J. Microbiol. Biotechnol. (2007), **17**(2), 297–304

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Influence of Aeration During Propagation of Pitching Yeast on Fermentation and Beer Flavor

CHEONG, CHUL[1], KARL WACKERBAUER[2], AND SOON AH KANG[1]*

[1]*Department of Fermented Food Science, Seoul University of Venture & Information, Seoul 137-070, Korea*
[2]*Department of Biotechnology, Technical University of Berlin, Berlin, Germany*

Received: August 29, 2006
Accepted: October 30, 2006

Abstract The effect of yeast propagated at different aeration conditions on yeast physiology, fermentation ability, and beer quality was investigated using three strains of *Saccharomyces cerevisiae*. It was shown that yeast cells grown under continuous aeration conditions during propagation were almost two times higher as compared with discontinuous aeration conditions. The maximum of cell growth of all samples reached between 36 h and 48 h. The concentration of trehalose was increased under continuous aerated yeasts, whereas glycogen was decreased. It was also observed that the concentration of glycogen and trehalose in yeast cells had no direct effect on subsequent fermentation ability. The effect of yeast propagated under different aeration conditions on subsequent fermentation ability was different from yeast strains, in which the influence will be most pronounced at the first fermentation. Later, the yeasts might regain its original characteristics in the following fermentations. Generally, continuously propagated yeast had a positive effect on beer quality in subsequent fermentation. Hence, the concentration of aroma compounds obtained with yeast propagated under 6 l/h for 48 h aeration was lower than those grown under other aeration conditions in the bottom yeasts; in particular, the amounts of phenylethyl alcohol, ester, and fatty acids were decreased.

Key words: Propagation, fermentation ability, glycogen, trehalose, beer analysis

Oxygen during propagation of brewing yeast has a profound effect on yeast metabolism, particulary on yeast growth and activity [5, 6, 8, 9, 12, 22]. In the traditional beer process, pitching yeast is propagated under weakly aerated conditions or yeast is taken from the sediment of earlier fermentations [2]. Oxygen is needed for the synthesis of sterols and unsaturated fatty acids, which are vital elements of the cell membranes [10]. Without a supply of these lipids, the cells cannot reproduce and their viability is lessened. Furthermore, the presence of oxygen is required for aerobic respiraton, with its very high energy yield and growth potential [9]. Wilson and McLeod [24] reported that the greatest effect of aeration conditions on the chemical composition of yeast cells was their fatty acid and sterol content. Free radicals and reactive oxygen species are generated from wort oxygenation and intracellularly during the limited period of yeast aerobic metabolism. These components result in damage to cellular components and potentially represent a significant stress to yeast during fermentation, as described by Martin *et al.* [15]. It has been reported that pitching technology has a considerable influence on the progress of fermentation, maturation, and beer quality [3, 4, 11, 16, 23, 26]. Ahvenainen *et al.* [2] found that the use of aerated pitching yeast, which is rich in lipids, shortens fermentation time and decreases the level of esters in final beer. Kringstad and Rasch [13] demonstrated that the method of preparation of the pitching yeast can have a considerable influence on the production of diacetyl and acetoin and that different yeast strains respond differently in this respect. There is clear need for a standardized experimental procedure for defining the oxygen requirements of brewing yeasts and thus providing information useful for control of industrial beer production. Lack of quantitative information on the oxygen requirements of brewing yeasts prompted the present investigations. Hence, in the present study, yeasts were propagated under different conditions of aeration, and intracellular levels of storage sugars, glycogen and trehalose, were compared. Thereafter, fermentation tests were performed with these yeasts and their effects

*Corresponding author
Phone: 82-2-3470-5273; Fax: 82-2-523-6767;
E-mail: sakang@suv.ac.kr

on the performance of fermentation and beer flavor were investigated.

MATERIALS AND METHODS

Strain of Yeast

Three strains of *Saccharomyces cerevisiae* were used in this study. Strain Rh is a bottom flocculent fermentation brewing yeast, strain Frank is a bottom powdery fermentation brewing yeast, and strain 160 is a top fermentation brewing yeast. All strains were obtained from the Brewing and Research and Teaching Institute (VLB) in Berlin.

Preculture

All yeasts were inoculated into 200-ml Erlenmeyer flasks containing 50 ml of wort and incubated statically at 25°C for 48 h.

Propagation of Yeast

Fifty ml of preculture was inoculated in a 10-l stirred conical glass flask (Schott Duran) that contained 5 l of sterile wort, and propagated with agitation at 100 rpm at 20°C for 48 h. The sterile wort contained (mg/l) total nitrogen 1,018, and free amino nitrogen 186, zinc 0.10, and the content of fermentable extract was 12%. Under aerobic conditions, sterile air was supplied continuously to the conical glass flask at the rate of 3 l/h for 48 h and 6 l/h for 48 h, respectively. Under low-aeration conditions, sterile air was supplied at the rate of 1.0 l/h for 10 min and 1.0 l/h for 3 h, respectively, and no air was supplied subsequently.

