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Influence Of Yeast Propagation In High-Gravity Wort On Subsequent Fermentation Performance

The vitality of the yeast, e.g. the physiological condition and activity, has great influence on the quality of the manufactured beer. Therefore yeast propagation in brewing processes - like high gravity brewing - that put additional stress on the yeast needs special attention. A brewery practising high gravity brewing has only wort with a high original gravity at its disposal. The wort can be used diluted or undiluted for the cultivation of yeast.

A series of experiments were conducted to examine the influence of propagation with high gravity wort on yeast and the resulting consequences for the fermentation, as well as the produced beer. The Yeast was cultivated, while varying the original gravity and composition of the propagation wort. The growth rate of the yeast decreased with increasing original gravity of propagation wort. At the same original gravity an increasing glucose content results in slower growth. Analysis of the activity of key enzymes leads to the conclusion that the growth rate is mainly influenced by a change in the yeast metabolism called the crabtree effect.

To evaluate the fermentation performance of the harvested yeast, it was used to ferment wort with 12° Plato. The yeasts propagated in wort with different gravities showed no significant difference in the final gravity. Yeast propagated in wort with the addition of glucose syrup had an unsatisfactory fermentation performance.

The concentration of volatiles and the foam stability in the produced beer were within a normal range. The bitterness and flavour of the beer produced with yeast propagated in wort with the addition of glucose syrup were not satisfying.

The conducted experiments showed that a brewery, using all malt high gravity in the brewing process, can use their wort undiluted for yeast cultivation. The exception is the usage of glucose syrup as a wort additive. It can lead to a severely decreased fermentation performance of the yeast if the wort is used for propagation purposes.

Descriptors: propagation, yeast, high-gravity, fermentation, enzyme

1 Introduction

High Gravity Brewing is a widespread method to increase the capacity of a brewery and to save energy [1]. But, this process subjects the yeast to additional stress, like high gravity and increased ethanol content of the wort [2]. Yeast is a important resource for brewing beer, the vitality of the yeast, e.g. the physiological condition and activity, has great influence on the quality of manufactured beer. Therefore yeast propagation in brewing processes - like high gravity brewing - that put additional stress on the yeast need special attention. A brewery practising high gravity brewing has only wort with a high original gravity at its disposal. If the wort is used undiluted as medium in the cultivation of yeast, the yeast - because of the medium - is subjected to additional stress. On the other hand if the wort is diluted, there is a certain risk of biological contamination.

To investigate the influence of propagation with high gravity wort on the cultivated yeast a series of propagations were conducted, varying the original gravity and the composition of the wort. The harvested yeast was then used for beer production.

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Figures see Appendix

2 Materials and Methods

All propagations were conducted in a computer-controlled (Siemens Simatic 5) industrial grade fermenter with a capacity of 60 litres. The aeration unit is controlled by an inline oxygen sensor, the air is sterile filtered. Temperature is regulated by cooling and heating zones, using water as coolant and steam for heat. In all experiments lager wort was used. For final gravities above 12° Plato, wort first runnings were diluted and boiled with hops to achieve about 20 bitter units. The worts with sugar syrup were made by adding the respective sugars to lager wort. The yeast strain 34/78 Hefebank Weihenstephan was employed in all experiments. All propagations where carried out at a wort temperature of 15°C and an oxygen level of 2 mg/l. Pressure in the fermenter was set to 0.8 bar.

The experiments were conducted in two runs, only in the first run the propagation was accompanied analytically. In the second run the yeast was propagated under the same conditions as the first run until a cell count of 120 million cells per ml was reached. The harvested yeast was used to ferment lager wort at 15°C in the research fermentation plant of the Lehrstuhl für Technologie der Braurei II. The beer matured one week at 4° C and three weeks at 0° C. Final gravity, alcohol, pH and the degree of attenuation were measured with a SCABA according to MEBAK II 2.13.6.2 and 2.17 [3]. The yeast cell count was determined using a Thoma Chamber (MEBAK III 10.4.3.1. and 10.11.4.4 [4]). The volatiles in the produced beer were measured by headspace chromatography (MEBAK III 1.1.1 and 1.2.1)[4]. With the produced beer a sensorial analysis was conducted following the DLG method (MEBAK II 2.8.4.4) [3].

