*International Food Research Journal 17: 309-318 (2010)*

# Effect of Tween 80 and ergosterol supplementation on fermentation performance of the immobilized yeast in high gravity brewing

[1]Tran, Q. H., [1]Nguyen, T. T., [1]Le V. V. M. and [2]Hoang, K. A.

[1] *Department of Food Technology, Ho Chi Minh City University of Technology, Vietnam*
[2] *Institute of Tropical Biology, Vietnam*

**Abstract:** This paper deals with the effect of Tween 80 and ergosterol supplementation to the 24°Pt wort with 30% maltose syrup adjunct on the fermentation performance of the immobilized yeast in calcium alginate gel. The higher the content of supplements in the wort, the higher the yeast growth, the sugar and free amino nitrogen consumption rates and the ethanol production rate but the lower the ethanol content in the green beer. The star distance of central composite circumscribed design was used to study the influence of Tween 80 and ergosterol on the ethanol concentration in the green beer. The optimal content of Tween 80 and ergosterol supplemented to the wort were 0.3%v/v and 18mg/L, respectively. In these conditions, the primary fermentation time reduced 22.2% and the ethanol concentration in the green beer was similar in comparision with those of the control sample.

**Keywords: Ergosterol, high gravity brewing, immobilization, Tween 80, *Saccharomyces cerevisiae***

## Introduction

High gravity brewing (HGB) involves preparation and fermentation of wort with a specific gravity higher than 12°P (degree Plato), which is the weight of the sugar equivalent in 100 g of the solution at 20°C. By increasing the wort specific gravity, the higher levels of the ethanol per given plant capacity can be achieved and substantial savings can be attained by the brewer; the plant efficiency and capacity are increased; labour, energy and capital costs are reduced (Casey *et al.*, 1984; Dragone *et al.*, 2004; Silva *et al.*, 2008). On the other hand, production of beer using immobilized cells has also been considered as a very promising technology. The use of immobilized cells in the fermentation process and their potential advantages over the free cell systems have been widely studied and reviewed (Patkova *et al.*, 2000; Kourkoutas *et al.*, 2004; Tran *et al.*, 2008a).

According to Casey *et al.* (1984), a factor limiting the production of high levels of ethanol by brewing yeasts is nutritional deficiency. Different researches on metabolic activities of the free yeasts in brewing showed that nutrient supplementation to the wort can alleviate the problems associated with high gravity fermentation, improving the fermentation performance of yeast (Cunningham and Stewart, 2000). In fact, the most success in enhancing the yeast's performance and tolerance for ethanol has been achieved by supplementing the high gravity wort with source of assimilable nitrogen, unsaturated fatty acids and sterols (Dragone *et al.*, 2004). The addition of lipids, especially ergosterol and unsaturated fatty acids has a pronounced effect on the growth and metabolism of free yeast (Guimaraes *et al.*, 2006). Sterols and unsaturated fatty acids, which are essential membrane components, are normally reduced in the high gravity wort with maltose syrup adjunct (Boulton and Quain, 2001a). However, until present there have been no studies on effect of Tween 80 and ergosterol supplementation on metabolic activities of the immobilized yeast in the high gravity brewing.

In this study, the influence of Tween 80 and ergosterol supplementation on the fermentation performance of the immobilized yeast in calcium

*Corresponding author.
Email: lvvman@hcmut.edu.vn
Tel:(00) (84) (8) 38 64 62 51; Fax: (00) (84) (8) 38 63 75 04

alginate gel in the high gravity brewing was examined.

## Materials and Methods

*Materials*

*Saccharomyces cerevisiae* (lager strain) used in this study was supplied by Tien Giang Foster Company. Na-alginate was supplied by Biotechnology Center, Nha Trang University. The ratio of mannuronic acid to guluronic acid (M/G) was 1.2. The viscosity (2% alginate solution, 25°C) was 423.6cp. Barley malt was supplied by Duong Malt Company; the extraction yield was 80%. High maltose syrup was supplied by Bibica Company. It was used as adjunct. Maltose content in the high maltose syrup was approximately 42% w/w.

Tween 80 and ergosterol were originated from Sigma Chemical Co. Tween 80 served as a source of unsaturated fatty acid. It contained approximately 70% oleic acid. Ergosterol was dissolved in a mixture of ethanol-Tween 80 (volume rario: 1/1) before supplementing to the high gravity wort (Huei-Fung, 2004). Other chemicals used in this study were supplied by Merck and Co., Inc.

*High gravity wort*

The brewer's worts were prepared from barley malt using conventional brewing techniques (Boulton and Quain, 2001a). In this study, the 24°Pt wort was used. The extract of 24°Pt wort was originated from 70% barley malt and 30% maltose syrup.

