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Strain specific performance of active dry yeast for fermentation of very high gravity wort

Active dry brewing yeast strains were characterized for fermentation of very high gravity wort from 18–36 °P. In higher gravity worts, metabolism of maltose and maltotriose decreases, resulting in lower attenuated beers. In general, all active dry yeast strains performed well with only minor changes in attenuation and metabolism of maltose and maltotriose up to 24–26 °P. Strain-specific differences in attenuation, sugar metabolism and ethanol production were identified allowing for the identification of strains that maintain consistent metabolism of maltose and maltotriose up to 36 °P. These differences highlight the importance of yeast strain selection for high gravity brewing applications.

Descriptors: high gravity, fermentation, yeast strain

1 Introduction

High gravity brewing (HGB) refers to the process of fermenting concentrated wort to produce a stronger beer. While there is no formal definition, it generally refers to wort with an original gravity (OG) of 15–20 °P that ferments to produce a beer >6 % abv [1]. HGB is used in larger breweries to increase production capacity and minimize cost. Strong beer produced by HGB is diluted with water after fermentation allowing for a greater volume of finished beer to be produced from each brew [1]. More recently, HGB has become popular with craft brewers to produce strong beers that are not diluted. Very high gravity brewing (VHGB) refers to fermentations above 20 °P, and there are commercial styles that push the limits of the gravity range including Barleywines (OG 19–28 °P) and Wee Heavy (OG 17–30 °P) [2] and there are examples of commercial beers push these limits above 30 °P [3].

The cellular environment imposed by HGB is stressful to the yeast cell. Yeast is pitched into a high density wort, which results in osmotic stress [4, 5]. High ethanol levels increase membrane fluidity and permeability and are toxic to the yeast cell [6–8]. Yeast cell growth is inhibited at ethanol concentrations between 4–12 % abv, but many industrial strains are able to produce ethanol levels above 20 % abv [8, 9, 11] and fermentation activity has been observed in the presence of ethanol concentrations up to 38 % abv [8, 10]. High osmotic pressure and high alcohol have been shown to affect yeast viability and morphology [1, 4, 5, 7, 12]. HGB can result in decreased metabolism of maltose and maltotriose [1] and the resulting longer fermentations can lead to nutrient deficiency [13], which may in turn affect flocculation [14]. Due to these challenges, the potential of very high gravity brewing (>20 °P) has not yet been realized [6].

Tolerance of yeast to conditions of HGB is dependent on strain, physiological state and cellular environment [6]. Since oxygen is less soluble in high gravity wort, yeast cell growth can be limited due to an inability to synthesize adequate levels of sterols and unsaturated fatty acids [13]. Cell growth inhibition caused by lower oxygen solubility in high gravity wort may be less significant when using active dry yeast, which is rich in sterols and unsaturated fatty acids and is therefore insensitive to wort aeration levels. Active dry yeast may be more tolerant to the stress of HGB due to the high levels of trehalose, which is an important cell membrane stabilizer and osmoprotectant [15]. Conversely, since the membrane of active dry yeast becomes transiently leaky during rehydration [16], it is possible that the additional stress on the cell membrane due to high osmotic stress may have a more significant impact on dry yeast.

This study aims to characterize the fermentation performance of selected active dry brewing yeast strains in very high gravity worts (18–36 °P) and to determine the strain-specific limits of fermentation under these conditions. Addition of adjuncts is common in HGB, and higher maltose levels have been associated with higher cell counts, higher fermentability and better flavor profile [1, 17]. In order to achieve higher maltose levels while maintaining high nutrient levels, we prepared very high gravity wort using 100 % dry malt extract (no adjuncts). Yeast strains were produced by Lallemand Inc and were selected to represent a range of beer styles

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Table 1 Malt recipe for preparing wort from 18–36 °P

ºPlato	pH	g H_2O	g DME	FAN (mg/L)
18	5.51	420	94	460
20	5.57	420	107	515
22	5.57	420	120	568
24	5.54	420	135	627
26	5.52	420	150	684
28	5.50	420	165	739
30	5.47	420	182	799
32	5.45	420	200	860
34	5.42	420	218	918
36	5.39	420	238	981

Table 2 Description of yeast strains used for inoculation of very high gravity wort (18–36 °P)

Commercial Name	Style	Description
Abbaye	Belgian Ale	*S. cerevisiae*
Belle Saison	Saison	*S. cerevisiae var. diastaticus*
Diamond	Lager	*S. pastorianus*
Munich Classic	Wheat beer	*S. cerevisiae*
Munich	Wheat beer	*S. cerevisiae*
Nottingham	Ale	*S. cerevisiae*
BRY-97	Ale	*S. cerevisiae*
Windsor	Ale (maltotriose –ve)	*S. cerevisiae*
London ESB	Ale (maltotriose –ve)	*S. cerevisiae*

Yeast strains selected are produced by Lallemand Inc.