To messure the enzyme activity yeast was mechanically cracked (French Press). The enzyme activity was determined photometrically in the attained yeast extract [5].

To give an optimal overview of the fermentation characteristics, a nonlinear regression model was used to optimize the fermentation chart [6]. The authors [6] claim a maximum deviation of one percent for their model.

3 Results and discussion

Propagation

The growth rate of the yeast in different propagation worts shows significant differences. In all malt worts higher gravity resulted in slower growth, in the log-phase it took approximately two to three hours longer to reach the same maximum cell count if the gravity was increased by 2° P (see figure 1). The time difference is composed of a prolonged lag phase and a slower growth in the exponential phase.

It is well known, that temperature, aeration and composition of the wort have the most impact on yeast growth [7]. In the conducted series of experiments only the composition of the wort was changed, i. e. sugar content and ratio. High ethanol and sugar content are lagging factors of yeast growth [8]. All propagation runs showed no significant difference in the produced amount of ethanol. Thus the change in growth rate is a result of the gravity i.e. the sugar content of the wort.

This was confirmed by changing the sugar composition using different syrups as wort additives, yet maintaining the same original gravity (16° P). In both experiments differences in maximum cell count reached were small but growth rates differed significantly, with the all-malt wort having the highest and the wort with addition of glucose syrup the lowest maximum growth rate. Apparently sugar concentration and composition influence the yeast metabolism. A lack of yeast nutrients can be excluded as an influence factor, because all propagation wort was based on lager wort of 12° Plato providing a sufficient supply. The amount of the sugar syrups added was below five percent, therefore dilution of the lager wort was insignificant.

During propagation in breweries the crabtree effect appears usually consistently - more or less distinct - because of the relatively high gravity [9,10]. The suppression of the respiration is energetically disadvantageous for the yeast, resulting in slower growth and formation of biomass. To gain an insight of the yeast metabolism during propagation, the activity of enzymes with key roles in the sugar metabolism were measured. Fermentation and respiration follow the same biochemical pathway until the formation of the intermediate pyruvate. On this level respiration competes with fermentation in form of the pyruvate dehydrogenase complex (PDH) and the pyruvate decarboxylase (PDC) [8, 9]. Therefore the change in their activity between lag- and log-phase was monitored by processing yeast samples at cell count of approximately 30 m/ml and 60 m/ml. A decrease of growth with increasing gravity and with addition of glucose syrup could be observed due to the increase in the sugar concentration. In wort of 12° Plato the PDC activity ($+7.14*10^{-2}$ U/mg) as well as the PDH activity ($+7.80*10^{-3}$ U/mg) of the yeast increase between lag and log phase, showing a high pyruvate metabolism resulting in the fastest growth. The yeast propagated in wort with the addition of glucose syrup had the lowest growth rates, an increase in PDC activity and the highest decrease in PDH activity between lag and log Phase (see figure 2). This showed that the yeast metabolism more strongly follows the fermentation pathway via acetaldehyde instead of acetyl-CoA. Regarding the activities of the alcohol dehydrogenase (ADH), to our surprise, no essential differences were found. We came to the conclusion that the formation of acetyl-CoA required for the formation of biomass occurred via the PDH-Bypass. The flow via this pathway strongly influences yeast growth during propagation. The bypass contains two enzymes (acetaldehyde dehydrogenase (AcDH) and acetyl-CoA synthetase (AsCoA) and should be looked at in further studies in this field [5,11].