Fermentation

Various propagated yeasts were pitched in a 5-l glass tube fermentor containing 2.5 l of 12% wort for fermentation tests and then fermented at 20°C for 6 days. After each fermentation, the yeast cells were harvested by centrifugation and used again for the next fermentation test.

Viability Test

Viable cells were measured with a microscope after staining with Mg-ANS (1-aniline-8-naphthalene-sulfuric acid, Sigma catalog No. A5144, U.S.A.). Viability was calculated by dividing the number of viable cells by the total number of cells, with results given as percentages.

Glycogen and Trehalose Analyses

Glycogen and trehalose concentrations were determined by the method described by Quain [18] and Winkler *et al.* [25]. For the determination of the glycogen, the alkali- and acid-soluble fractions were treated with amyloglucosidase originated from *Aspergillus niger* (EC.3.2.1.3.; Sigma catalog No. A7420) and the amount of glucose released was estimated using a enzymatic colorimetric kit (Boehringer Mannheim catalog No. 716251). For the determination of trehalose, the yeast pellets extracted with water were treated with trehalase (EC.3.2.1.28.; Sigma catalog No. T8778) and the amount of glucose released was estimated.

Analytical Methods

The standard analysis for wort and beer was determined according to the method reported by Pfenninger [17]. The original gravity, apparent extract, and alcohol in the beer were analyzed using a beer analyzer (Anton Paar with auto-sampler, Model SP-1). The extract was determined during the fermentation using Biegeschwinger (Model Anton Paar, DPR Y), which determines the extract by measuring its density. The aromatic compounds were analyzed by GC using a Hewlet Packard 6890 gas chromatograph fitted with a flame ionize detector (FID). Two µl of the solution was injected onto a carbowax column (50 m×0.2 mm i.d., 1/4 inch o.d., Perkin-Elmer, USA) with the split ratio set at 1:60. The oven temperature program was 65°C isothermally for 4 min, then increased by 9.5°C/min to 180°C, and held at 180°C for 25 min. The injection temperature and detector temperature were 200°C. Nitrogen was used as the carrier gas (1 ml/min).

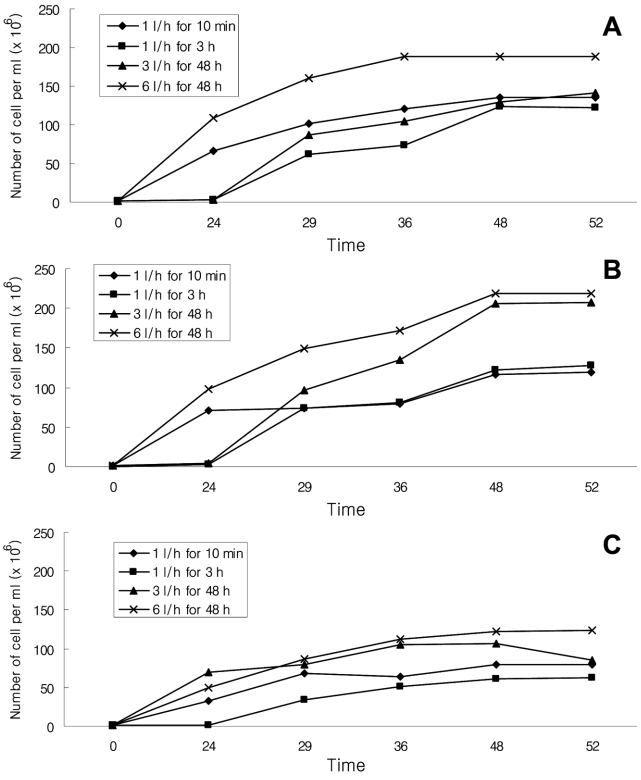

Fig. 1. Changes in the number of cells of bottom flocculent fermentation yeast (**A**), bottom powdery fermentation yeast (**B**), and top fermentation yeast (**C**).

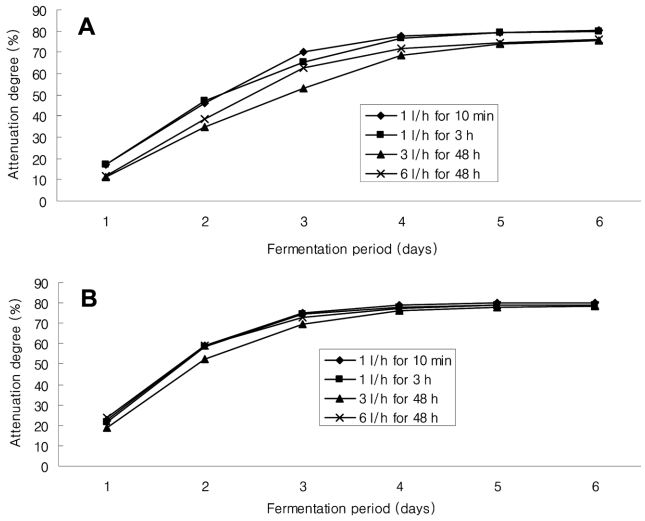

Fig. 2. Changes of fermentation ability during 1st fermentation (**A**) and 2nd fermentation (**B**) using pitching yeasts propagated under various aeration conditions in bottom flocculent fermentation yeast.

