Food Sci. Biotechnol. Vol. 17, No. 4, pp. 739 ~ 744 (2008)

Food Science and Biotechnology

Optimal Conditions for Propagation in Bottom and Top Brewing Yeast Strains

Chul Cheong, Karl Wackerbauer[1], Si Kyung Lee[2], and Soon Ah Kang*

Department of Fermented Food Science, Seoul University of Venture & Information, Seoul 135-090, Korea
[1]Department of Biotechnology, Technical University of Berlin, Berlin, Germany
[2]Department of Applied Biological Science, Konkuk University, Seoul 143-701, Korea

Abstract The method of yeast propagation has an influence on yeast physiology, fermentation ability, flocculation rate, and taste stability of beer. In order to find optimal conditions for propagation, several parameters were investigated in combinations. The bottom brewing yeast grown at 10°C indicated that a higher flocculation capacity during the 1st fermentation. However, the taste stability and the aroma profile were not affected by parameters of propagation investigated. The beer quality was rather affected by storage duration. In addition, a correlation between tasting and chemiluminescence was found at the beer, which was produced using bottom brewing yeast. The propagation at 10-25°C with addition of zinc ion indicated the best condition to improve fermentation ability, flocculation rate, and filterability for bottom brewing yeast, whereas the propagation at 30°C with addition of zinc ion showed the best condition to increase fermentation ability for top brewing yeasts.

Keywords: flocculation, pure culture, fermentation ability, beer analysis

Introduction

The correct and modern propagation of pure culture yeast are a base condition for a faultless beer quality (1). Hence, the propagation is an important procedural step in an industrial brewery because confident fermentation, prevention of contamination, and production of constant beer quality can be guaranteed only by means of vital yeast (2-6). In the brewing industry, it is observed problems during the fermentation and aging, which lead to prolongation of fermentation time or to deficient degradation of by-products of fermentation. Sometimes a poor foam stability and deterioration of beer flavour can be occurred (1,7). It is also often reported that a strongly flocculating yeast strain will be become a weakly flocculating yeast in the 1st fermentation proportional compared to further fermentations. Weak flocculation yeast strains are not suitable in conventional beer production because they cause unwanted flavors and filtration difficulties (6,7). These total problems are attributed to bad quality of pitching yeast, which is connected with the propagation of pure culture yeast. The characteristic of yeast is fixed genetically, on the other hand the chemical and physical environment can also cause the changes of yeast character during propagation (8, 10-12). The environmental factors that report to affect yeast physiology are wort composition, aeration rate, temperature, and CO_2 level (10,13-17). Consequently it is important how the each yeast strains should be propagated so that a strong pitching yeast is produced which maintains its original character and leads to constant fermentation process, and to faultless beer quality (18,19). Thus it is needed a standardized propagation procedure of brewing yeast. The aim of this study is to find optimal conditions of propagation method for bottom and top brewing yeast strains and thus providing information useful for control of industrial beer production. The applied method of propagation based on batch method (1) in which the yeast is cultured and used for pitching whole yeast propagated.

*Corresponding author: Tel: +82-2-3470-5273; Fax: +82-2-523-6767
E-mail: sakang@suv.ac.kr
Received October 5, 2007; Revised December 12, 2007;
Accepted December 17, 2007

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 160 is a top fermentation brewing yeast (called alt brewing yeast). Strain 127 is also a top fermentation brewing yeast (called wheat brewing yeast). All strains were obtained from the Brewing and Research and Teaching Institute (VLB) in Berlin.

Preculture All yeasts were inoculated into of 200-mL Erlenmeyer flask containing 50 mL of wort and incubated at 25°C for 48 hr.

Propagation of yeast Fifty mL of preculture were inoculated in a 10-L stirred conical glass flask that contained 5 L of sterile wort, and propagated with agitation at 100 rpm at 20°C for 48 hr. 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%.

Fermentation Under various propagated yeasts were pitched 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 fermentation, the yeast was harvested and used again for the next fermentation test.

Viability test Viable cells were measured with a microscope after staining with Mg-1-aniline-8-naphthalene-sulphuric acid (ANS, Sigma-Aldrich, St. Louis, MO, USA). Viability was calculated by dividing the number of viable cells by the total number of cells, with results given as percentages.