*Inoculum preparation*

Precultures were prepared by two successive inoculations: 1) in 100 ml Erlenmeyer Shake-flask containing 15 ml of 8%(w/w) malt wort; and 2) in 500 ml Erlenmeyer Shake-flask containing 150 ml of 8%(w/w) malt wort. For both periods, the preculture was grown at 30°C and 100 rpm for 24h.

*Yeast immobilization in alginate gel*

A volume of yeast suspension ($100.10^6$ cell $mL^{-1}$) was added to an equal volume of sodium alginate solution (50 g/L) and homogenized. This mixture was then dropped into a 3% (w/v) $CaCl_2$ solution for formation of calcium alginate gel beads. The cell concentration in the gel bead was $50x10^6$ cell/$cm^3$. The residence time of the gel beads in calcium chloride solution was 4 hours at 4°C for increasing the gel strength. Then the gel beads were washed in sterile water (Tran *et al.*, 2008b).

**Table 1.** Factor levels according to the star distance of CCC design

| Variables | Levels | | |
|---|---|---|---|
| | -1 | 0 | +1 |
| $X_1$: Content of supplemented Tween 80 (%v/v) | 0.18 | 0.24 | 0.30 |
| $X_2$: Content of supplemented Ergosterol (mg/L) | 6 | 12 | 18 |

*Beer fermentation by immobilized yeast in alginate gel*

Batch fermentation was carried out in laboratory stainless steel bioreactors containing 1.8L of 24°Pt wort. The fermentation temperature was 17°C. The inoculation rate was $10x10^6$ cells/mL of wort. Firstly, the effect of each supplement on the fermentation performance of the immobilized yeast in high gravity brewing was examined. The content of Tween 80 and ergosterol supplemented to the wort was varied: 0, 0.12, 0.24, 0.36 and 0.48% (v/v); and 0, 0.12, 0.24 and 0.36 (mg/L), respectively.

The fermentation was considered as it was completed when the degree of attenuation reached 75%. The degree of attenuation was calculated by the reducing sugar content in the initial wort and in the green beer (Patkova *et al.,* 2000).

Then the combined effect of Tween 80 and ergosterol supplementation to the wort on the fermentation performance of the immobilized yeast was studied. Star distance of central composite circumscribed design (star distance of CCC design) with the software Moode-Design of experiments and optimization (version 5.0, Umetrics Inc., USA) was used. The contents of Tween 80 and ergosterol added to the wort were considered as the variables in this experimentation (Table 1). The ethanol concentration (%v/v) in the green beer after 84 fermenting hours was selected as the dependent variable-response.

*Analytical methods*

The Ca-alginate gel beads containing yeast

**Table 2.** Effect of Tween 80 supplementation on primary fermentation parameters of high gravity brewing using immobilized yeast

| Content of supplemented Tween 80 (%v/v) | 0 | 0.12 | 0.24 | 0.36 | 0.48 |
|---|---|---|---|---|---|
| Fermentation time (h) | $108^{a1}$±1.23 | $108^{a1}$±0.97 | $96^{b1}$±1.57 | $96^{b1}$±1.83 | $84^{c1}$±0.88 |
| Viable cell concentration after fermentation ($10^6$ cell/mL) | $74.86^{c2}$±1.68 | $77.08^{c2}$±0.83 | $87.64^{b2}$±1.46 | $890.72^{b2}$±1.46 | $97.78^{a2}$±1.04 |
| Sugar consumption rate (g/L.h) | $1.28^{c3}$±0.02 | $1.29^{c3}$±0.10 | $1.44^{b3}$±0.08 | $1.44^{b3}$±0.12 | $1.69^{a3}$±0.05 |
| FAN consumption rate (mg/L.h) | $2.35^{c4}$±0.08 | $2.39^{c4}$±0.04 | $3.12^{b4}$±0.06 | $3.15^{ab4}$±0.08 | $3.27^{a4}$±0.08 |
| Ethanol concentration in the green beer (% v/v) | $8.47^{a5}$±0.05 | $8.41^{a5}$±0.05 | $7.95^{b5}$±0.21 | $7.90^{b5}$±0.12 | $7.29^{c5}$±0.04 |
| Ethanol production rate (g/L.h) | $0.62^{c6}$±0.003 | $0.62^{c6}$±0.003 | $0.65^{b6}$±0.017 | $0.65^{b6}$±0.010 | $0.68^{a6}$±0.004 |

*Various small letters in row represent statistically significant difference at the level of p=0.05*

cells were dissolved in a 3% (w/v) Ethylenediamine tetraacetic acid disodium salt (EDTA) solution. The yeast concentration was then determined by haemocytometry using Thoma counter chamber. Yeast viability was determined by methylene blue test (Patkova *et al.,* 2000).

Reducing sugar and free amino nitrogen (FAN) were measured by spectrophotometric method, using 3.5 dinitrosalicylic acid and ninhydrin, respectively (Jones *et al.,* 2007).