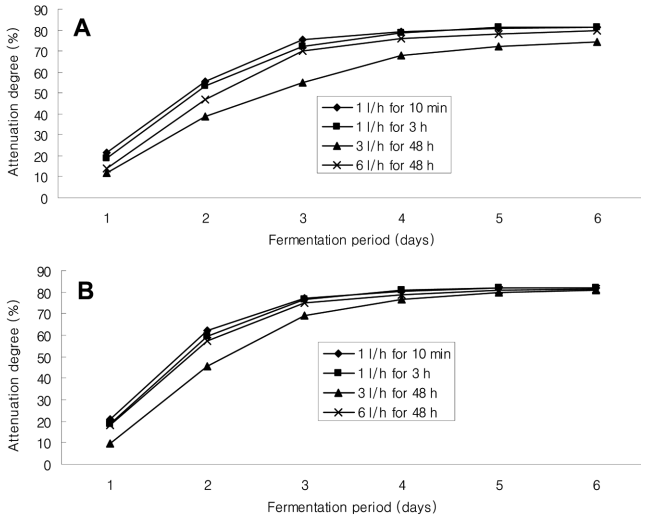

Fig. 4. Changes of fermentation ability during 1st fermentation (**A**) and 2nd fermentation (**B**) using pitching yeasts propagated under various aeration conditions in top fermentation yeast.

RESULTS AND DISCUSSION

Cell Growth of Yeast Propagated Under Various Aeration Conditions

Changes of cell growth during propagation under various aeration conditions for different yeasts are shown in Fig. 1. As shown in Fig. 1, a considerable difference in the cell growth among the samples under various aeration conditions was seen, in which the sample of 6 l/h for 48 h-aeration showed the fastest cell growth during propagation. The maximum of cell growth of all samples reached between 36 h and 48 h, which were similar to the results of Maemura *et al.* [14] and Schmidt [21]. In addition, the maximum number of yeast cells of the sample of 6 l/h for

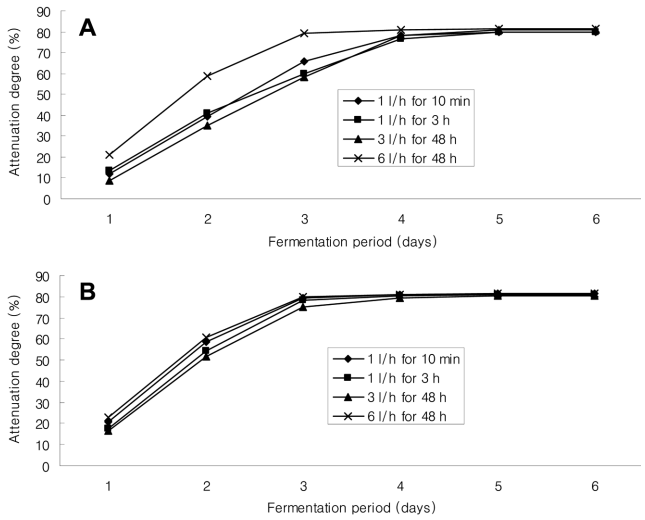

Fig. 3. Changes of fermentation ability during 1st fermentation (**A**) and 2nd fermentation (**B**) using pitching yeasts propagated under various aeration conditions in bottom powdery fermentation yeast.

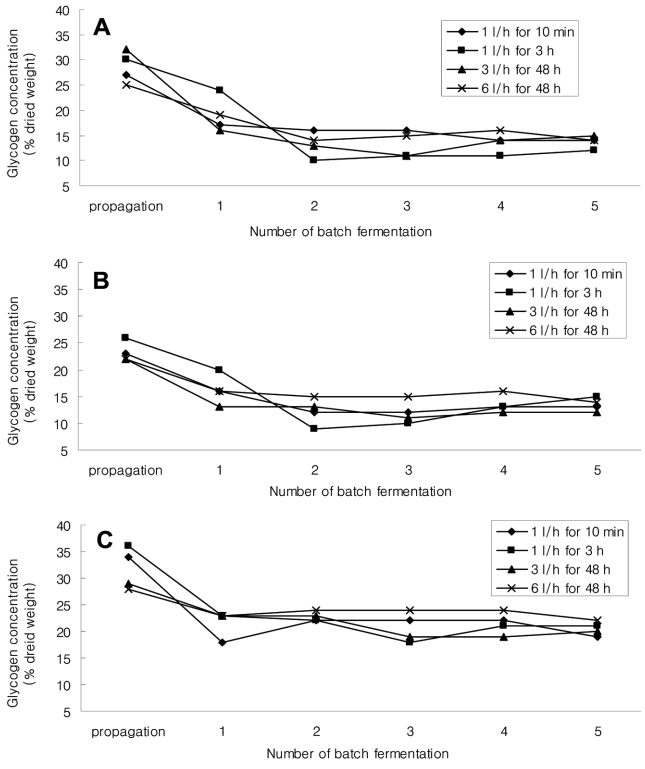

Fig. 5. Changes of glycogen concentration after propagation and each fermentation using pitching yeasts propagated under various aeration conditions in bottom flocculent fermentation yeast (**A**), bottom powdery fermentation yeast (**B**), and top fermentation yeast (**C**).