Taste analysis The tasting was accomplished according to the method reported by Pfenninger (20). This method is based on Mitteleuropaeische Brauerei Analytik Kommision (MEBAK), in which is demonstrated about taste analysis.

Chemiluminescence The test was based on modified method of Wackerbauer *et al.* (21). Each light content was detected which is released by chemiluminescence at oxidative radical reaction in the beer deterioration. It is obtained a graphic diagram of intensity allocation over reaction time. The point of appearance of second intensity maximum is important. It was measured using CLD-100 as chemiluminescence detector and CLC-10 as impulse counter, and chemiluminescence amplifier (Tohoku Electronic Industry, Japan). Absorption and converting of data were carried out over IBM compatible PC (type PC-9801 VM). Unit of display was occurred over digital plotter VP-6803A (Panasonic, Osaka, Japan). Experimentally, 50 mL of beer is decarbonated under cooling and elimination of light in ultra sonic bath. The sample remains masked for 4 min for back-formation of foam. Thereafter 12 mL is pipetted into the measuring cell. The measurement is carried out in measuring cell at 60°C.

Absorption integral The analytical method is modification of the method presented by Klein *et al.* (22). It was predominantly to detect furan and furanose. The 100 g chilled beer is weighed and 0.5 mL silicon antifoam (Ruehl puromer, Germany) is added. The sample is given into the distillation equipment of water vapour. Distillate is catched in chilled volumetric flask (50-mL) and temperated, and filled up to marker. It is measured using spectral photometer (Spectronic AquaMate; Merck, Damstadt, Germany) at 200-350 nm.

Analytical methods The analysis for wort and beer was determined according to the method reported by Pfenninger (20). The original gravity, apparent extract and alcohol in beer were analyzed using beer analyser of Anton Paar with autosampler, (SP-1; Burkard Scientific, Middx, UK). The extract was determined during the fermentation using Biegeschwinger (DPR Y; Anton Paar, Toledo, IL, USA), which determines the extract by measuring its density. The aromatic compounds such as ester, higher alcohols were analyzed gas chromatography (GC) using a Hewlett Packard 6890 GC fitted with a flame ionize (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, Lombard, IL, USA) with 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).

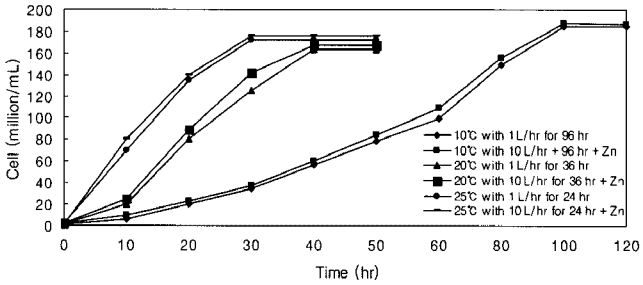

Fig. 1. Changes of yeast growth under various propagation conditions in bottom brewing yeast.

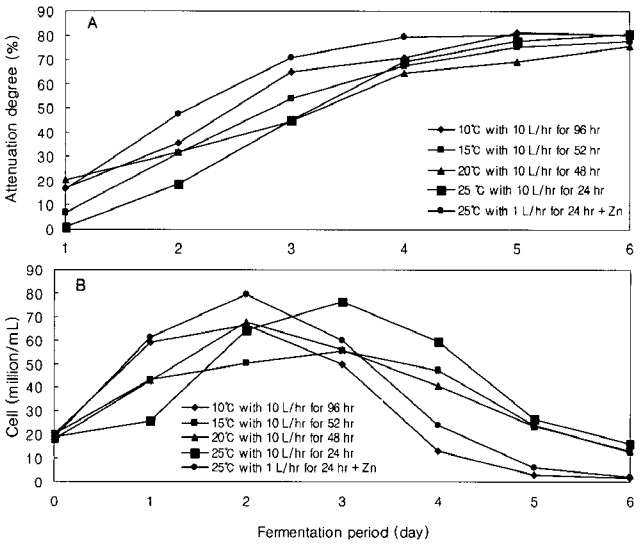

Fig. 2. Changes of fermentation ability (A) and number of cells in suspension (B) under various propagation conditions in bottom brewing yeast.