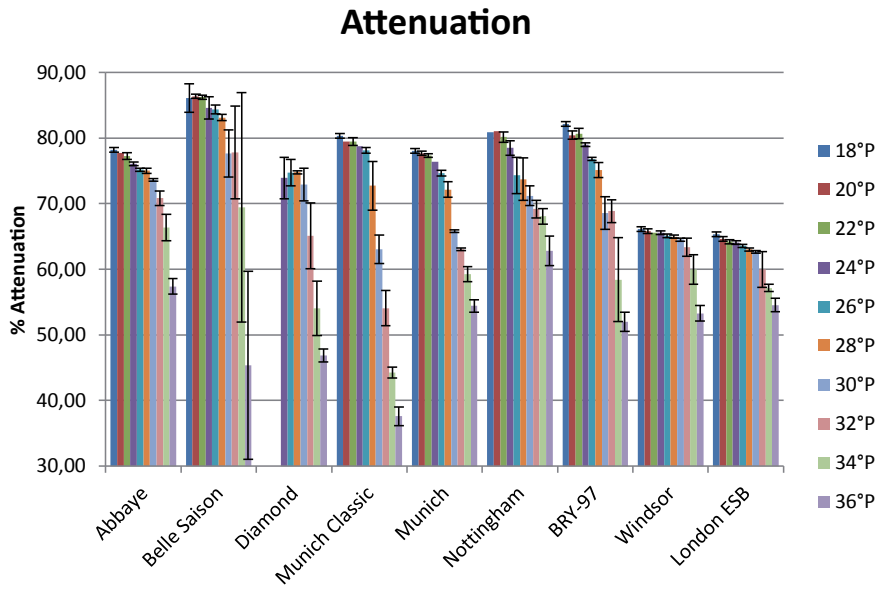

Fig. 1 Attenuation of nine different yeast strains fermenting in high gravity wort (18–36 °P). Each data point is the average of three replicate shake flask fermentations from the same wort. Error bars represent 95 % confidence intervals

including ales, Belgian ales, wheat beers and lagers. Fermentation performance was assessed through measurement of attenuation, sugar metabolism (glucose, maltose, maltotriose, dextrins) and ethanol production.

2 Materials and Methods

2.1 Shake flask set-up

For each strain tested, 30 x 250 ml Erlenmeyer flasks were fitted with rubber stoppers pierced with a syringe needle to allow gas to escape during fermentation. Syringes were covered with aluminum foil and flasks were autoclaved before being filled with 150 ml of wort.

2.2 Wort preparation

Briess CBW® Sparkling Amber Dry Malt Extract (DME) was used to prepare very high gravity wort with densities ranging from 18–36 °P. According to the manufacturer, a typical 100 g sample of this product contains 13 g glucose, 45 g maltose, 13 g maltotriose, 17 g higher saccharides, 8.5 g of protein and 3 g of water. The free amino nitrogen (FAN) content of the DME was measured using a ninhydrin-based dying assay. A dilute wort sample was prepared by dissolving a 1.5 g sample of DME in 1 L of distilled water. In a 2 ml microfuge tube, 1 ml of dilute wort was mixed with 0.5 ml of ninhydrin reagent (3.743 g sodium phosphate dibasic, 3 g potassium phosphate monobasic, 0.25 g ninhydrin, 0.15 g fructose dissolved in distilled water in a 50 ml volume) and incubated in a heat block for 16 minutes at 100 °C. Samples were cooled to room temperature and a 0.75 ml volume of the sample was diluted with 1.25 ml of dilution solution (1 g potassium iodide dissolved in 300 ml distilled water plus 200 ml of 96 % ethanol) and absorbance was measured at 570 nm. The FAN content of the DME (mg FAN per g) was calculated by comparison to a standard curve of glutamine using a concentration range from 0–78 mg/L. The FAN content of the wort was calculated based on the weight of DME used to prepare the wort at each density and is shown in Table 1.