48-h aeration was about two times higher than that under other aerobic conditions. Similar results were obtained from the other yeast strains (Figs. 1B and 1C, respectively). The cell growth of yeast with regard to aeration conditions during propagation was strongly dependent on the yeast strains used. The results of the present study also supported that the increase of cell growth was dependent on the aeration volume, where the larger, the aeration volume, the larger the increase in cell growth which was mainly due to the synthesis of sterols and unsaturated fatty acids in the yeast membrane [1, 7, 26].

Effect of Aeration Conditions on Fermentation Ability

Changes of fermentation ability during fermentation using pitching yeasts propagated at different aeration conditions are shown in Figs. 2, 3 and 4, respectively. The samples of 1 l/h for 10 min and 1 l/h for 3 h aeration showed a higher attenuation degree, which indicates a degree of fermentation, in bottom flocculent fermentation yeast than those samples of 3 l/h for 48 h and 6 l/h for 48 h aeration over all 1st fermentation periods (Fig. 2A), but there were no significant differences regarding the attenuation degree among all the samples during the 2nd fermentation (Fig. 2B).

The time needed to reach maximal attenuation degree (ca. 80%) was only 3 days in the sample of 6 l/h for 48 h aeration compared with 5 days with samples of other aeration conditions in bottom powdery fermentation yeast (Fig. 3A). However, the fermentation ability among the samples during the 2nd fermentation was almost the same (Fig. 3B).

The samples of 1.0 l/h for 10 min, 1.0 l/h for 3 h, and 6 l/h for 48 h aeration showed a higher fermentation ability during the 1st fermentation in top fermentation yeast than that of 3 l/h for 48 h aeration (Fig. 4A), which also showed lower fermentation ability during the 2nd fermentation up to 4 days of fermentation and then indicated a similar fermentation ability at the end of the fermentation like the other samples (Fig. 4B). According to Ahvenainen and Maikinen [2], significant reduction in fermentation time was obtained when aerated pitching yeast was used. In the present study, it was noticed that the aeration conditions during propagation had an influence only on the 1st fermentation and no effect on up to following 5th fermentation (data not shown). It must be pointed out also that the results dependent on aeration conditions during propagation for the subsequent fermentation ability could differ considerably depending on the yeast strain used.

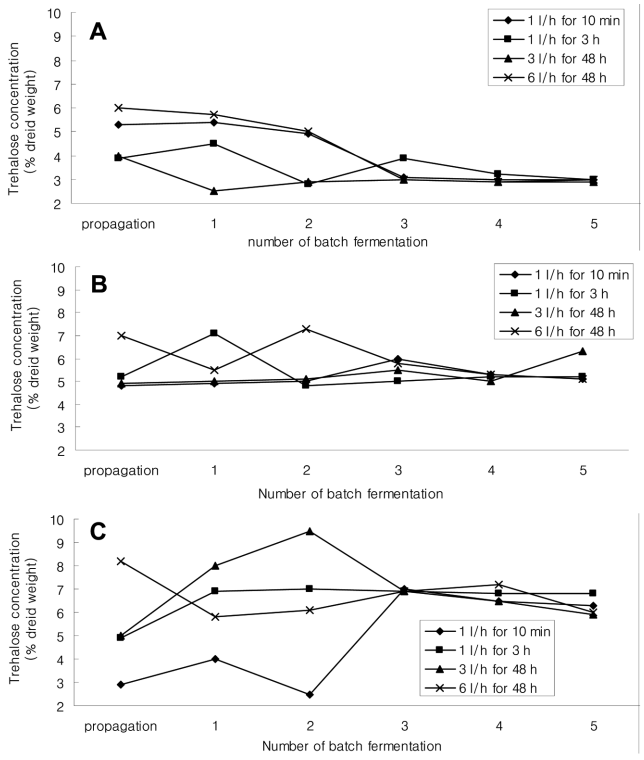

Fig. 6. Changes of trehalose concentration after propagation and each fermentation using pitching yeasts propagated under various aeration conditions in bottom flocculent fermentation yeast (**A**), bottom powdery fermentation yeast (**B**), and top fermentation yeast (**C**).