Fermentation - Produced beer

As described above yeast was harvested from the propagations at a cell count of 120 million per ml as pitching yeast for fermentation of lager wort. In the beginning of the fermentation (first 48 hours) the starting fermentation rate of the yeast, comparing yeast harvested from all malt worts, increases in respect to the gravity of the propagation wort. The yeast cultivated in wort with the addition of maltose syrup starts slightly slower than than yeast cultivated in all-malt wort of the same gravity. The yeast harvested from wort with the addition of glucose syrup has the worst degree of attenuation. The differences in the final gravity and final fermentation grade were marginal. The only exception here is the yeast propagated in wort with the addition of glucose syrup, the fermentation proceeded significantly slower, and the yeast had an unsatisfactory fermentation performance (see figure 3).

All fermentations were conducted under the same conditions using wort of comparable composition. This premise leads to the conclusion that the differences in fermentation performance are the result of the physical condition of the yeast influenced by the prior propagation. This leads to the conclusion that the discrepancies are likely caused by a shortening, or an elongation respectively of the adaptation phase of the yeast to its new milieu [2]. Higher growth activity of the yeast during propagation leads to a slower initial fermentation rate, this is self-evident by the comparison of the yeasts harvested from wort of 16° Plato and wort with the addition of maltose syrup. The later showed a higher growth activity during propagation but a slower initial fermentation rate, the elongation of the adaptation phase is caused by the change from biomass production to fermentation metabolism.

The flocculation of the yeast slowed with the increase of the gravity of the propagation wort, matching previous examinations in literature [12]. With the produced beer a sensory analysis was conducted and the content of volatiles as well as the foam stability was measured. Neither the foam stability nor the content of volatiles showed any significant difference between the samples. The small variations shown are most likely caused by fermentation and maturing conditions and cannot be referred to the yeast used. All samples were rated sensory between "four" and "five" in flavour and bitterness (according to DLG). Except beer produced from yeast propagated in wort with addition of glucose syrup, which was rated a "three".

4 Conclusion - Summary

The yeast growth rate decreases with increasing orginal gravity of the propagation wort. At the same original gravity a higher glucose content results in slower growth. This is caused by change in the yeast metabolism, mainly the PDH-bypass.

The harvested yeasts show differences in fermentation performance but not in final gravity, yeast propagated in higher gravity ferments faster. The yeast propagated in wort with the addition of glucose syrup shows unsatisfactory fermentation performance.

The concentration of volatiles and the foam stability were within a normal range, all produced beer had normal analytical results. The unsatisfactory fermentation performance of the yeast propagated in wort with glucose syrup resulted in a weak rating during sensory analysis.

Brewers using the high-gravity method may use their wort undiluted for propagation purposes without significant impact on the subsequent fermentation performance, thus reducing the risk of yeast and wort contamination. The exception is the use of glucose to raise the gravity of the wort. The use of such wort in propagation may lead to unsatisfactory fermentation performance and a reduced quality in the beer produced.