Wort was prepared in 1 L glass bottles using the quantities listed in Table 1 and the initial density and pH were measured. The density was adjusted if necessary by adding more DME or water, but no pH adjustments were made. For each wort sample, three replicate shake flasks were filled with 150 ml of wort.

2.3 Yeast inoculation and fermentation

A selection of nine different yeast strains produced by Lallemand Inc were tested for performance in very high gravity fermentations. Strains selected represent different beer styles including two Belgian strains (one diastaticus), a lager strain, two wheat beer strains and four ale strains (two maltotriose negative). While liquid yeast is typically

pitched by number of viable cells, dry yeast is typically pitched by weight since the number of viable cells is consistent and stable over time. Active dry yeast was rehydrated by adding one part yeast to nine parts of sterile tap water and incubating for 15 minutes in a 30 °C water bath. Each shake flask containing 150 ml of wort was inoculated with 1.5 ml of rehydrated yeast for a pitch rate of 1 g/L (>5 million viable cells per ml).

The inoculated shake flasks were incubated at 25 °C and 150 rpm. The original and final gravity were measured directly using an Anton Paar DMA 35 density meter. The progression of fermentation was monitored by measuring the weight of the shake flasks instead of taking samples to measure density directly. Since no samples were being removed from the flasks during fermentation, the decrease in weight corresponds to the production and loss of CO_2. The shake flasks were weighed daily and fermentation was considered complete when the change in weight was less than 0.2 g in 24 hours.

2.4 Quantification of sugars and ethanol by HPLC

Samples were filtered using a Phenex 0.2 µm nylon membrane syringe filter and analyzed via high pressure liquid chromatography (HPLC). The concentrations of dextrins, maltotriose, maltose, glucose and ethanol were measured with an Agilent 1200 series HPLC equipped with a Bio-Rad Aminex HPX-87H ion exclusion column. Samples were eluted with 5 mM H_2SO_4 at a flow rate of 0.6 ml/min at 65 °C. All metabolites were detected with an Agilent 1260 Infinity II Refractive Index Detector.

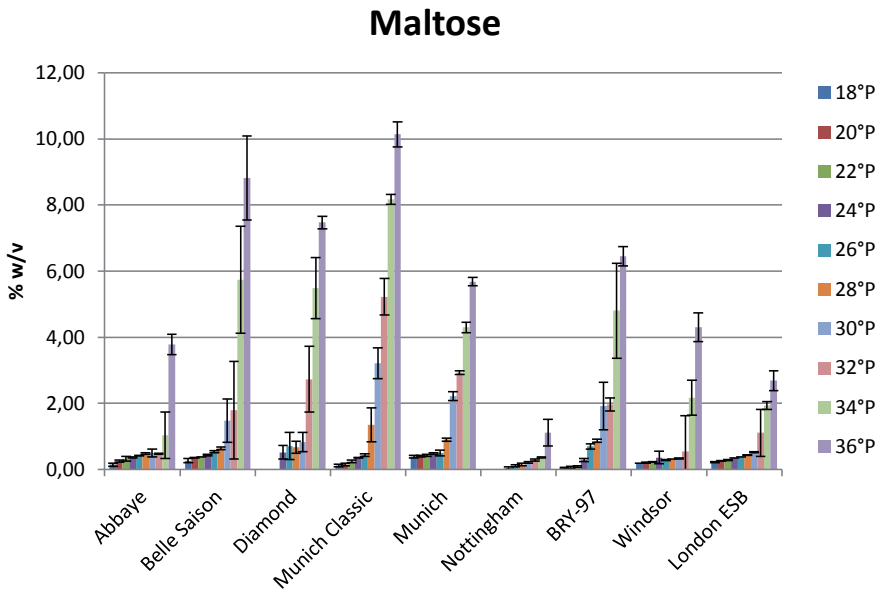

Fig. 2 **Maltose levels in beer fermented by nine different yeast strains in high gravity wort (18–36 °P). Each data point is the average of three replicate shake flask fermentations from the same wort. Error bars represent 95 % confidence intervals**