Results and Discussion

Optimization of conditions for propagation by bottom brewing yeast *Yeast growth during propagation*: Changes of yeast growth under various propagation conditions in bottom brewing yeast are shown in Fig. 1. The temperature played a decisive role for cell growth rate, but had no effect on maximum yeast yield. The maximum yeast growth was seen within 48 hr at the samples of propagation above 20°C, whereas at 10°C this was achieved after 100 hr. This is supportive to the results of Maemura *et al.* (23). The addition of zinc ion (0.5 ppm) during propagation and the aeration rate had no influence either on speed of cell growth or on maximum cell yield. So the yeast growth was not affected by combination of various propagation factors. The viability of cells tested after propagation were 99%, which was the same as the results of Cheong *et al.* (13).

***Fermentation curve and changes of number of yeast cells in suspension*:** Supplementation of zinc ion to growth medium led to high growth and increased fermentation activity (Fig. 2), in which was showed an improved flocculation rate. At 10°C, the yeast growth were not

Table 1. Beer analysis after 1st fermentation under various propagation conditions in bottom brewing yeast

Parameters[1)]	10°C with 10 L/hr for 96 hr	15°C with 10 L/hr for 52 hr	20°C with 10 L/hr for 48 hr	25°C with 10 L/hr for 24 hr	25°C with 1 L/hr for 24 hr+Zn
Original gravity (%)	11.74	11.63	11.67	11.35	11.65
Apparent extract (%)	1.95	1.77	1.92	1.83	1.91
Real extract (%)	3.82	3.66	3.79	3.65	3.77
Alcohol (%vol)	5.20	5.23	5.17	5.04	5.16
Apparent attenuation (%)	83.30	84.70	83.50	83.90	83.50
pH value	4.28	4.23	4.24	4.42	4.31
Color (EBC)	5.90	6.10	6.10	4.70	5.60
Viscosity (12%)	1.45	1.63	1.65	1.65	1.65
Bitterness unit (BE)	34.00	32.60	34.40	29.50	34.90
Free amino nitrogen (mg/L)	89.00	87.00	83.00	78.00	85.00
Foam (R&C)	126.00	121.00	128.00	117.00	123.00
Diacetyl (ppm)	0.03	0.03	0.03	0.03	0.02
Higher alcohols (ppm)	85.00	95.80	85.90	84.70	89.60
Ester (ppm)	20.70	22.20	20.00	20.50	23.50
Fatty acids (ppm)	9.30	10.20	10.00	9.10	10.40

[1)]Original gravity, density of wort; apparent extract, apparent final gravity; real extract, real final gravity; apparent attenuation, apparent degree of fermentation.

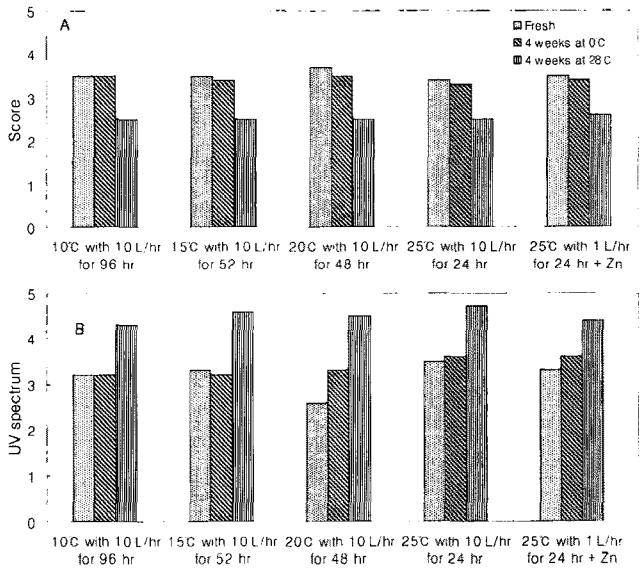

Fig. 3. Tasting (A) and absorption integral (B) over 4 weeks under various propagation conditions in bottom brewing yeast.

