

IMPROVEMENT OF FERMENTATION PERFORMANCE IN HIGH GRAVITY BREWING

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ABSTRACT: *High gravity brewing is a perspective technique for increasing brewing capacity without significant capital expenditure. In Vietnam, high gravity brewing has been carried out in some breweries, the specific gravity of wort is up to 16° Plato (Pt). In this paper, fermentation of the 16-22° Pt wort was examined. The obtained results showed that the higher the specific gravity of wort, the longer the fermentation time and the higher the residual substrate content in the green beer. The principal reason was due to high ratio of C/N content in wort. Then some nitrogen sources were alternatively added to the 22° Pt wort. It was showed that the fermentation was significantly accelerated and the alcohol content in the green beer increased. Yeast extract was considered as a suitable nitrogen source for supplementation in high gravity brewing. For the 22° Pt wort, the optimal content of supplementing yeast extract is 90mgN/litre.*

1. INTRODUCTION

Many breweries throughout the world are embracing high gravity brewing as part of their production strategy for increasing brewing capacity without significant capital expenditure. It is one of many techniques that are being employed for process intensification of primary fermentation and maturation in brewing (Stewart G.G. 2000). In Vietnam, up to now, high gravity brewing has been carried out in some breweries such as Saigon brewery, Foster Tien giang brewery... The specific gravity of wort is up to 16° Pt. Attempts to ferment worts with higher specific gravity have proven to be difficult, largely because of yeast viability, slow and incomplete fermentation. Some results show that both ethanol toxicity and high osmotic pressure levels have been implicated as the limiting factors (Casey G.P., Magnus C.A., Ingledew W.M., 1984).

In this paper, fermentation of the 16-22° Pt wort was examined for elucidating some difficulties in high gravity brewing. Then some nitrogen sources were alternatively added to the 22° Pt wort for improving the fermentation performance. Finally, the optimal supplementing nitrogen content for the high specific gravity wort was quantified.

2. MATERIALS AND METHODS

Wort: Wort was prepared from 75% malt and 25% rice by decoction method. Termamyl 120L (alpha-amylase) and Ceremix 2XL (complex: alpha-amylase, beta-glucanase and protease) - supplied by Novo-Nordisk - were used. The specific gravity of the obtained 15° Pt wort was then adjusted to 16, 18, 20 or 22° Pt by sucrose addition. In this research, malt was supplied by Barrett Burston Malting Co., rice - by My tuong Co. Ltd. and sucrose - by Bourbon Co., Ltd. $(\text{NH}_4)_2\text{HPO}_4$ and yeast extract (produced by a Chinese company) were used as supplementing source of nitrogen.

Fermentation: A strain of *Saccharomyces cerevisiae* species was used. The inoculating rate was 15 million viable cells per mL. The fermentation was carried out at 16°C in a 1.5L fermenter.

Analytical methods (EBC Analytica, 1987).

Specific gravity was determined by densimeter method (Anton Paar equipment). The precision of this equipment is $\pm 0.01^\circ\text{Pt}$.

Free amino nitrogen was determined by spectrophotometric method using ninhydrin reagent.

Ethanol was analyzed by gas chromatography using flame ionisation detector (FID)

Yeast cell concentration was determined by hemocytometry using Malassez counter. Methylene blue test was used for yeast viability evaluation.

3. RESULTS AND DISCUSSION

3.1. Fermentation of high specific gravity wort

The fermentation was carried out with four high specific gravity worts: 16, 18, 20 and 22 $^\circ\text{Pt}$. The concentrations of free amino nitrogen in the four media were similar (196mg/L). Figure 1 shows the kinetics of specific gravity during the fermentation.