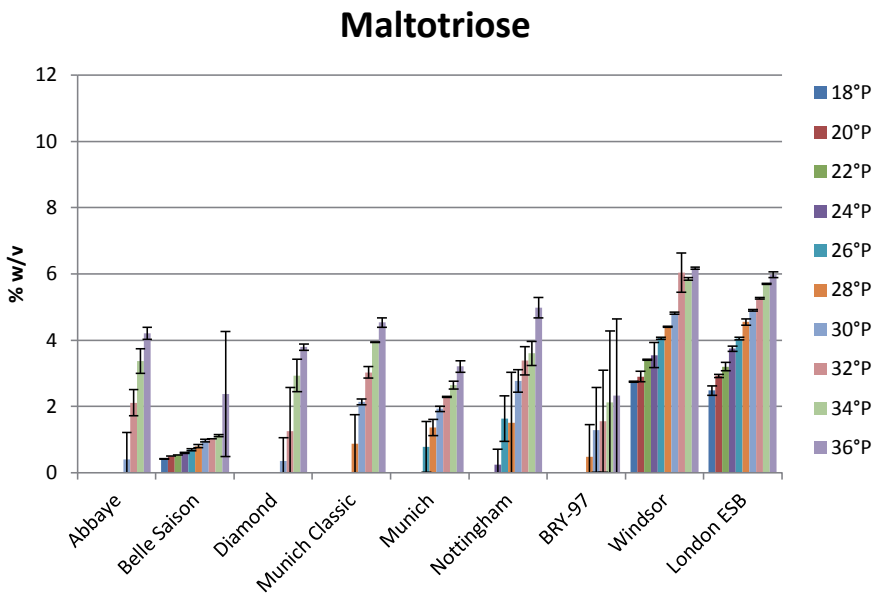

Fig. 3 **Maltotriose levels in beer fermented by nine different yeast strains in high gravity wort (18–36 °P). Each data point is the average of three replicate shake flask fermentations from the same wort. Error bars represent 95 % confidence intervals**

3 Results & Discussion

For all strains tested, attenuation decreases to some degree with increasing OG (Fig. 1). Attenuation decreases gradually as OG increases from 18 °P and more rapidly above 24–26 °P. Attenuation is not uniformly affected in all yeast strains. For example, Belle Saison, Munich Classic, Munich, BRY-97 and Diamond have precipitous drops in attenuation for OG above 26–28 °P. Windsor and London ESB do not lose much attenuation at higher OG, but have generally lower attenuation rates across the range of wort gravities tested due to an inability to metabolize maltotriose.

All yeast strains exhibit a reduced ability to metabolize maltose in wort with OG above 28 °P (Fig. 2). Again, the extent of this effect is strain dependant. Munich Classic, Belle Saison, BRY-97, Diamond and Munich do not metabolize maltose well above 28 °P. Windsor, London ESB and Abbaye retain some maltose metabolism above 32 °P. Nottingham metabolizes maltose completely even up to 34 °P.

Maltotriose metabolism decreases at OG above 24–28 °P for most strains (Fig. 3). The clear exceptions are London ESB and Windsor, which are maltotriose negative strains and showed a linear trend with increasing OG. Belle Saison (diastaticus) also showed this linear trend indicating incomplete maltotriose metabolism from 18–34 °P.

Glucose was completely metabolized by all strains tested from 18–36 °P (data not shown). While glucose is transported passively into the cell by facilitated diffusion, maltose and maltotriose require active transportation into the cell by transporters that are driven