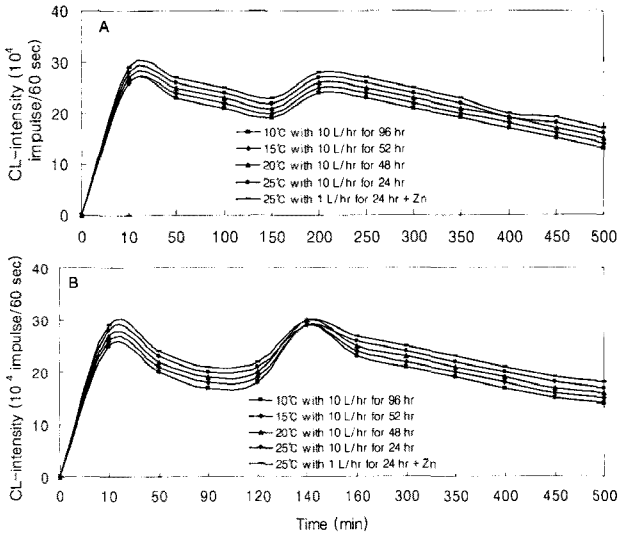

Fig. 4. Chemiluminescence curve from fresh beer (A) and after 4 weeks at 28°C (B) under various propagation conditions in bottom brewing yeast.

significantly changed, but showed a pronounced flocculation ability, which supported the results of Hamersveld *et al.* (24) and Garsoux *et al.* (25). The effectiveness of 10°C-propagation on the flocculation was found independent on variation of propagation conditions. The influence of temperature on the flocculation process may be complex. Low temperature cells had probably a more hydrophobic cell wall as was shown by Van Iersel *et al.* (7). These results are important for the brewing industry, because the filtration problem after 1st fermentation can be solved. The influence by addition of zinc ion or by cold temperature (10°C) on the fermentation activity and the flocculation rate were stronger than the variation of temperature, aeration rate, and aeration time. No interactions between propagation conditions investigated were found in optimal study.

Beer analysis: The beer analysis after the 1st fermentation yeast grown under different propagation conditions in bottom brewing yeast is shown in Table 1. It was observed that the sample grown under 10 L/hr aeration at 15°C for 96 hr indicated a slightly higher level of higher alcohol than those of other propagation conditions. The yeast grown under 1 L/hr aeration at 25°C for 24 hr with addition of zinc ion showed a higher level of ester and higher alcohol. The sample propagated under 10 L/hr without addition of zinc ion at 25°C indicated the worst foam stability. The difference among the samples was regarding aroma profile insignificant.

Taste stability: Samples grown under 10 L/hr aeration at 20°C for 48 hr preferred to other samples with regard to the propagation conditions in tasting (Fig. 3A). When samples aged for 4 weeks at 28°C, very low in tasting were seen,

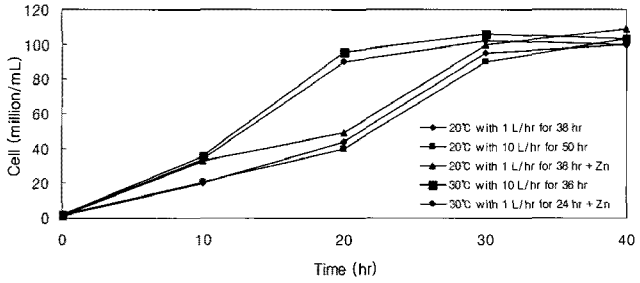

Fig. 5. Changes of yeast growth under various propagation conditions in alt brewing yeast.

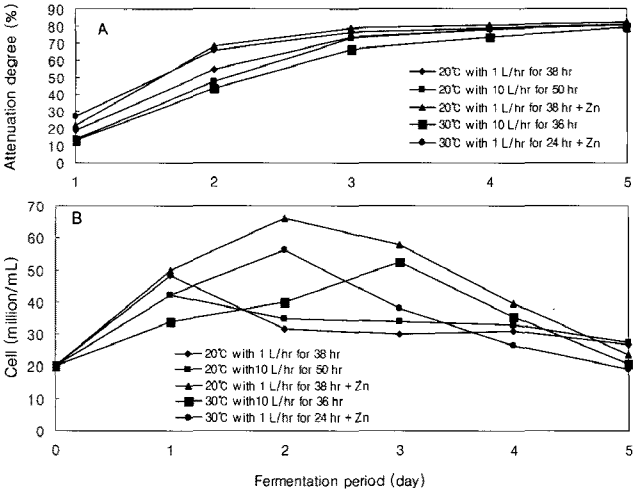

Fig. 6. Changes of fermentation ability (A) and number of cells in suspension (B) under various propagation conditions in alt brewing yeast.