Diacetyl was quantified by spectrophotometric method using o-phenylenediamine (European Brewery Convention, 1998).

Ethanol and volatile compounds were determined by gas chromatography (Agilent technologies 6890N) using a flame ionization detector (FID) and a HP-FFAP column (19091F-413), with 30m length, 0.25µm film thickness and 0.32mm internal diameter. The working conditions were as follows: injection temperature was 200°C; oven temperature was maintained at 45°C for 2 min, and then increased to 150°C with the rate of 7°C/min, hold for 2 min; detector temperature was 200°C. The carrier gas was hydrogen (Gil *et al.,* 2006).

*Reproducibility*

All results presented in this study are the average of three independent experiments

*Statistical analysis*

Statistical analysis of the results was done by Analysis of Variance (ANOVA). Means were compared by Multiple range tests ($p \leq 0.05$), statistical analysis was carried out using the software Statgraphics plus (version 3.2, StatPoint technologies, Inc., USA).

## Results and Discussion

*Effect of Tween 80 supplementation on fermentation performance of the immobilized yeast in high gravity brewing*

In this experiment, the content of Tween 80 supplemented to the wort was changed: 0 (control sample), 0.12, 0.24, 0.36 and 0.48% (v/v). The results presented in Table 2 show the effect of Tween 80 on the fermentation performance of the immobilized yeast in high gravity brewing. It can be noted that the higher the content of oleic acid in wort, the higher the maximum viable yeast cell concentration in the culture. The same phenomenon was also observed by Ohta and Hayashida (1983) who reported that an increase in content of Tween 80 added to the medium augmented the growth of the free sake yeast cells. In addition, Tween 80 supplementation to the high gravity wort improved the metabolic activities of the immobolized yeast. High content of Tween 80 added to the wort resulted in high substrate consumption rate and ethanol production rate of the immobilized yeast. However, when the Tween 80 concent in wort were 0% and 0.12% (v/v), the fermentation characteristics were similar. The same phenomenon was also observed when the Tween 80 content in wort were 0.24% and 0.36% (v/v).

The improvement in ethanol production rate was

**Table 3.** Effect of ergosterol supplementation on the fermentation characteristics of the immobilized yeast in calcium alginate gel in high gravity brewing

| Content of supplemented ergosterol (mg/L) | 0 | 12 | 24 | 36 |
|---|---|---|---|---|
| Fermentation time (h) | $108^{a1}$±1.57 | $96^{b1}$±1.39 | $96^{b1}$±0.98 | $96^{b1}$±1.05 |
| Viable cell concentration after fermentation ($10^6$ cell/mL) | $74.86^{c2}$±0.24 | $77.08^{b2}$±0.84 | $78.47^{ab2}$±0.96 | $78.61^{a2}$±0.87 |
| Sugar consumption rate (g/L.h) | $1.32^{b3}$±0.02 | $1.48^{a3}$±0.02 | $1.49^{a3}$±0.02 | $1.50^{a3}$±0.02 |
| FAN consumption rate (mg/L.h) | $2.61^{b4}$±0.02 | $2.82^{a4}$±0.06 | $2.82^{a4}$±0.01 | $2.87^{a4}$±0.02 |
| Ethanol concentration (% v/v) | $8.41^{a5}$±0.04 | $8.36^{a5}$±0.08 | $8.33^{a5}$±0.05 | $8.33^{a5}$±0.09 |
| Ethanol production rate (g/L.h) | $0.62^{b6}$±0.009 | $0.69^{a6}$±0.007 | $0.69^{a6}$±0.005 | $0.68^{a6}$±0.008 |

*Various small letters in row represent statistically significant difference at the level of p=0.05*

due to the reduction in time required to complete the fermentation. When the content of Tween 80 in wort rose from 0 to 0.48%v/v, the fermentation time decreased from 108h to 84h.

The ethanol concentration in the green beer was dependent on the initial oleic acid content in the wort. Contrary to the fermentation time, when the Tween 80 content added to the wort increased from 0 to 0.48% (v/v), the ethanol concentration decreased from 8.5% (v/v) to 7.3% (v/v). Higher initial Tween 80 content added to the wort led to the higher ethanol production rate but the lower ethanol concentration in the green beer. This could be explained that with the addition of unsaturated fatty acid in the wort, the growth and metabolic activities of yeast increased throughout the fermentation (Casey *et al.*, 1983; Dragone *et al.*, 2004). Additionally, carbohydrate source of the wort was utilised for increasing the yeast biomass and thereby the quantity available for ethanol formation was reduced.

From Table 2, it can be concluded that the suitable content of Tween 80 added to 24°Pt wort in high gravity brewing with the immobilized yeast was 0.24% (v/v). In this case, the fermentation time and ethanol concentration in the green beer reduced 11.1% and 6.1%, respectively in comparison with those of the control sample.