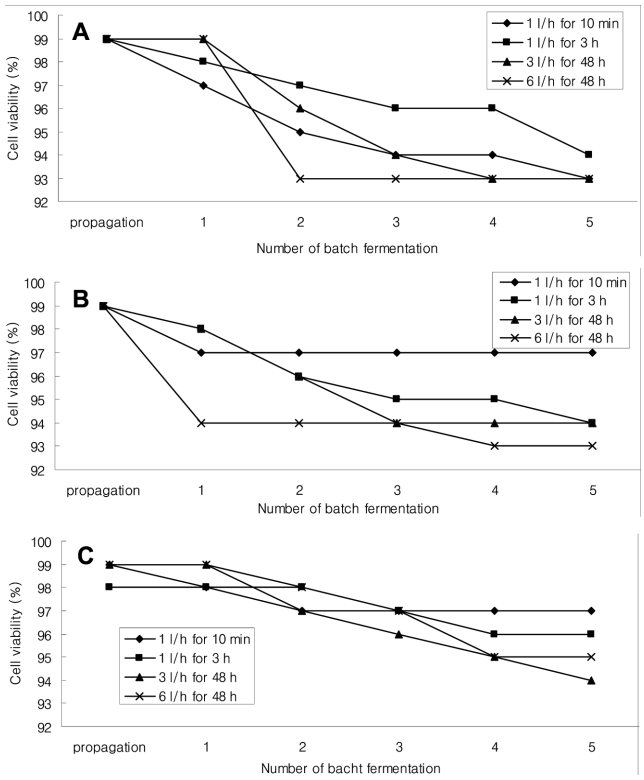

Fig. 7. Changes of viability after propagation and each fermentation using pitching yeasts propagated under various aeration conditions in bottom flocculent fermentation yeast (**A**), bottom powdery fermentation yeast (**B**), and top fermentation yeast (**C**).

Effect of Aeration Conditions on Level of Carbohydrates, Glycogen and Trehalose

Fig. 5 shows the changes of the intracellular levels of glycogen after propagation and each fermentation, respectively. As shown in Fig. 5A, the sample of 6 l/h for 48 h aeration showed the lowest level of glycogen, whereas the highest level of glycogen was found for the sample of 3 l/h for 48 h aeration, which is explained by the increased growth of yeast cells, which was similar to the results of Maemura *et al*. [14]. In bottom powdery fermentation yeast (Fig. 5B), the sample of 1.0 l/h for 3 h aeration showed the highest level of glycogen compared with other samples. In top fermentation yeast (Fig. 5C), the samples of 3 l/h for 48 h and 6 l/h for 48 h aeration showed a higher concentration of glycogen than those of 1 l/h for 10 min and 1 l/h for 3 h aeration. All of the yeast strains tested also showed, independent of aeration levels, the highest glycogen concentration after propagation and a marked decline after the 1st fermentation, and a little after then. It was also observed after propagation that more glycogen was accumulated in top fermentation yeast, when the yeast was propagated under low aeration conditions (1 l/h for 10 min and 1 l/h for 3 h, respectively), but the effect of aeration level on glycogen concentration was less clear in bottom fermentation yeasts. These different results among the yeast strains might be explained by a sensitivity for oxygen and different genetic characteristic of each yeast strain. According to Quain and Tubb [19], the relationship between glycogen dissimilated and sterol formed is stoichiometric. Fermentations of wort pitched with low-glycogen yeast were slower than those pitched with high-glycogen yeast. However, in the present study, there was no direct correlation between the glycogen concentration of pitching yeast and subsequent fermentation ability (Fig. 2–4), which was the same as the results of Wackerbauer *et al*. [23] and Sall *et al*. [20].

Fig. 6 shows the changes of the intracellular levels of trehalose after propagation and each fermentation, respectively. The samples of 6 l/h for 48 h aeration showed the highest level of trehalose. Martin *et al.* [15] and Imlay *et al*. [9] reported that free radicals (OH^-) and reactive forms

Table 1. Beer analysis after 1st fermentation under various aeration conditions in bottom flocculent fermentation yeast.

Parameters	1 l/h for 10 min	1 l/h for 3 h	3 l/h for 48 h	6 l/h for 48 h
Original gravity (%)	12.17	11.61	11.95	12.21
Apparent extract (%)	2.23	2.30	2.71	2.66
Alcohol (%)	5.29	4.94	4.92	5.10
Apparent attenuation (%)	81.60	80.10	77.30	78.20
Color (EBC)	26.00	25.00	25.00	22.00
pH-value	4.03	3.90	3.92	3.98
Bitterness unit (BE)	21.00	18.90	17.00	17.80
Total nitrogen (mg/l)	765.70	768.00	777.40	742.60
Free amino nitrogen (mg/l)	81.00	79.00	82.00	82.00
Total diacetyl (ppm)	0.11	0.10	0.11	0.10
Total pentandion (ppm)	0.05	0.07	0.10	0.08
Phenylethyl alcohol (ppm)	78.70	35.90	26.70	22.10
Acetaldehydes (ppm)	33.60	19.10	19.40	17.80
Σ Ester (ppm)	**16.8**	**15.6**	**9.4**	**8.6**
Ethyl formate (ppm)	<0.1	<0.1	<0.1	<0.1
Ethyl acetate (ppm)	14.00	13.90	8.80	8.10
Isoamyl acetate (ppm)	1.20	0.90	0.40	0.30
Phenylethyl acetate (ppm)	1.60	0.80	0.20	0.20
Σ Higher alcohols (ppm)	**109.4**	**94.1**	**93.6**	**89.4**
n-Propanol (ppm)	19.90	22.20	24.60	26.10
2-Methyl propanol (ppm)	12.90	11.20	11.00	10.30
2-Methyl butanol (ppm)	28.20	15.20	12.90	11.60
3-Methyl butano l(ppm)	48.40	45.50	45.10	41.40
Σ Fatty acids (ppm)	**5.76**	**8.77**	**7.26**	**4.51**
Butylic acid (ppm)	1.53	3.15	2.29	0.68
Isovaleric acid (ppm)	<0.01	1.25	1.24	0.99
Valeric acid (ppm)	0.07	0.03	0.04	0.06
Caproic acid(ppm)	1.53	1.30	1.08	1.02
Caprylic acid (ppm)	2.53	2.82	2.42	1.71
Capric acid (ppm)	0.07	0.18	0.16	0.05
Lauric acid (ppm)	0.03	0.04	0.03	<0.01