compared to other samples. The beer quality was not dependent on variation of propagation conditions, but was changed rather by storage duration. The samples which was stored fresh and for 4 weeks at 0°C, respectively, indicated no significant difference in the investigation using absorption integral (Fig. 3B). In addition, the sample which were stored over 4 weeks at 28°C showed a higher value compared with those of other samples independent on propagation conditions. The investigation using chemiluminescence showed that all samples indicated a same curve (Fig. 4A). Samples which were stored over 4 weeks at 28°C was lost resistance power opposite to oxidation compared to other samples (Fig. 4B), which one can find maximum of intensify. A similar result was also observed by another researcher (11,26,27). From the results of this experiments, it was concluded that the propagation for bottom brewing yeast should be carried out under continuous aeration with addition of zinc ion at 10-25°C. Thereby vital pitching yeast which has a positive effect on fermentation can be produced without any influence on beer quality.

Optimization of conditions for propagation by alt brewing yeast ***Yeast growth during propagation of yeast*****:** Changes of yeast growth at various propagation conditions for alt brewing yeast are investigated. As shown in Fig. 5, a considerable difference in the yeast growth among the samples was seen, in which the samples with 30°C propagation showed the fastest cell growth during propagation as expected. The samples grown at 20°C showed the maximum of yeast growth between 30 and 40 hr in propagation, whereas this was reached already between 20 and 30 hr in propagation at the samples propagated at 30°C. The maximum of cell yield reached was among the samples very similar (100-120 million/mL). It was also obtained that the addition of zinc ion had no effect on cell yield.

Fermentation curve and changes of number of yeast cells in suspension**:** The samples with addition of zinc ion during propagation showed a higher fermentation activity during 1st fermentation from 1 day up to 5 days in which was also obtained a higher yeast growth (Fig. 6A). The

Table 2. Beer analysis after 1st fermentation under various propagation conditions in alt brewing yeast

Parameters[1)]	20°C with 1 L/hr for 38 hr	20°C with 10 L/hr for 50 hr	20°C with 1 L/hr for 38 hr+Zn	30°C with 10 L/hrfor 36 hr	30°C with 1 L/hr for 24 hr+Zn
Original gravity (%)	11.48	11.61	11.57	11.57	11.32
Apparent extract (%)	1.38	1.48	1.41	1.51	1.45
Real extract (%)	3.31	3.41	3.35	3.49	3.34
Alcohol (%vol)	5.34	5.37	5.37	5.33	5.22
Apparent attenuation (%)	87.90	87.20	87.80	86.90	87.10
pH value	4.38	4.57	4.29	4.32	4.56
Color (EBC)	6.00	5.80	6.40	5.30	5.30
Viscosity (12%)	1.62	1.61	1.61	1.61	1.61
Bitterness unit (BE)	26.80	28.50	28.60	29.40	22.80
Free amino nitrogen (mg/L)	79.30	97.20	75.10	104.30	89.80
Foam (R&C)	108.00	106.00	117.00	109.00	101.00
Diacetyl (ppm)	0.04	0.05	0.04	0.03	0.06
Higher alcohols (ppm)	109.30	100.50	114.20	90.40	96.70
Ester (ppm)	25.10	21.70	21.20	24.50	27.50
Fatty acids (ppm)	11.80	10.20	11.60	10.50	12.10

[1)]Original gravity, density of wort; apparent extract, apparent final gravity; real extract, real final gravity; apparent attenuation, apparent degree of fermentation.