*Effect of erogosterol supplementation on fermentation performance of the immobilized yeast in high gravity brewing*

In this experiment, the ergosterol content added to the wort was varied: 0 (control sample), 12, 24 and 36 mg/L. The experimental results are seen in Table 3. The results show that the fermentation performance of the immobilized yeast in high gravity brewing changed when the ergosterol was supplied to wort. When 12 mg/L ergosterol was added to the wort, the fermentation time decreased 11.1%, but the ethanol concentration in the green beer was similar to that in the control sample. In addition, maximum viable yeast cell concentration, substrate consumption rate and ethanol production rate were improved. It can be explained that ergosterol increased the growth, fermentation activity and ethanol endurability of yeast (Chen *et al.,* 1990). Similar results were reported by other researchers. According to Ohta and Hayashida (1983), sake yeast growth was significantly promoted in the nitrogen gas-sparged anaerobic culture supplemented with ergosterol at a level of 1 mg/mL. The sake yeast cell concentration increased 170x$10^6$ cells/mL compared with that of the control sample. Meanwhile, a study by Chen *et al.* (1990) showed that when 0.002 mg/L ergosterol was supplemented to the defined medium, the cell concentration of *Kluyveromyces fragilis* NRRL 2415 immobilized on small cubes (5 mm) of natural sponge increased slightly compared with that of the control sample.

However, the results in Table 3 show no significant difference in the metabolic activities of the immobilized yeast in the calcium alginate gel when the content of ergosterol added to the wort increased from 12 to 36mg/L.

**Table 4.** Experimental design and results according to the star distance of CCC design

| Assay | Independent variable | | Coded Variable | | Response |
|---|---|---|---|---|---|
| | Tween80 (%v/v) | Ergosterol (mg/L) | $X_1$ | $X_2$ | E (% v/v) |
| 1 | 0.18 | 6 | -1 | -1 | 8.01 |
| 2 | 0.30 | 6 | +1 | -1 | 7.90 |
| 3 | 0.18 | 18 | -1 | +1 | 8.10 |
| 4 | 0.30 | 18 | +1 | +1 | 8.25 |
| 5 | 0.16 | 12 | $-2^{1/2}$ | 0 | 8.10 |
| 6 | 0.32 | 12 | $+2^{1/2}$ | 0 | 8.20 |
| 7 | 0.24 | 3.5 | 0 | $-2^{1/2}$ | 7.80 |
| 8 | 0.24 | 20.5 | 0 | $+2^{1/2}$ | 8.20 |
| 9 | 0.24 | 12 | 0 | 0 | 8.12 |
| 10 | 0.24 | 12 | 0 | 0 | 8.13 |
| 11 | 0.24 | 12 | 0 | 0 | 8.12 |
| 12 | 0.24 | 12 | 0 | 0 | 8.14 |

E: Ethanol concentration in the green beer (% v/v)

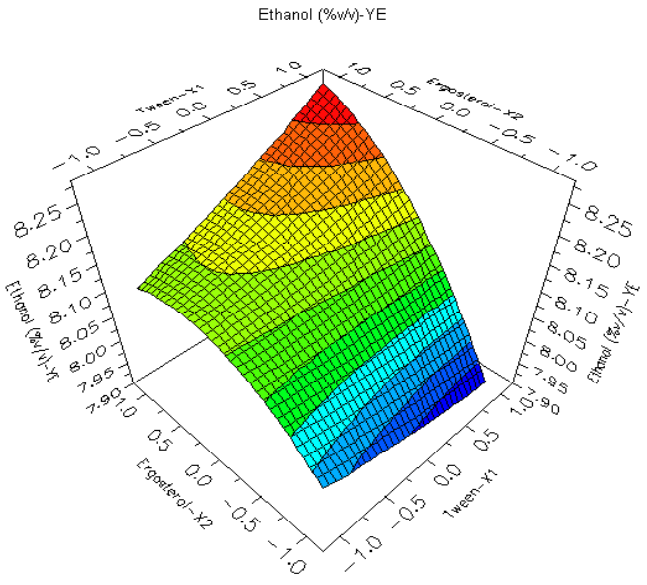


**Figure 1.** Response surface plot described by the quadratic model for ethanol concentration ($Y_E$), $X_1$: Tween 80 content, $X_2$: ergosterol content added to the wort.