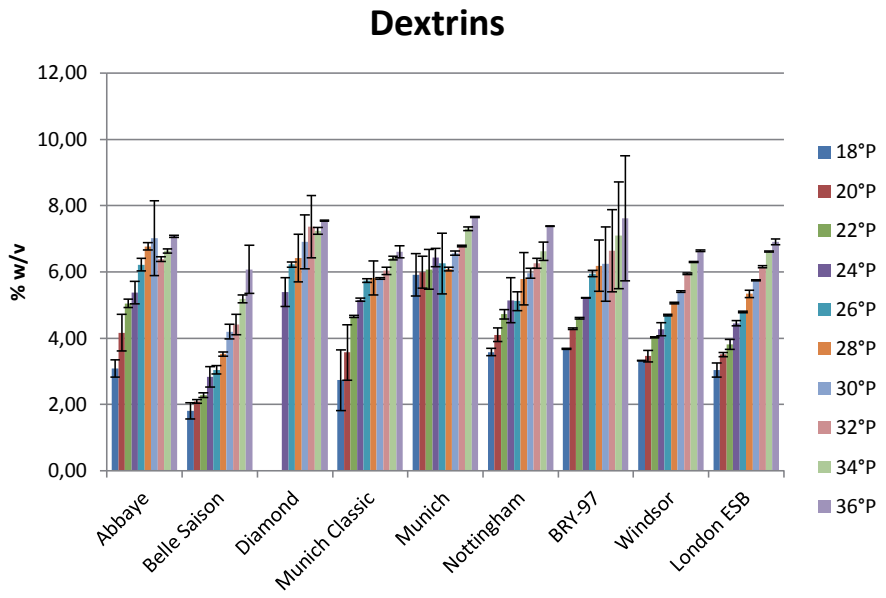

Fig. 4 **Dextrin levels in beer fermented by nine different yeast strains in high gravity wort (18–36 °P). Each data point is the average of three replicate shake flask fermentations from the same wort. Error bars represent 95 % confidence intervals**

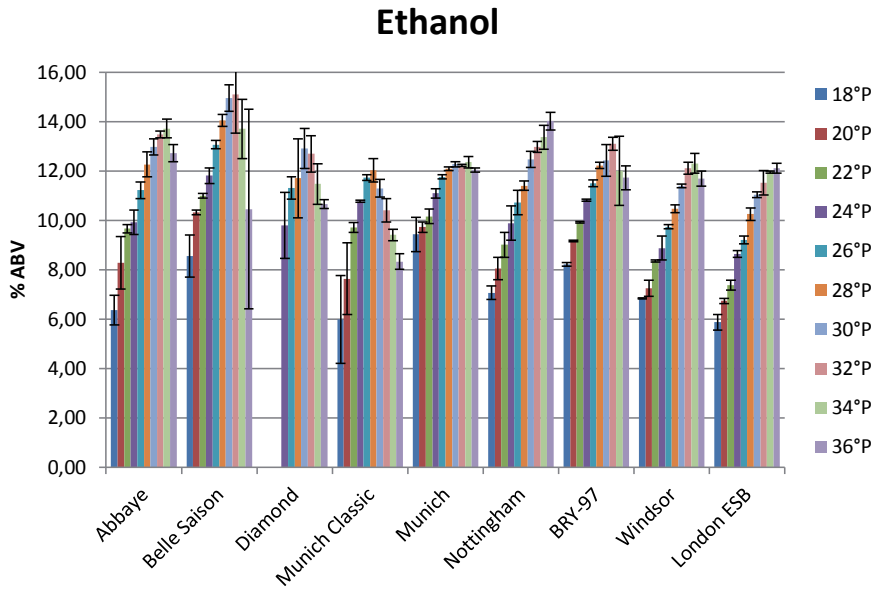

Fig. 5 **Ethanol levels in beer fermented by nine different yeast strains in high gravity wort (18–36 °P). Each data point is the average of three replicate shake flask fermentations from the same wort. Error bars represent 95 % confidence intervals**

by the proton gradient across the cell membrane [18, 19]. These results support the hypothesis that during high gravity fermentation, the active transport of maltose and maltotriose into the cell is inhibited, whereas the passive transport of glucose is unaffected.

The strain-specific decrease in attenuation at high OG shown in figure 1 corresponds to changes in maltose and maltotriose metabolism for these strains (Fig. 2 and 3). This suggests that the decrease in attenuation is the result of a loss of ability to metabolize maltose and maltotriose.

Dextrins are not metabolized well by any of the yeast strains and shows a relatively linear trend with increasing OG (Fig. 4). Belle Saison is a known diastaticus strain and the beer produced from this strain has lower dextrin levels than other strains, but dextrins were not completely metabolized during the time course of these experiments.