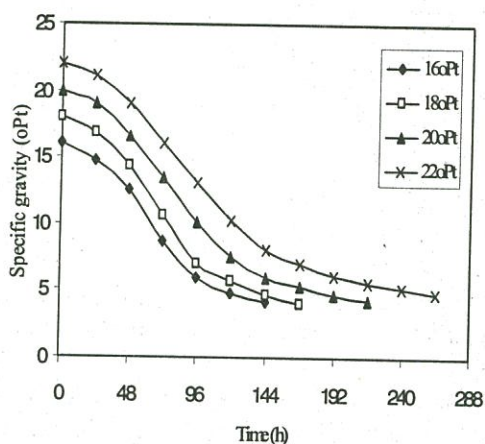


Fig 1. Kinetics of wort specific gravity

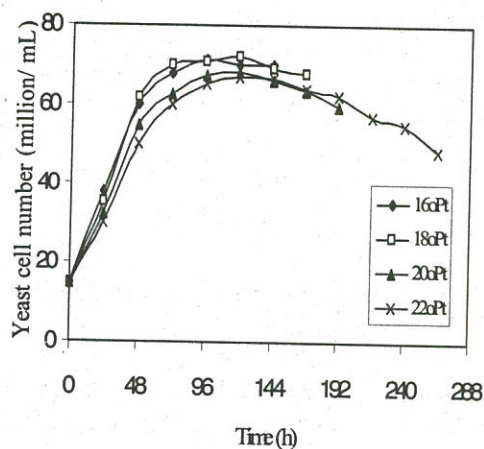


Fig 2. Kinetics of yeast growth

The higher the specific gravity of wort, the longer the fermentation time. In addition, the substrate assimilation rate of the 4 media were not the same. For 16 $^\circ\text{Pt}$ and 18 $^\circ\text{Pt}$ worts, it was about 0.083 $^\circ\text{Pt/L.h}$. However, for 20 $^\circ\text{Pt}$ and 22 $^\circ\text{Pt}$ wort, the substrate assimilation rates were 0.073 $^\circ\text{Pt/L.h}$ and 0.065 $^\circ\text{Pt/L.h}$ respectively. Perhaps high osmotic pressure of high gravity wort decreased yeast metabolic activities.

The higher the specific gravity of wort, the higher the residual substrate content in the green beer. However this phenomenon did not affect to the quality of the final product due to beer dilution by carbonized water after the fermentation and maturation.

The kinetics of yeast growth is visualized in figure 2. In the first 72 hours, the growth rate of yeast in the 16 $^\circ\text{Pt}$ and 18 $^\circ\text{Pt}$ media was higher than that in the 20 $^\circ\text{Pt}$ and 22 $^\circ\text{Pt}$ media. In addition, the maximal cell concentrations in the 16 $^\circ\text{Pt}$ and 18 $^\circ\text{Pt}$ worts were the highest. It is likely that high osmotic pressure of the medium decreased yeast growth. Moreover, high sugar and low assimilable nitrogen contents reduced the C/N ratio in high gravity wort. Some authors affirmed that this phenomenon affects negatively to the yeast reproduction during the fermentation (O'Connor-Cox E.S.C., Ingledew W.H. 1989).

With regard to ethanol formation, our results showed that the higher the specific gravity of wort, the higher the ethanol concentration in the green beer. The ethanol formation rates in the four 16, 18, 20 and 22°Pt media were 0.037, 0.037, 0.031 and 0.027 g/L.h.

3.2. Nitrogen supplementation to high gravity wort

The C/N ratio in high gravity wort is not balanced due to high sugar and low assimilable nitrogen contents. In this experiment, three 22°Pt media were used. The first medium is the control. Its free amino nitrogen content was 190mg/L. $(\text{NH}_4)_2\text{SO}_4$ and yeast extract were added to the second and the third media respectively for supplementation of nitrogen source (60mgN/L). Figure 3 shows the specific gravity evolution during the fermentation.

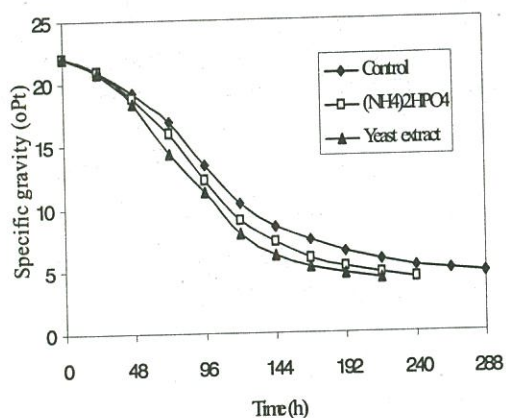


Fig 3. Kinetics of specific gravity in the 22°Pt wort with nitrogen addition

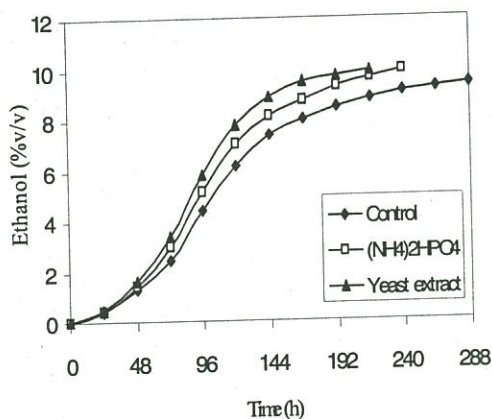


Fig 4. Kinetics of ethanol formation in the 22°Pt wort with nitrogen addition

Nitrogen addition to high specific gravity wort reduced significantly the fermentation time in brewing. The fermentation time of medium supplemented by $(\text{NH}_4)_2\text{SO}_4$ or yeast extract was 91% or 82% respectively in comparison with that of the control sample. Therefore, yeast extract gave better result than $(\text{NH}_4)_2\text{SO}_4$. Decrease in fermentation time increases the fermenter capacity and reduces the energy, labor and capital costs.

Figure 4 indicates that nitrogen supplementation to wort increased significantly the ethanol production rate. The ethanol production rate increased 18.5% (0.032g/L.h) and 33.3% (0.036g/L.h) in worts added by $(\text{NH}_4)_2\text{SO}_4$ and yeast extract respectively over that seen in the control sample (0.027g/L.h). It appeared that these supplements ameliorated the metabolic activities of yeast. The ethanol concentration in green beer from wort with nitrogen supplementation was a little higher than that from the control medium.