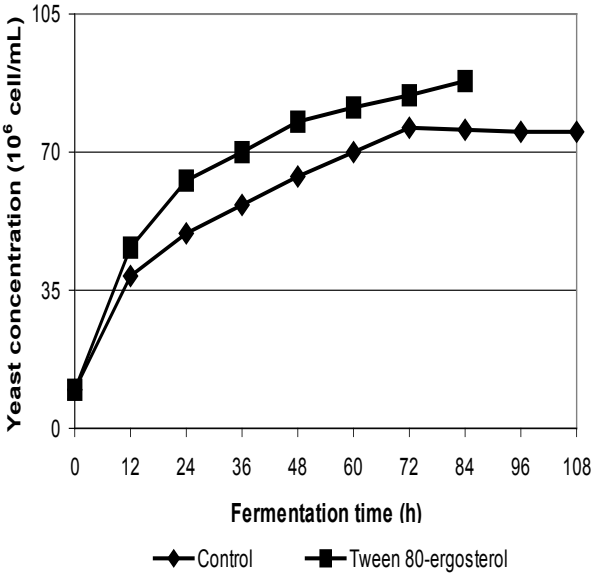


**Figure 2.** The effect of Tween 80 and ergosterol supplementation on viable cell concentration during high gravity brewing using immobilized yeast.

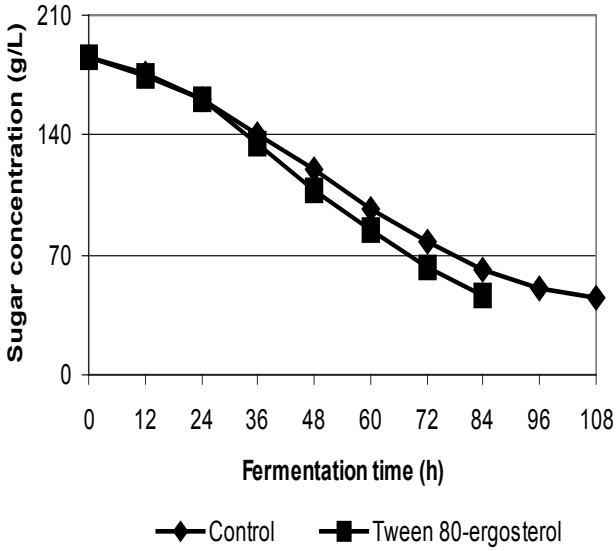


**Figure 3.** The effect of Tween 80 and ergosterol supplementation on sugar concentration during high gravity brewing using immobilized yeast.

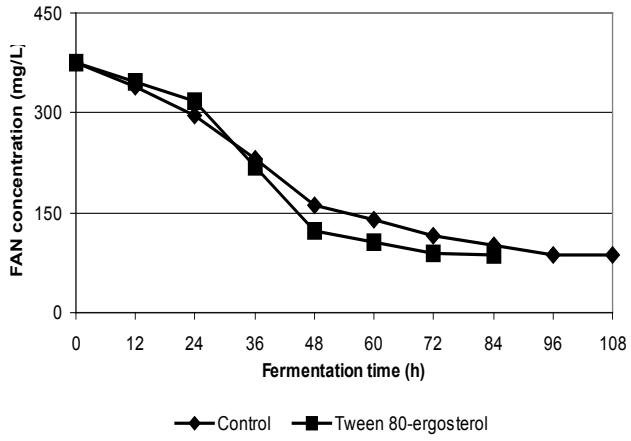


**Figure 4.** The effect of Tween 80 and ergosterol supplementation on FAN concentration during high gravity brewing using immobilized yeast

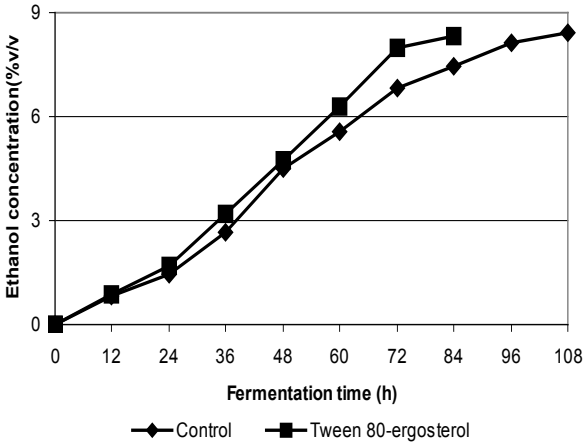


**Figure 5.** The effect of Tween 80 and ergosterol supplementation on ethanol concentration during high gravity brewing using immobilized yeast

**Table 5.** Coefficients, effects, standard errors and P-value for ethanol concentration ($Y_E$) using the star distance of CCC design

| Ethanol (%v/v) | Coeff. | Effect | Std. Err. | *p* |
|---|---|---|---|---|
| Constant | 8.128 | | 0.013 | 8.8e4-016 |
| Tween 80 | 0.023 | 0.046 | 0.008 | 0.042 |
| Ergosterol | 0.126 | 0.252 | 0.008 | 7.48e-006 |
| Tween 80 * Tween 80 | 0.009 | 0.019 | 0.009 | 0.409 |
| Ergosterol * Ergosterol | -0.066 | -0.128 | 0.009 | 0.005 |
| Tween 80 * Ergosterol | 0.065 | 0.130 | 0.013 | 0.002 |