(H_2O_2) of oxygen is formed during the metabolite phase. These intermediate products cause damage to intracellular components and represent a stress factor of yeast, which can be the reason for the increased accumulation of trehalose under continuous aeration conditions. In the present study, the sample of 6 l/h for 48 h aeration showed the highest trehalose level after propagation, which supported the hypothesis of Imay *et al.* [9]. It was noticed that all the yeast strains investigated, shown to be independent on aeration conditions, had an irregular concentration of trehalose after each fermentation tests, and so the concentration of trehalose had no effect on subsequent fermentation ability, which was found at all yeasts tested up to the 5th fermentation tests.

Effect of Aeration Conditions on Viability of Yeasts

The viability of cells grown at different aeration conditions after propagation is shown in Fig. 7. It was observed that cells of all samples tested after propagation were 99% viable and the viability decreased as fermentation subsequently progressed, of which the sample of 6 l/h for 48 h aeration showed the highest decline of viability for bottom flocculent and powdery fermentation yeasts, whereas the sample of 3 l/h for 48 h aeration indicated the highest decline of viability for top fermentation yeast. However, all of the yeasts tested also showed very high viabilities up to 5th fermentation tests independent on aeration conditions, which are also strongly related to the treatment of yeast harvested after each fermentation. A similar result was also observed by another researcher [14], who tested the viability only with one yeast strain. In this experiment, it did not observe any relationship between level of trehalose and viability under various aeration conditions.

Beer Analysis

The beer analysis after the 1st fermentation with yeast propagated under different aeration conditions for the bottom flocculent yeast is shown in Table 1. Schmidt [21] reported that the concentration of higher alcohols and ethyl acetate of fermented yeast using pitching yeast propagated under continuous aeration was decreased, whereas acetaldehyde

Table 2. Beer analysis after 1st fermentation under various aeration conditions in bottom powdery fermentation yeast.

Parameters	1 l/h for 10 min	1 l/h for 3 h	3 l/h for 48 h	6 l/h for 48 h
Original gravity (%)	12.19	11.88	11.72	12.02
Apparent extract (%)	2.23	2.22	2.19	2.19
Alcohol (%)	5.30	5.14	5.06	5.23
Apparent attenuation (%)	81.60	81.30	81.30	81.80
Color (EBC)	23.00	24.00	25.00	21.00
pH-value	4.07	3.99	3.95	4.01
Bitterness unit (BE)	20.20	18.50	18.00	18.20
Total nitrogen (mg/l)	751.40	768.60	760.00	786.20
Free amino nitrogen (mg/l)	82.00	83.00	70.00	78.00
Total diacetyl (ppm)	0.18	0.10	0.08	0.12
Total pentandion (ppm)	0.09	0.06	0.06	0.06
Phenylethyl alcohol (ppm)	35.40	27.30	29.20	26.00
Acetaldehydes (ppm)	11.50	6.60	3.60	7.20
Σ Ester (ppm)	**16.7**	**19.0**	**13.1**	**11.8**
Ethyl formate (ppm)	0.70	<0.1	<0.1	<0.1
Ethyl acetate (ppm)	14.70	17.40	12.10	10.90
Isoamyl acetate (ppm)	0.80	1.00	0.60	0.40
Phenylethyl acetate (ppm)	0.50	0.60	0.40	0.50
Σ Higher alcohols (ppm)	**99.2**	**87.0**	**93.5**	**97.3**
n-Propanol (ppm)	23.30	17.40	22.50	25.70
2-Methyl propanol (ppm)	9.20	10.00	9.80	9.80
2-Methyl butanol (ppm)	17.90	12.10	10.20	16.20
3-Methyl butanol (ppm)	41.80	47.50	51.00	45.60
Σ Fatty acids (ppm)	**8.08**	**9.85**	**8.13**	**5.45**
Butylic acid (ppm)	1.81	1.65	1.55	0.56
Isovaleric acid (ppm)	0.82	1.27	1.48	0.89
Valeric acid (ppm)	0.05	0.03	0.06	0.03
Caproic acid (ppm)	1.84	2.02	1.46	1.34
Caprylic acid (ppm)	3.44	4.68	3.35	2.56
Capric acid (ppm)	0.10	0.19	0.20	0.05
Lauric acid (ppm)	0.02	0.01	0.03	0.02