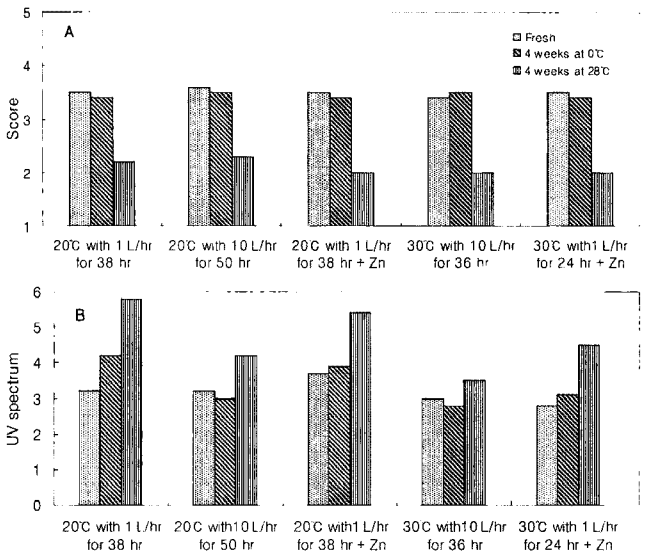

Fig. 7. Tasting (A) and absorption integral (B) over 4 weeks under various propagation conditions in alt brewing yeast.

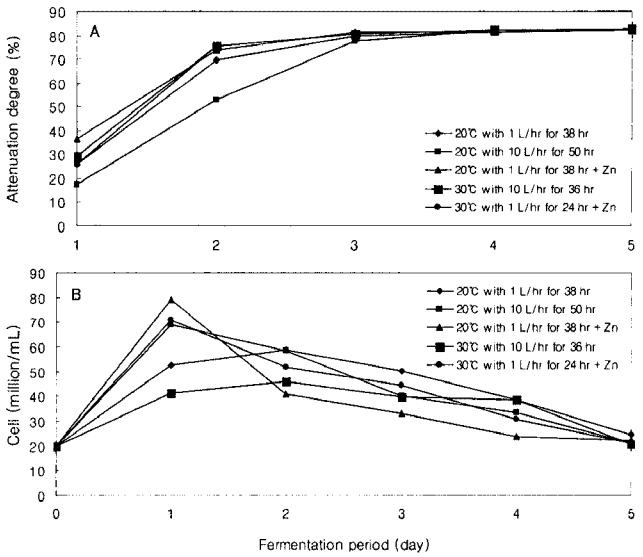

Fig. 8. Changes of fermentation ability (A) and the number of cells in suspension (B) under various propagation conditions in wheat brewing yeast.

effectiveness of zinc ion was also here confirmed. The variation of temperature, aeration rate, and aeration time did not play role on fermentation activity. The samples with addition of zinc ion during propagation indicated a later sedimentation and sedimented fast at the end of fermentation (Fig. 6B).

Beer analysis: The samples grown at 20°C led to higher level of higher alcohols compared to those of the samples propagated at 30°C (Table 2). It was also noted that the samples which was aerated strongly led to low level of fatty acids independent on temperature of propagation. The sample grown with addition of zinc ion at 20°C indicated the best foam stability. However, there was no significant difference among the samples regarding aroma component and non-biological stability.

Taste stability: There was no significant difference among the samples of fresh beer regarding propagation conditions in tasting (Fig. 7A). The samples which were stored for 4 weeks at 0 and 28°C, respectively, indicated also no significant difference among the samples by means of propagation conditions. However, the sample which was stored over 4 weeks at 28°C showed a higher value compared to those of other samples independent on propagation conditions using absorption integral (Fig. 7B). From the results of this experiment, it was concluded that die propagation for alt brewing yeast should be accomplished under continuous aeration with addition of zinc ion at 30°C. Thereby vital pitching yeast which has a positive effect on fermentation can be produced without any influence on beer quality.

Table 3. Beer analysis after 1st fermentation under various propagation conditions in wheat brewing yeast

Parameters[1)]	20°C with 1 L/hr for 38 hr	20°C with 10 L/hr for 50 hr	20°C with 1 L/hr for 38 hr+Zn	30°C with 10 L/hr for 36 hr	30°C with 1 L/hr for 24 hr+Zn
Original gravity (%)	11.55	11.81	11.93	11.58	11.94
Apparent extract (%)	1.98	1.97	2.05	1.71	1.96
Real extract (%)	3.80	3.85	3.94	3.60	3.86
Alcohol (%vol)	5.08	5.22	5.25	5.23	5.30
Apparent attenuation (%)	82.90	83.20	82.80	85.20	83.60
pH value	4.10	4.12	4.05	4.28	4.11
Color (EBC)	4.50	4.80	5.50	4.80	5.40
Viscosity (12%)	1.65	1.66	1.66	1.64	1.64
Bitterness unit (BE)	25.20	26.50	28.70	25.90	28.70
Free amino nitrogen (mg/L)	32.00	40.00	37.00	58.00	47.00
Foam (R&C)	120.00	125.00	136.00	123.00	132.00
Diacetyl (ppm)	0.11	0.15	0.09	0.18	0.14
Higher alcohols (ppm)	228.30	212.70	240.50	207.10	223.60
Ester (ppm)	36.80	14.00	28.40	18.00	30.10
Fatty acids (ppm)	7.40	5.90	7.00	7.00	7.30

[1)]Original gravity, density of wort; apparent extract, apparent final gravity; real extract, real final gravity; apparent attenuation, apparent degree of fermentation.