**Table 6.** Analysis of variance (ANOVA) for the model representing the ethanol concentration ($Y_E$)

| Ethanol (%v/v) | DF | SS | MS | *F* | *p* |
|---|---|---|---|---|---|
| Total | 12 | 785.398 | 65.4498 | | |
| Constant | 1 | 785.215 | 785.215 | | |
| Total corrected | 11 | 0.1824 | 0.0166 | | |
| Regression | 5 | 0.1787 | 0.0357 | 57.3942 | 0.000 |
| Residual | 6 | 0.0037 | 0.0006 | | |
| Lack of fit | 3 | 0.0034 | 0.0012 | 12.5899 | 0.033 |
| Pure error | 3 | 0.0003 | 9.167e-005 | | |

From Table 3, it can be concluded that the suitable ergosterol content supplemented to the wort in high gravity brewing with the immobilized yeast was 12 mg/L.

*Combined effect of Tween 80 and ergosterol supplementation on ethanol concentration in the green beer fermented by the immobilized yeast in high gravity brewing*

The combined effect of Tween 80 and ergosterol supplementation to the 24°Pt wort on ethanol biosynthesis of the immobilized yeast in high gravity brewing was investigated. A star distance of CCC design was used. Content of Tween 80 –$X_1$ (%v/v) and content of ergosterol-$X_2$ (mg/L) added to the wort were chosen as independent factors in the experimental design. Thus, experiments were planned to obtain a quadratic model of 2 x 2 trials plus star configuration ($\alpha = \pm 2^{1/2}$) and four replicates at the central point. The response was ethanol concentration in the green beer after 84 fermenting hours. The experimental matrix and results are shown in table 4.

From Table 5, the fact that both examined variables showed a significant effect at the 95% confidence level was verified. After fitting the experimental data (Table 5), the results showed that the linear coefficients ($X_1$, $X_2$), the pure quadratic coefficient ($X_2^2$) and the interaction coefficient ($X_1X_2$) were significant, but the pure quadratic coefficient ($X_1^2$) was not ($p$=0.409). Table 5 showed that both contents affected the ethanol concentration in green beer but the effect of ergosterol content was higher. The statistical significance of the quadratic model equation was evaluated by the analysis of variance (ANOVA) in Table 6. The influence of Tween 80 content and ergosterol content added to the wort on the ethanol concentration in the green beer ($Y_E$) was calculated and expressed in a quadratic model.

$$Y_E = 8.128 + 0.023X_1 + 0.126X_2 - 0.066X_2^2 + 0.065X_1X_2$$

The graphic illustration of the quadratic model is shown in Figure 1. It can be confirmed that the ethanol concentration in the green beer increased at higher level of ergosterol and Tween 80. The optimal conditions suggested by the obtained mathematical model were $X_1$=1, $X_2$=1, respectively. Actual values of the two factors against critical values were 0.3% (v/v) for Tween 80 and 18 mg/L for ergosterol, respectively. In these conditions, the ethanol concentration in the green beer reached maximum: 8.28 % (v/v).

*Fermentation performance of the immobilized yeast in wort supplemented with optimum content of Tween 80 and Ergosterol*

**Table 7.** Concentration of some volatile compounds (mg/L) in the green beer fermented by the immobilized yeast (24°P wort was added with Tween 80 (0.3% v/v) and ergosterol (18 mg/L). The green beer was diluted by water to 5% (v/v) ethanol content)

| | Diacetyl | Ethylacetate | Propanol | Isoamylalcohol | Acetaldehyde |
|---|---|---|---|---|---|
| **Control sample** | $0.68^{a1}$ | $27.95^{a2}$±1.36 | $17.53^{a3}$±2.84 | $67.22^{b4}$±0.87 | $18.88^{a5}$±1.15 |
| **Sample supplemented withTween 80 and ergosterol** | $0.88^{b1}$ | $19.71^{b2}$±0.71 | $26.06^{b3}$±0.77 | $95.84^{a4}$±0.04 | $19.51^{a5}$±0.49 |

*Various small letters in column represent statistically significant difference at the level of p=0.05*

Kinetics of yeast growth, substrate assimilation and ethanol formation in wort supplemented with optimal content of Tween 80 and ergosterol are shown in Figures 2, 3, 4 and 5.

Figure 2 presents the evolution of viable yeast cell concentration in the culture. The cell number attained maximum (76.1 x$10^6$ cells/mL) after the first 72 h of fermentation in the culture without supplementation of Tween 80 and ergosterol (control sample). Meanwhile, in the culture supplemented with Tween 80 and ergosterol, yeast biomass synthesis continued to increase throughout the fermentation and maximum viable yeast cell concentration reached 87.8x$10^6$ cells/mL. This value was 17.6% higher than that in the control sample. This observation was in agreement with the results of Guimaraes *et al.* (2006). According to these authors, in the culture added with Tween 80 (0.1%v/v) and ergosterol (15 mg/L), cell number of the free yeast was 94.9% higher than that in the control sample. Meanwhile, the results of Casey *et al.* (1983, 1984) showed that adding Tween 80 and ergosterol to the medium improved the viability of the free yeast throughout the fermentation.