was increased considerably. Kringstad and Rasch [13] found that aerobically grown pitching yeast was rich in diacetyl after fermentation compared with anaerobic yeast. In this study, it was noticed that the concentration of aroma compounds obtained with yeast propagated under 6 l/h for 48 h aeration was lower than those grown under other aeration conditions; in particular, the amount of phenylethyl alcohol, acetaldehydes, ester, higher alcohols, and fatty acids was decreased, which has a positive effect on beer quality. It was also observed that there was no difference of level of diacetyl dependent on aeration conditions. In bottom powderly fermentation yeast (Table 2), it was observed that the yeast propagated under 6 l/h for 48 h aeration was lower than those grown under other aeration conditions. In particular, the amount of phenylethyl alcohol, ester, and fatty acids was decreased, whereas other aroma compounds indicated no relationship among the samples depended on the aeration conditions. In top fermentation yeast (Table 3), the byproducts of fermentation using yeast propagated at different aeration conditions indicated that the fermentation with continuous aerated yeast (3 l/h for 48 h and 6 l/h for 48 h, respectively) showed lower levels of ester and fatty acids than those with low aerated yeasts (1 l/h for 10 min and 1 l/h for 3 h, respectively). Other by products were not uniform in relation to aeration conditions during propagation.

From the results of these experiments, it was concluded that the continuous aeration during propagation of yeast had an effect on yeast metabolism, yield of yeast, and beer quality, but yeasts strains respond differently to the level of aeration during propagation. From the practical point of view, it is of importance to consider how long these characteristics of yeast induced by the method of aeration during propagation will remain in the yeast. It is assumed that the level of aeration during propagation influenced only the first fermentation with regard to yeast physiology, fermentation ability, and beer quality. In further fermentation, the yeast may regain its original characteristics. Quantitatively, the amount of oxygen needed is determined by the amount consumed by the wort and the amount required by the yeast strains.

Table 3. Beer analysis after 1st fermentation under various aeration conditions in top fermentation yeast.

Parameters	1 l/h for 10 min	1 l/h for 3 h	3 l/h for 48 h	6 l/h for 48 h
Original gravity (%)	12.26	11.93	12.46	12.54
Apparent extract (%)	2.10	2.01	2.51	2.25
Alcohol (%)	5.41	5.27	5.31	5.50
Apparent attenuation (%)	82.80	83.10	79.80	82.10
Color (EBC)	24.00	25.00	25.00	26.00
pH-value	4.21	4.11	3.93	4.06
Bitterness unit (BE)	22.00	19.30	16.60	19.20
Total nitrogen (mg/l)	832.10	775.40	691.70	741.60
Free amino nitrogen (mg/l)	103.00	88.00	77.00	85.00
Total diacetyl (ppm)	0.15	0.07	0.10	0.12
Total pentandion (ppm)	0.05	0.03	0.03	0.03
Phenylethyl alcohol (ppm)	31.00	23.40	29.00	23.10
Acetaldehydes (ppm)	30.40	7.80	10.00	23.70
Σ Ester (ppm)	**20.0**	**23.2**	**14.1**	**15.8**
Ethyl formate (ppm)	0.20	0.20	<0.1	0.20
Ethyl acetate (ppm)	18.30	21.10	13.70	15.00
Isoamyl acetate (ppm)	1.00	1.40	0.30	0.40
Phenylethyl acetate (ppm)	0.50	0.50	0.10	0.20
Σ Higher alcohols (ppm)	**90.5**	**88.2**	**97.9**	**92.1**
n-Propanol (ppm)	15.50	16.30	21.80	19.40
2-Methyl propanol (ppm)	9.70	9.30	11.90	10.80
2-Methyl butanol (ppm)	16.50	9.10	9.90	16.60
3-Methyl butanol (ppm)	48.80	53.50	54.30	45.30
Σ Fatty acids (ppm)	**9.43**	**10.19**	**6.94**	**7.59**
Butylic acid (ppm)	1.48	1.89	0.88	1.40
Isovaleric acid (ppm)	0.63	0.70	1.20	0.69
Valeric acid (ppm)	0.06	0.04	0.06	0.08
Caproic acid (ppm)	2.46	2.25	1.56	1.93
Caprylic acid (ppm)	4.73	5.17	3.24	3.23
Capric acid (ppm)	0.05	0.14	<0.01	0.12
Lauric acid (ppm)	0.02	<0.01	<0.01	0.14

Acknowledgment

This study was supported by the Technology Development Program for Agriculture and Forestry, Ministry of Agriculture and Forestry, Republic of Korea.