In all cases, the ethanol levels are associated with the efficiency of maltotriose and maltose metabolism (Fig. 5). For Abbaye, Belle Saison, Munich Classic, Diamond and BRY-97, maximum ethanol levels of 11–15 % abv are reached in worts between 28–34 °P. As the OG increases above this maximum ethanol level, these strains begin to succumb to the stress of the harsh high gravity fermentation, which may be a combination of ethanol toxicity and nutrient and oxygen deficiency. Munich, London ESB and Windsor reach a plateau ethanol concentration, but do not decrease from this maximum ethanol level even up to 36 °P. For these strains, maltotriose and maltose metabolism is inhibited. Nottingham is notable in that it maintains a linear trend of ethanol levels with increasing OG, which is consistent with the fact that this strain maintains maltose metabolism even up to 34–36 °P. Further study would be warranted for Nottingham, Windsor, London ESB and Munich at OGs >36 °P to determine their maximum ethanol production levels.

Since nutrient deficiency is a stress for yeast during HGB, these experiments should be repeated using wort supplemented with nutrients. In this case, high gravity wort was prepared from DME instead of by addition of adjuncts and FAN levels were relatively high, but vitamins, minerals and oxygen may be limiting. Also, since yeast cell membranes have been shown to be affected by high gravity wort and high ethanol levels, it is possible that rehydration may play a greater role in HGB compared to brewing more standard beers. It would be worthwhile to test whether rehydration of active dry yeast is important during HGB, including rehydration of the yeast in the presence of sterols and polyunsaturated fatty acids to promote membrane integrity. Since these fermentations were performed in shake flasks, cells were prevented from settling and any effect of HGB on flocculation was not assessed. These results should be validated in the context of a beer fermentation in a traditional fermenter without agitation. A single pitch rate of 1 g/L was used for all fermentations. Increasing the pitch rate for higher gravity fermentations may improve fermentation performance.

Since maltose and maltotriose metabolism varies by yeast strain, it may be possible to design a yeast blend that is optimized for HGB. For example, it is possible that Nottingham (an excellent maltose metabolizer) could be blended with Belle Saison (a good

maltotriose metabolizer) to achieve a higher attenuation and ethanol level than either strain alone.

While HGB has many advantages, it is not useful if it does not produce good beer. Flavor and aroma analysis was not done for these fermentations. It would be worth studying the formation of esters and fusel alcohols since higher levels have been previously associated with HGB [20, 21].

4 Conclusions/Summary

Active dry yeast exhibits a remarkable resilience when challenged with the harsh fermentation environment of very high gravity wort. Most strains tested perform very well in worts less than 24–26 °P with only small decreases in attenuation. As the OG of the wort increases above 26 °P, many strains experience a more substantial decline in attenuation. In all cases, lower attenuation in higher OG worts was associated with a reduced ability to metabolize maltose and maltotriose and lower total ethanol concentrations. Although all strains showed decreased attenuation, certain strains were identified (notably Nottingham and Abbaye) that are capable of maintaining higher levels of maltose metabolism up to (and possibly above) 36 °P. Maltotriose metabolism was more strongly inhibited than maltose at OG greater than 24 °P for most strains, but Abbaye, Diamond, Munich Classic and BRY-97 maintained high maltotriose metabolism up to 28–30 °P. Maximum ethanol levels were achieved at 30–34 °P for most strains, but Nottingham may continue to produce higher ethanol levels above 36 °P. These results highlight the importance of yeast strain selection for high gravity brewing applications.