The evolution of reducing sugar and FAN contents in the immobilized yeast culture is showed in Figure 3 and 4, respectively. It can be affirmed that the presence of Tween 80 and ergosterol in the wort stimulated substrate utilization. The primary fermentation time reduced by 24h compared with that of the control sample. Reduction in the fermentation time was due to higher biomass synthesis and higher metabolic activities of the immobilized yeast in the culture supplemented Tween 80 and erogsterol. This was consistent with the results of Casey and Ingledew (1984, 1985) who reported that supplementation of 0.4% Tween 80 and 40mg/L ergosterol to the 27°Pt wort dramatically influenced the fermentation kinetics. The fermentation time was reduced to 6 days compared with 14 days in the control sample.

Figure 5 presents the kinetics of ethanol formation of the immobilized yeast. Statistical analysis of the results showed that the difference in ethanol concentration in the green beer from the two cultures was not significant.

However, culture supplemented with optimal content of Tween 80 and ergosterol resulted in significantly faster ethanol production rate. The improvement in ethanol production rate was due to the reduction in time required to complete the fermentation.

*Formation of volatile products*

Table 7 illustrates the concentration of some volatile compounds in the green beer diluted to 5% (v/v) ethanol. It is apparent that with the optimal content of supplements, the diacetyl concentration in the green beer was higher than that of the control sample. Increase of diacetyl concentration in the green beer was due to a reduction of primary fermentation time that led to a short period of diacetyl reduction by yeast (Jones, 2007; Dragone *et al.,* 2007). However, the diacetyl concentration in the green beer can be reduced by the supplementation of diacetyl reductase in the culture. With the supplementation of this enzyme, diacetyl converted to acetoin and further to 2,3-butanediol. These reduced compounds have much higher flavor thresholds and have no impact on the beer flavor.

Acetaldehyde flavor is considered as beer defect when this compound presents in excessive concentration. With optimal content of supplements, its content in the green beer was similar to that in the control sample, the obtained results was below the flavor threshold (25 mg/L) (Jones, 2007).

The concentrations of higher alcohol appeared to be significantly influenced by Tween 80 and ergosterol supplementation. The concentration of propanol and isoamyl alcohol in the green beer increased 48.56% and 42.58% compared with those

in the control sample, respectively. In addition, the level of isoamy alcohol in the green beer reached 95.8 mg/L and exceeded the flavour threshold (70 mg/L) (Jones, 2007). However, that level was lower than the damage threshold (100 mg/L) for the flavor of beer (Moonjai *et al*., 2002). This was consistent with the results of Casey *et al.* (1985). With supplementation of 0.4% Tween 80 and 40 mg/L ergosterol to the 27°Pt wort, the level of isoamyl alcohol in the green beer was approximately 50% higher than the flavor threshold.

With optimal content of supplements, the reduced level of ethylacetate was 29.5% compared with that of the control sample. This could be explained that a large proportion of the acetyl-CoA concentration inside the cells was utilised for assimilation reactions and thereby the quantity available for ester formation was reduced (Pfisterer *et al.*, 1976; Boulton and Quain, 2001b). In addition, the activity of alcohol acetyltransferase was inhibited by increasing the content of unsaturated fatty acid in the cell membrane (Yoshioka and Hashimoto, 1981; Fujii *et al.*, 1997; Moonjai *et al*., 2002).This observation was in agreement with the results of Casey *et al.* (1985). With the supplementation of Tween 80 (0.40%v/v) and ergosterol (40 mg/L) in the 27°Pt wort, the level of ethylacetate in the green beer was approximately 26.6% lower than that in the control sample.

## Conclusions

The results of the present study demonstrated that supplementation of Tween 80 and ergosterol to 24 °Pt wort prepared from 70% malt and 30% maltose syrup improved the ethanol production rate of the immobilized yeast in high gravity brewing. With the supplementation content of Tween 80 of 0.3%v/v and ergosterol of 0.18 mg/L, the primary fermentation time reduced 22.2% in comparision with those of the control sample and the ethanol concentration in the green beer was similar. The supplements had effects on formation of some volatile products in the green beer. The levels of propanol and isoamyl alcohol increased 48.6% and 42.6%, but the content of ethylacetate was reduced 29.5% compared with those of the control sample.

## References

Boulton, C. and Quain, D. 2001. The brewing process. Brewing yeast and fermentation, p. 19-68. London: Blackwell Science.

Boulton, C. and Quain, D. 2001. Biochemistry of fermentation. Brewing yeast and fermentation, p. 69-142. London: Blackwell Science.