5 References

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Appendix

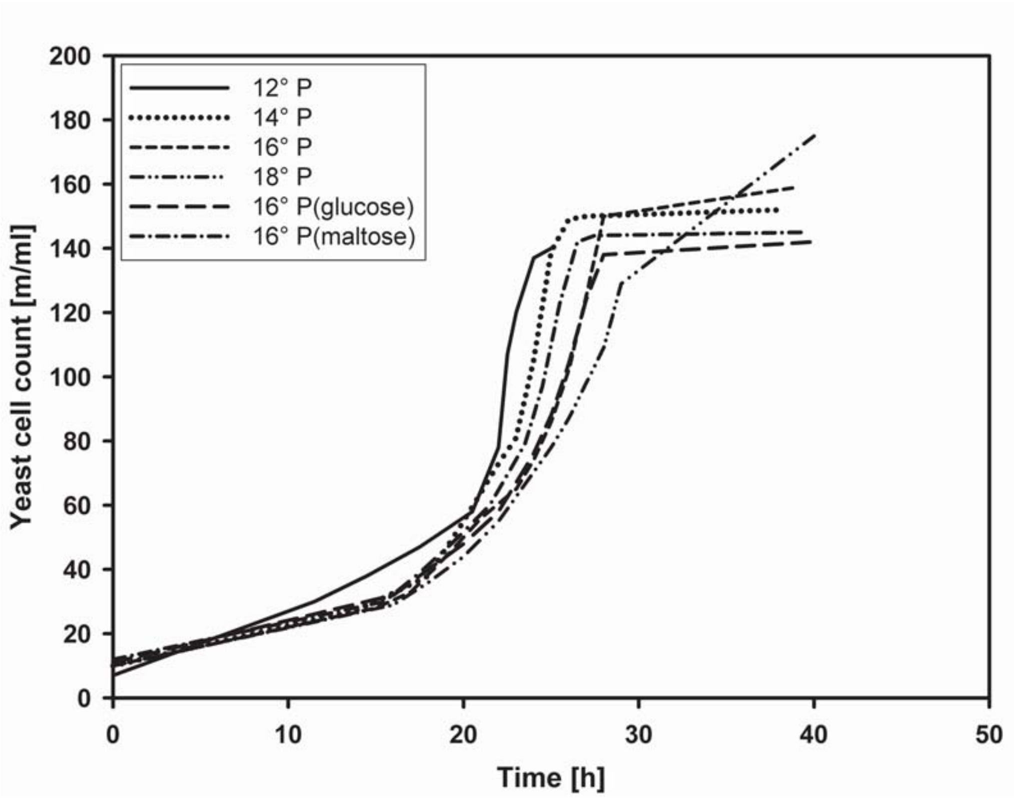

Fig. 1 Yeast cell count during propagation (Temperature 15° C, Oxygen 2 mg/l) in all malt worts with an original gravity of 12° P, 14° P, 16° P and 18° P as well as worts with the addition of glucose and maltose syrup (16° P)