5 References

1. Stewart, G.G.: High-Gravity Brewing and Distilling – Past Experiences and Future Prospects, Journal of the American Society of Brewing Chemists, **68** (2010), no. 1, pp. 1-9.
2. Beer Style Guidlines, Beer Judge Certification Program, https://www.bjcp.org/docs/2015_Guidelines_Beer.pdf, accessed 1 March 2019.
3. Anchorage Brewing Co.'s A Deal With the Devil Clone Recipe, BYO, https://byo.com/recipe/anchorage-brewing-co-s-deal-devil-clone/.
4. Pratt-Marshall, P.L.; Brey, S.E.; De Costa, S.D.; Stewart, G.G. and Bryce, J.H.: High gravity brewing – an inducer of yeast stress, Brewer's Gurdian, **121** (2002), pp. 22-26.
5. Pratt, P.L.; Bryce, J.H. and Stewart, G.G.: The Effects of Osmotic Pressure and Ethanol on Yeast Viability and Morphology, Journal of the Institute of Brewing, **109** (2003), no. 3, pp. 218-228.
6. Gibson, B.R.: 125th Anniversary Review : Improvement of Higher Gravity Brewery Fermentation via Wort Enrichment and Supplementation, Journal of the Institute of Brewing, **117** (2011), no. 3, pp. 268-284.
7. Zhuang, S.; Smart, K. and Powell, C.: The impact of extracellular osmolality on *Saccharomyces* yeast populations during brewing fermentations, Journal of the American Society of Brewing Chemists, **75** (2017), no. 3, pp. 244-254.
8. Casey, G.P. and Ingledew, W.M.: Reevaluation of Alcohol Synthesis and Tolerance in Brewer's Yeast, J. Am. Soc. Brew. Chem., **43** (1985), no. 2, pp. 75-83.
9. Ingledew, W.M.: Biographical Review: Wallowing with the Yeasts Used to Make Alcohol, Journal of the American Society of Brewing Chemists, **73** (2015), no. 3, pp. 209-222.
10. W. Brown, S.; G. Oliver, S.; E. F. Harrison, D. and Righelato, R.: Ethanol inhibition of yeast growth and fermentation: Differences in the magnitude and complexity of the effect, Applied Microbiology and Biotechnology, **11** (1981), no. 3, pp. 151-155.
11. Hayashida, S. and Ohta, K.: Formation of High Concentrations of Alcohol by Various Yeasts, Journal of the Institute of Brewing, **87** (1981), no. 1, pp. 42-44.
12. Casey, G.P. and Ingledew, W.M.: High-Gravity Brewing: Influence of Pitching Rate and Wort Gravity on Early Yeast Viability, Journal of the American Society of Brewing Chemists, **41** (1983), no. 4, pp. 148-152.
13. Casey, G.P.; Magnus, C.A. and Ingledew, W.M.: High-Gravity Brewing: Effects of Nutrition on Yeast Composition, Fermentative Ability, and Alcohol Production, Applied and Environmental Microbiology, **48** (1984), no. 3, pp. 639-646.
14. Smit, G.; Straver, M.H.; Lugtenberg, B. and Kijne, J.W.: Flocculence of *Saccharomyces cerevisiae* cells is induced by nutrient limitation, with cell surface hydrophobicity as a major determinant, Applied and Environmental Microbiology, **58** (1992), no. 11, pp. 3709-3714.
15. Majara, M.; O'Connor-Cox, E.S.C. and Axcell, B.C.: Trehalose – An osmoprotectant and stress indicator compound in high and very high gravity brewing, Journal of the American Society of Brewing Chemists, **54** (1996), no. 3, pp. 149-154.
16. Crowe, J.; F. Carpenter, J. and M. Crowe, L.: The role of vitrification in anhydrobiosis, Annual Review of Physiology, **60** (1998), pp. 73-103.
17. Piddocke, M.P.M.P.; Kreisz, S.; Heldt-Hansen, H.P.; Nielsen, K.F. and Olsson, L.: Physiological characterization of brewer's yeast in high-gravity beer fermentations with glucose or maltose syrups as adjuncts, Applied Microbiology and Biotechnology, **84** (2009), no. 3, pp. 453-464.
18. Alves, S.L.; Herberts, R.A.; Hollatz, C.; Trichez, D.; Miletti, L.C.; de Araujo, P.S. and Stambuk, B.U.: Molecular analysis of maltotriose active transport and fermentation by *Saccharomyces cerevisiae* reveals a determinant role for the AGT1 permease., Applied and Environmental Microbiology, **74** (2008), no. 5, pp. 1494-1501.
19. Alves-jr, S.L.; Herberts, R.A.; Hollatz, C.; Miletti, L.C. and U, B.: Maltose and Maltotriose Active Transport and Fermentation by *Saccharomyces cerevisiae*, Journal of the American Society of Brewing Chemists, **65** (2007), no. 2, pp. 99-104.
20. Saerens, M.G.; Verbelen, P.J.; Vanbeneden, N.; Thevelein, J.M. and Delvaux, F.R.: Monitoring the influence of high-gravity brewing and fermentation temperature on flavour formation by analysis of gene expression levels in brewing yeast., Applied Microbiology and Biotechnology, **80** (2008), no. 6, pp. 1039-51.
21. Lei, H.; Zhao, H.; Yu, Z. and Zhao, M.: Effects of Wort Gravity and Nitrogen Level on Fermentation Performance of Brewer's Yeast and the Formation of Flavor Volatiles, Applied biochemistry and biotechnology, **166** (2012), no. 6, pp. 1562-1574.

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