Casey, G. P., Magnus, C. A. and Ingledew, W. M. 1983. High-gravity brewing: nutrient enhanced production of high concentrations of ethanol by brewing yeast. Biotechnology letters 5(6): 429-434.

Casey, G. P., Magnus, C. A. and Ingledew, W. M. 1984. High-gravity brewing: effects of nutrition on yeast composition, fermentation ability and alcohol production. Applied and Environmental Microbiology 48(3): 639-646.

Casey, G. P. and Ingledew, W. M. 1985. Reevalution of alcohol synthesis and tolerance in brewer's yeast. Journal of the American Society of Brewing Chemists 43(2): 75-83.

Chen, C., Dale, M. C. and Okos, M. R. 1990. Minimal nutritional requirements for immobilized yeast. Biotechnology and Bioengineering 36: 993-1001.

Cunningham, S. and Stewart, G. 2000. Acid washing and serial repitching a brewing ale strain of *Saccharomyces cerevisiae* in high gravity wort and the role of wort oxygenation condition. Journal of the Institute of Brewing 106(6): 389-402.

Dragone, G., Silva, D. P. and Silva, J. B. A. 2004. Factor influencing ethanol production rates at high-gravity brewing. Lebensm.-Wiss. U.-technology 37: 797-802.

European Brewery Convention 1998. Method 9.24.2. Vicinal diketones in beer: spectrophotometric method. Analytica-EBC.

Fujii, T., Kobayashi, O., Yoshimoto, H., Furukawa, S., Tamai, Y. 1997. Effect of aeration and unsaturated fatty acid on expression of the *Saccharomyces cerevisiae* alcohol acetyltranferase gene. Applied and Environmental Microbiology 63 (5): 910-915.

Gil, M., Cabellos, J. M., Arroyo, T. and Prodanov, M. 2006. Characterization of the volatile fraction of young wines from the Denomination of Origin "Vinos de Madrid" (Spain). Analytica. Chimica. Acta 563 (1-2): 145-153.

Guimaraes, P. M. R., Virtanen, H. and Londesborough, J. 2006. Direct edivence that maltose transport activity is affected by lipid composition of brewer's yeast. Journal of the Institute of Brewing 112(3): 203-209.

Huei-Fung, T., Bard M., Izumikawa, K., Krol, A. A., Sturm, A. M., Culbertson, N. T., Pierson, C. A. and Bennett, J. E. 2004. *Candida glabrata* erg1 mutant with increased sensitivity to a zoles and to low oxygen tension. Antimicrobial Agents and Chemotherapy 48 (7): 2483–2489.

Jones, H. L., Margaritis, A. and Stewart, R. 2007. The combined effects of oxygen supply strategy, inoculum size and temperature profile on very-high-gravity beer fermentation by *Saccharomyces cerevisiae*. Journal of the Institute of Brewing 113(2): 168-184.

Kourkoutas, Y., Bekatorou, A., Banat, I. M., Marchant, R. and Koutinas, A. A. 2004. Immobilization technologies and support materials suitable in alcohol beverages production: a review. Food Microbiology 21: 377-397.

Moonjai, N., Verstrepen, K. J., Delvaux, F. R., Derdelinckx, G. and Verachtert, H. 2002. The effect of linoleic acid supplementation of cropped yeast on its subsequent fermentation performance and acetate ester synthesis. Journal of the Institute of Brewing 108(2): 227- 235.

Ohta, K. and Hayashida, S. 1983. Role of Tween 80 and monoolein in a lipid-sterol-protein complex which enhances ethanol tolerance of sake yeasts. Applied and Environmental Microbiology 46(4): 821-825.

Patkova, J., Smogrovicova, D., Domeny, Z. and Bafrncova, P. 2000. Very high gravity wort fermentation by immobilised yeast. Biotechnology Letters 22: 1173–1177.

Pfisterer, E., Hancock, I. and Garrison, I. 1976. Effects of fermentation environment on yeast lipid synthesis. Journal of the American Society of Brewing Chemists 35(2): 49-54.

Silva, D. P., Brankyik, T., Dragone, G., Vicente, A. A., Teixeira, J. A. and Silva, J. B. A. 2008. High gravity batch and continuous processes for beer production: Evaluation of fermentation performance and beer quality. Chemical Papers 62 (1): 34–41.

Tran, Q. H., Le, V. V. M. and Hoang, K. A. 2008a. Comparison of the fermentation performance by free and immobilized yeast in high gravity brewing. Proceeding of 4th national scientific conference, p.290-293. Hanoi: Science and Technics Publishing House.

Tran, Q. H., Le, V. V. M. and Hoang, K. A. 2008b. Influence of yeast and alginate concentrations in alginate gel beads on the fermentation characteristics of immobilized yeast in high gravity brewing. Proceeding of 4th national scientific conference, p.294-297. Hanoi: Science and Technics Publishing House.