Low

[†]Lag time of liquid yeast shorter than comparative dried control and higher ethyl ester concentration.
[‡]Where pitching rate is not sufficiently low that fermentation is not conducted to completion.

Liquid yeast pitching rates were studied across a range between 0.04-200 x10^6 cells/mL. The results reflected those seen with dried yeast with some key differences. As observed with dried yeast, increasing the pitching rate reduced the lag time of fermentation, however the maximum consumption rate, V_{max}, also increased with increasing yeast pitching rate. It is postulated that this is because liquid yeast is less dependent on wort nutrition as it is pitched during exponential phase. Due to a reduction in lag time and an increase in V_{max}, overpitched liquid yeast fermentations are much quicker than those that are underpitched. It is suggested that this effect at plant scale would be emphasised due to the increased bacterial load in such fermentations, which will compete with underpitched fermentations for the same wort nutrients.

The reduction in lag time for overpitched fermentations of both dried and liquid formats is likely to be caused by the increased rate at which the yeast consumes the growth limiting wort components. These include dissolved oxygen and free amino nitrogen, although it is not clear what the limiting factor for yeast cell growth is in malt whisky fermentation. Once these are consumed, the yeast will enter the exponential phase where wort carbohydrates are consumed.

By reducing the time to complete primary fermentation using liquid yeast, higher pitching rate allows secondary fermentation by bacteria and other metabolic conversions to take place sooner. Anecdotally, in the Scotch whisky industry, long fermentation times are considered to produce fruity and waxy style distillates. Thus, methods employed to reduce the lag phase of fermentation can allow a distiller to run shorter fermentations but still achieve the desired flavour characteristics in the final distillate.

Similarly, to comparisons between yeast formats, the differences in ethyl ester concentration in new make spirit were impacted by yeast pitching rate. For dried yeast, the most underpitched (0.5g/L) fermentations demonstrated the highest concentrations of these esters. The opposite trend was observed for phenylethyl acetate, which was highest in distillates from the most overpitched (2g/L) distillates.

The effects of ester concentration in new distillate varied across the fermentations pitched with liquid yeast. The concentration of these esters was particularly high for fermentations with a pitching rate of 0.4 x10^6 cells/mL. It was concluded that ethyl ester production was optimised by low pitching rates, until this resulted in incomplete attenuation. The trend in concentration of ethyl esters was similar across both formats where the liquid yeast pitching rates were between 0.04-2 x10^6 cells/mL. These results demonstrate that a distiller can maximise the production of ethyl esters in new make spirit by using a liquid yeast format in conjunction with the minimum pitching rate required for complete carbohydrate metabolism.

In this study, an industrially relevant yeast strain was tested at laboratory scale. The specific effects vary with both dried and liquid format and pitching rate. This reflects the robustness of the strain and performance in the dried format, which can vary across strains of *S. cerevisiae*. Through variation of both yeast format and pitching rate, distillers may fine tune the fermentation process to produce new make spirit to specification. This may include parameters such as fermentation throughput and flavour which can be altered by the yeast format and pitching rate.

Author contributions

Struan J Reid: Investigation, visualisation, writing (original draft, review and editing).
R Alex Speers: writing (review and editing).
William B Lumsden: funding acquisition, resources, supervision.
Nicholas A Willoughby: writing (review and editing).
Dawn L Maskell: conceptualisation, supervision, resources, writing (review and editing).

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Conflict of Interest

The authors declare there are no conflicts of interest.

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