3.3. Determination of the supplemented nitrogen content

Yeast extract was chosen as a suitable nutritive source for yeast in high gravity brewing. In this experiment, five media were used. The first one was the control sample. Its free amino nitrogen content was 195mg/L. Different contents of yeast extract: 30, 60, 90 and 120mgN/L was alternatively added to the four media. Figure 5 shows that increase in nitrogen content in wort decreased the fermentation time.

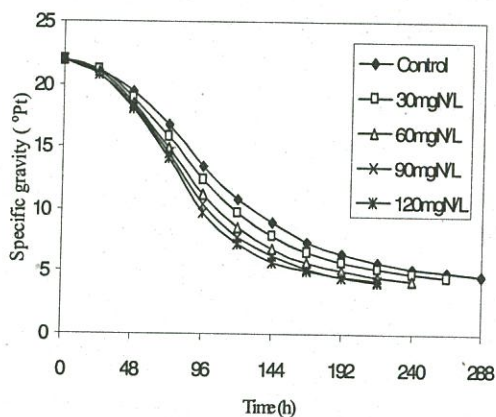


Fig 5. Influence of yeast extract supplementation to the evolution of specific gravity in the 22°Pt wort

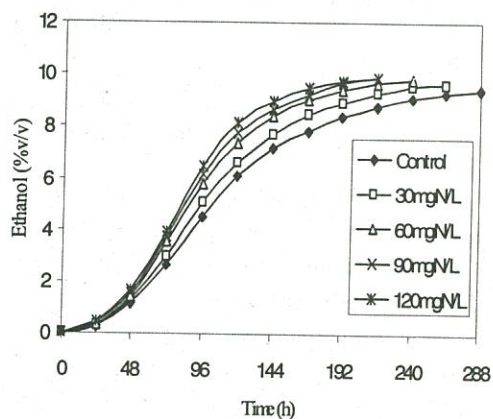


Fig 6. Influence of yeast extract supplementation to the ethanol formation in the 22°Pt wort

With regard to yeast growth, it was observed that the higher the nitrogen content in wort, the higher the growth rate of yeast and the higher the cell concentration.

The kinetics of ethanol formation were seen on figure 6. If the supplemented content increased from 30mgN/L to 90 mgN/L, the ethanol production rate increased from 11.5% to 38.5% in comparison with that in the control medium. However, the ethanol production rates were similar in the two media supplemented by 90mgN/L and 120mgN/L. Therefore, an optimal C/N ratio is essential for sugar transformation to ethanol in brewing. Moreover, our results showed that nitrogen supplementation increased slightly ethanol concentration in the green beer.

4. CONCLUSION

In high gravity brewing, the fermentation time was longer in comparison with traditional brewing. High osmotic pressure and non-optimal C/N ratio of wort decreased the growth rate, cell concentration in the fermenting medium and metabolic activity of yeast during the fermentation. Nitrogen supplementation to high gravity wort was a simple technique for ameliorating the fermentation performance. Yeast extract was considered as a suitable nitrogen source. For 22°Pt wort brewed from 75% malt, 25% rice and sucrose (70g/L), the optimal content of yeast extract for supplementation was approximately 90mgN/L. In this case, the fermentation time decreased 25% and the ethanol production rate increased 38.5% in the comparison with the control medium without nitrogen supplementation.

NGHIÊN CỨU CẢI THIỆN QUÁ TRÌNH LÊN MEN NỒNG ĐỘ CAO TRONG SẢN XUẤT BIA

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TÓM TẮT: lên men nồng độ cao là một kỹ thuật nhiều triển vọng trong sản xuất bia vì nó làm tăng năng suất sản xuất của các nhà máy mà không đòi hỏi nhiều chi phí đầu tư. tại Việt Nam, kỹ thuật lên men nồng độ cao đã được áp dụng tại một số nhà máy sản xuất bia, nồng độ chất khô của dịch nha trước khi lên men có thể tăng đến 16°pt. nghiên cứu này khảo sát quá trình lên men dịch nha có nồng độ chất khô thay đổi từ 16-22°pt. nếu nồng độ chất khô của dịch nha càng cao thì thời gian lên men càng dài và hàm lượng đường sót trong bia non cũng càng cao. nguyên nhân chính là do tỉ lệ hàm lượng các hợp chất c/n trong dịch nha quá cao. tiếp theo, chúng tôi thử bổ sung lần lượt một số hợp chất có chứa nitơ vào dịch nha 22°pt. kết quả thực nghiệm cho thấy quá trình lên men được rút ngắn và nồng độ cồn trong bia non tăng lên. chất chiết nấm men được xem là nguồn nitơ tốt nhất để bổ sung vào dịch nha trong sản xuất bia với kỹ thuật lên men nồng độ cao. đối với dịch nha 22°pt, hàm lượng chất chiết nấm men cân bổ sung là 90mg/lít.

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