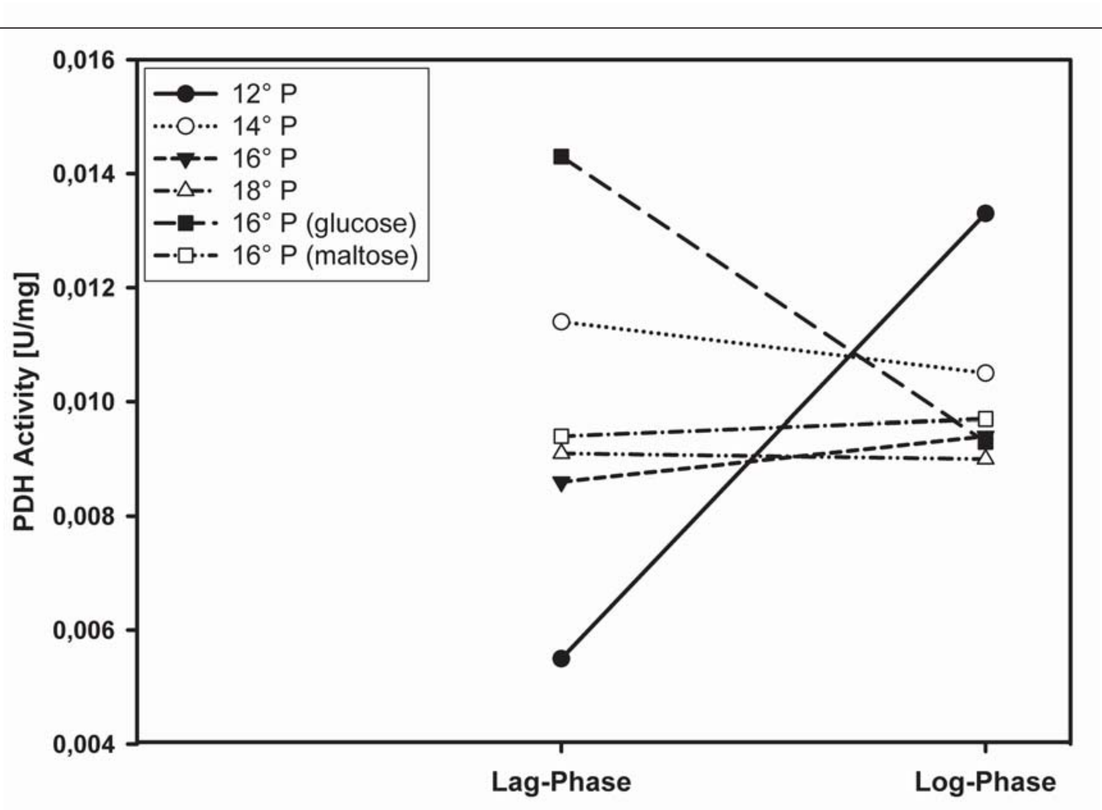

Fig. 2 PDH activity during propagation propagation (Temperature 15° C, Oxygen 2 mg/l) in all malt worts with an original gravity of 12° P, 14° P, 16° P and 18° P as well as worts with the addition of glucose and maltose syrup (16° P). Lag-Phase approx. cell count 30 m/ml, Log-Phase approx. 60 m/ml

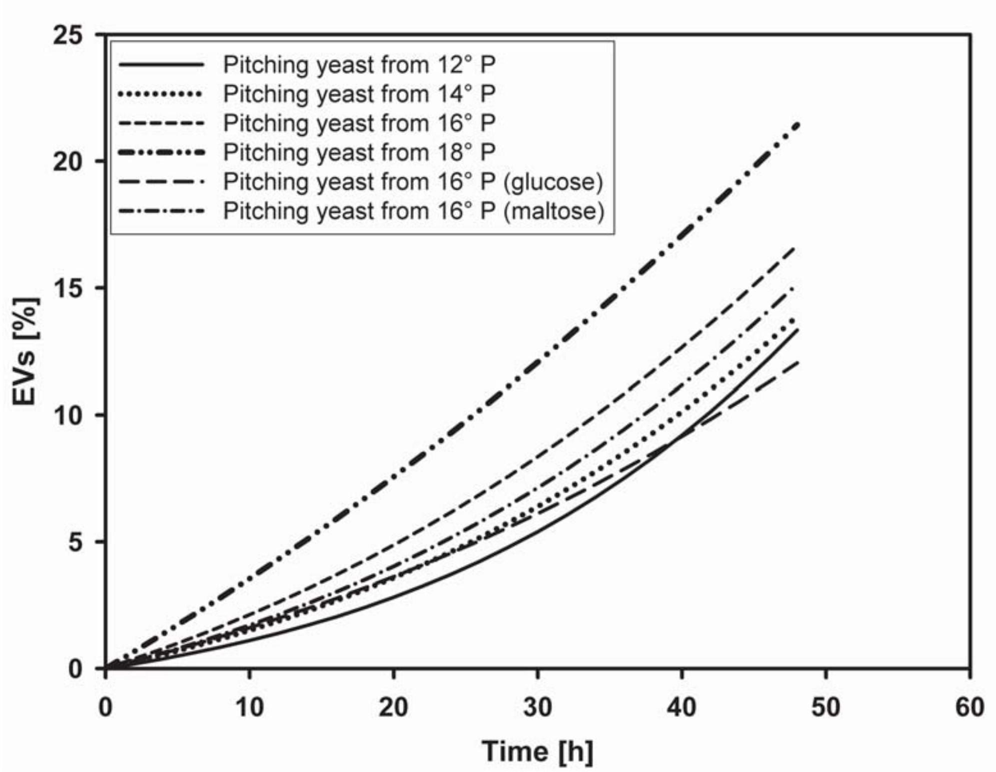

Fig. 3 Fermentation chart of lager wort at 15° C, pitching yeast harvested from propagation in all-malt worts with an original gravity of 14° P, 16° P and 18° P as well as pitching yeast from wort with the addition of glucose and maltose syrup (16° P). A non linear regression model was used to optimize the fermentation chart [6].