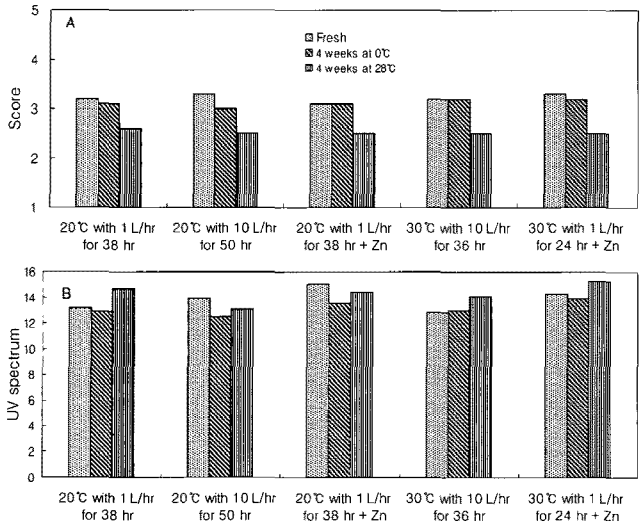

Fig. 9. Tasting (A) and absorption integral (B) over 4 weeks under various propagation conditions in wheat brewing yeast.

Optimization of conditions for propagation by wheat brewing yeast ***Yeast growth during propagation of yeast*****:** The progress of yeast growth dependent on various propagation conditions was occurred like at alt brewing yeast (data not shown). So the addition of zinc ion and aeration rate during propagation had no influence on yeast growth. Interaction among the propagation conditions was not also obtained.

Fermentation curve and changes of number of yeast cells in suspension**:** It was noted that the sample propagated with addition of zinc ion showed higher yeast growth and increased fermentation activity during 1st fermentation (Fig. 8A). This trend was also obtained during 2nd fermentation (data not shown). Hence the positive effect of zinc ion on the fermentation activity was observed for wheat brewing yeast. The samples indicated a different sedimentation rate during fermentation, but sedimented at the end of fermentation the same (Fig. 8B).

Beer analysis**:** The wheat beer yeast indicated a typical and characteristical value with a content of higher alcohols (Table 3). The sample grown by aeration rate increased during propagation led to a low level of ester and fatty acids. The sample grown with addition of zinc ion at 20°C showed the best foam stability like in the alt brewing yeast. The difference among the samples regarding aroma component and non-biological stability were insignificant.

Taste stability**:** It was showed in tasting that the propagation conditions had on direct effect on beer quality (Fig. 9A). This was determined not only in the fresh beers but also in stored beers. In addition, the quality of beer which was stored for 4 weeks at 28°C was deteriorated considerably compared to those of the other samples. No significant difference was found among the samples regarding propagation conditions and store duration in the investigation by absorption integral (Fig. 9B). From a practical point of view, it was concluded that the wheat brewing yeast should be propagated under continuous aeration with addition of zinc ion at 30°C.