REFERENCES

1. Ahvenainen, J. 1982. Lipid composition of aerobically and anaerobically propagated Brewers bottom yeast. *J. Inst. Brew.* **88:** 367–370.
2. Ahvenainen, J. and V. Maekinen. 1981. The effect of pitching yeast aeration on fermentation and beer flavour. *EBC Congress* 285–291.
3. Back, W., I. Bohak, and T. Ackermann. 1993. Optimierte Hefewirtschaft. *Brauwelt* **39:** 1960–1963.
4. Back, W. and C. Forster. 1999. Hefemanagement und Anstelltechnologie mit ihren Auswirkungen auf Geschmack und Geschmacksstabilitaet. *EBC Congress* 627–636.
5. Boulton, C. A. and D. E. Quain. 1999. A novel system for propagation of brewing yeast. *EBC Congress* 647–654.
6. Brandl, J. 1994. Hefereinzucht, Hefefuehrung, Hefeaufbewahrung. *Brauwelt* **45:** 2470–2473.
7. Brown, C. M. and B. Johnson. 1971. Influence of oxygen tension on physiology of *Saccharomyces cerevisiae* in continuous culture. *Antonie Van Leeuwenhoek* **37:** 477–487.
8. Gruetzmacher, J. 1991. Bedeutung des Sauerstoffs fuer die Bierhefe. *Brauwelt* **23:** 958–9668.
9. Imay, J. A., S. M. Chin, and S. Linn. 1988. Toxic DNA damage by hydrogen peroxide through the Fenton reaction *in vivo* and *in vitro*. *Science* **240:** 640–642.
10. Jabkobsen, M. and S. W. Thorne. 1980. Oxygen requirements of brewing strains of *Saccharomyces uvarium* (*carlsbergensis*)-bottom fermentation yeast. *J. Inst. Brew.* **86:** 284–287.
11. Kang, H. Y., Y. S. Kim, G. J. Kim, J. H. Seo, and Y. W. Ryu. 2005. Screening and characterization of flocculent yeast, *Candida* sp. HY 200, for the production of xylitol from D-xylose. *J. Microbiol. Biotechnol.* **15:** 362–367.
12. Kim, H. M., S. W. Kim, H. J. Hwang, M. K. Park, A. G. M. Yehia, J. W. Choi, and W. Y. Yun. 2006. Influence of agitation intensity and aeration rate on production of antioxidative exopolysaccharides from submerged mycelial culture of *Ganoderma resinaceum*. *J. Microbiol. Biotechnol.* **16:** 1240–1247.
13. Kringstad, H. and S. Rasch. 1966. The influence of the method of preparation of pitching yeast on its production of diacetyl and acetoin during fermentation. *J. Inst. Brew.* **72:** 56–61.
14. Maemura, H., S. Morimura, and K. Kida. 1998. Effects of aeration during the cultivation of pitching yeast on its characteristics during the subsequent fermentation of wort. *J. Inst. Brew.* **104:** 207–211.
15. Martin, V., D. E. Quain, and K. A. Smart. 1999. The oxidative stress response of ale and lager yeast strains. *EBC Congress* 679–686.
16. Maule, D. R. 1980. Propagation and handling of pitching yeast. *Brewer's Digest* February: 36–40.
17. Pfenninger, H. 1993. Brautechnische Analysenmethoden Band II. *Selbstverlag der MEBAK*, 3. Auflage, Freising.
18. Quain, D. E. 1981. The determination of glycogen in yeasts. *J. Inst. Brew.* **87:** 289–291.
19. Quain, D. E. and R. S. Tubb. 1982. The importance of glycogen in brewing yeasts. *MBAA Technical Quarter* **19:** 29–33.
20. Sall, C. J., J. F. Seipp, and A. T. Pringle. 1988. Changes in Brewers yeast during storage and the effect of these changes on subsequent fermentation performance. *ASBC* **46:** 23–25.
21. Schmidt, H. J. 1993. Beschleunigte Hefefuehrung von Reinzuchthefen. *Brauwelt* **44/45:** 2254–2274.
22. Thakker, C., B. Suresh, and R. Dilip. 2006. Formation of succinic acid by *Klebsiella pneumoniae* MCM B-325 under aerobic and anaerobic conditions. *J. Microbiol. Biotechnol.* **16:** 870–879.
23. Wackerbauer, K., T. Tayama, M. Fitzner, and S. Kunerth. 1997. Zeitgemaesses Management der Anstellhefe. *Brauwelt* **3:** 80–87.
24. Wilson, K. and B. J. McLeod. 1976. The influence of conditions of growth on the endogenous metabolism of *Saccharomyces cerevisiae*: Effect on protein, carbohydrate, sterol and fatty acid content and on viability. *Antonie Van Leeuwenhoek* **42:** 397–410.
25. Winkler, K., I. Kienle, M. Burgert, J. C. Wagner, and H. Holzer. 1991. Metabolic regulation of the trehalose content of vegetative yeast. *FEBS* **291:** 269–272.
26. Zepf, M., E. Geiger, and I. Nieten. 2000. Parametersteuerung bei der aeroben Hefevermehrung. *Brauwelt* **44:** 1849–1851.