References

1. Wackerbauer K, Tayama T, Fitzner M, Kunerth S. Proper management of pitching yeast. Brauwelt 3: 80-87 (1997)
2. Martin V, Quain DE, Smart KA. The oxidative stress response of ale and lager yeast strains. EBC Congress 79: 679-686 (1999)
3. Thurston PA, Taylor R, Ahvenainen J. Effects of linoleic acid supplements on the synthesis by yeast of lipids and acetate esters. J. Inst. Brew. 87: 92-95 (1981)
4. Ahvenainen J. Lipid composition of aerobically and anaerobically propagated brewer's bottom yeast. J. Inst. Brew. 88: 367-370 (1982)
5. Cho WI, Choi JB, Lee KP, Cho SC, Park EJ, Chung MS, Pyun YR. Antimicrobial activity of medical plants against *Bacillus subtilis* spore. Food Sci. Biotechnol. 16: 1072-1077 (2007)
6. Lee EJ, Kim SG, Yoo SR, Oh SS, Hwang IG, Kwon GS, Park JK. Microbial cantamination by *Bacillus cereus*, *Clostridium perfringens*, and *Enterobacter sakazakii* in *sunsik*. Food Sci. Biotechnol. 16: 948-953 (2007)
7. Van Iersel MFM, Meersman E, Arntz M, Rombouts FM, Abee T. Effect of environmental conditions on flocculation and immobilization of brewer's yeast during production of alcohol free beer. J. Inst. Brew. 104: 131-136 (1998)
8. Alex speers R, Tung MA, Durance TD, Stewart GG. Biochemical aspects of yeast flocculation and its measurement: A review. J. Inst. Brew. 98: 293-300 (1992)
9. Van Hamersveld EH, Van der Lans RGJM, Caulet PJC, Luyben KChAM. Modeling brewer's yeast flocculation. Biotechnol. Bioeng. 57: 331-341 (1998)
10. Kringstad H, Rasch S. 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 (1996)
11. Pascale B, Dengis D, Rouxhet G. Flocculation mechanisms of top and bottom fermentation brewing yeast. J. Inst. Brew. 103: 257-261 (1997)
12. Van der Aar PC, Strava MH, Teunssen WRH. Flocculation of brewer's lager yeast. EBC Congress 73: 259-266 (1993)
13. Cheong C, Wackerbauer K, Kang SA. Influence of aeration during propagation of pitching yeast on fermentation and beer flavour. J. Microbiol. Biotechn. 17: 297-304 (2007)
14. Donhauser S, Wagner D. Possible effect of zinc contents in wort. Monatsschr. Brauwiss. 6: 223-230 (1986)
15. Brown CM, Johnson B. Influence of oxygen tension on the physiology of *Saccharomyces cerevisiae* in continuous culture. Anton Leeuw. Int. J. G. 37: 477-487 (1971)
16. Kruger L, Pickerell TW, Axcell B. The sensitivity of different brewing yeast to carbon dioxide inhibition: Fermentation and production of flavour-active volitile compounds. J. Inst. Brew. 98: 133-138 (1992)
17. Knatchbull FB. The effect of low CO_2 pressures on the absorption of amino acids and production of flavour-active volitiles by yeast. J. Inst. Brew. 93: 420-424 (1987)
18. Collinson LP, Dawes IW. Inducibility of the response of yeast cells to peroxide stress. J. Gen. Microbiol. 138: 329-335 (1992)
19. Lehmann J, Back W. Practical change of yeast assimilation. Brauindustrie 4: 225-228 (1997)
20. Pfenninger H. Brewing Technical Analysis Method II. 3rd ed. Freising, Germany. pp. 203-207 (1993)
21. Wackerbauer K, Zufall C, Legand J. Discover of taste instability of beer. Brauwelt 40/41: 1796-1803 (1999)
22. Klein H, Krammer R, Natter M. Determination for predict of taste stability of beer. EBC Congress 77: 553-560 (1997)
23. Maemura H, Morimura S, Kida K. Effects of aeration during the cultivation of pitching yeast on its characteristic during the subsequent fermentation on wort. J. Inst. Brew. 104: 207-211 (1998)
24. Van Hamersveld EH, Van Loosdrecht MCM, Van der Lans RGJM, Luyben KChAM. On the measurement of the flocculation characteristics of brewer's yeast. J. Inst. Brew. 102: 333-342 (1996)
25. Garoux G, Haubursin H, Bilbault S, Dufour JP. Yeast flocculation: Biochemical characterization of yeast cell wall components. EBC Congress 73: 275-281 (1993)
26. Kang MH, Saraswat JL, Park YH. Production of lyophilizied culture of *Lactobacillus acidophilus* with preserving cell viability. J. Microbiol. Biotechn. 11: 1055-1060 (1990)
27. Park JE, Lee KH, Jahang DJ. Effect of trehalose on bioluminescence and viability of freeze-dried baterial cells. J. Microbiol. Biotechn. 12: 349-353 (2002)