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Effect of High Pressure Low Temperature Treatment on the Inactivation of *Saccharomyces cerevisiae*

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Introduction

Currently some studies have found that greater microbial inactivation can be achieved by high pressure treatment and many microorganisms are lethally damaged by pressure. The destruction of microorganisms by high pressure was reported 100 years ago (Hite, 1899). A fundamental key for the understanding of bacterial inactivation is the combination of biological and mathematical studies (K.V. Kilimann, 2006). Therefore, the objective of this study was to show the inactivation of *Saccahromyces cerevisiae* in saline solution under different pressurization time (10, 20 and 30 min) to elucidate the influence of HPLT. For the description of HPLT inactivation of *S. cerevisiae*, the activation energy was calculated. In order to show morphological changes of the cell wall, scanning electron microscopic images were observed after HPLT.

Materials and Methods

S. cerevisiae dry yeast was obtained from the Food Company (Ottugi, Korea) and used in the experiments. The cells were diluted respectively in 0.9% saline solution at the same concentration. HPLT was progressed with pressurization fluid at the pressure of 200 MPa and maintained the respective pressurization time (10, 20 and 30 min). Before pressure induction, the temperature of pressurization fluid was set by 4, 12 and 20℃ and the center temperature of the cell suspension was recorded respectively on the pressure treatment. After HPLT, yeast cell suspensions were immediately plated on yeast mold agar (YM) and counted after incubation for 48 h at 37℃. The inactivation was characterized by viable cell counts. Compression to 200 MPa resulted in a temperature upshift of 4℃ or less independent on the sample temperature. The yeast suspension was sealed into a germ-free plastic tube (volume = 2 mL) and kept at 4℃ until use. The yeast in sealed tubes was

exposed to high pressure treatment at 200 MPa at the same temperature and time. Adiabatic heat generated during pressurization was about 4, 12 and 20℃. Treatment temperature was monitored by a digital temperature controller (YOKOGAWA, Japan). Ethanol was used as the pressure medium. Surviving cells after pressurization was estimated by the viable count method using YM agar media (Difco, USA). The plates for *S. cerevisiae* were incubated at 37℃ for 48 h and then the colonies were enumerated. All experiments were carried out in duplicate and the standard deviations were calculated from the duplicate experiments. From the analysis using scanning electron microscopic (SEM) technique, the morphology of yeast cells was observed after HPLT.

Results and Discussions

Combined treatments of pressure and temperature for inactivation of *S. cerevisiae* were investigated within the range of 4~20℃ and 0.1~200 MPa. Effect of HPLT on pH was shown in Table 1.

All of pH-values in HPLT represented higher values than that of the control but not significantly different except 4℃. Depending on the pressure/temperature conditions applied, the activity loss of *S. cerevisiae* could increase up to 100% after the pressure build-up phase. Fig. 1 shows the inactivation behavior of *S. cerevisiae* subjected to the HPLT and time treatment.

The minimum log reduction showed only about 1.0-log reduction after HPLT at 12℃ for 10 min, whereas the maximum log reduction showed about 3.3-log reduction after HPLT at 12℃ for 30 min. Therefore, Fig. 1 shows that the inactivation effect of HPLT for *S. cerevisiae* was higher for 30 min compared to 10 min at any temperature.

The activation energy of *S. cerevisiae* was calculated 8.97 kJ mol^{-1} after HPLT by using Arrenius relations (Fig. 2.). An increase of temperature resulted in a decease of the activation energy. From the results of the present study, it is concluded that *S. cerevisiae*

Table 1. Effect of HPLT time and temperature on pH-value of *S. cerevisiae* at 200MPa.

Temperature (℃)	Treatment time (min)		
	10	20	30
Control	5.92±0.01^{c}	5.91±0.01^{a}	5.89±0.02^{a}
4	6.34±0.01^{a}	6.22±0.04^{a}	6.09±0.26^{a}
12	6.17±0.02^{b}	6.23±0.04^{a}	6.14±0.24^{a}
20	6.11±0.02^{b}	6.17±0.21^{a}	6.04±0.19^{a}

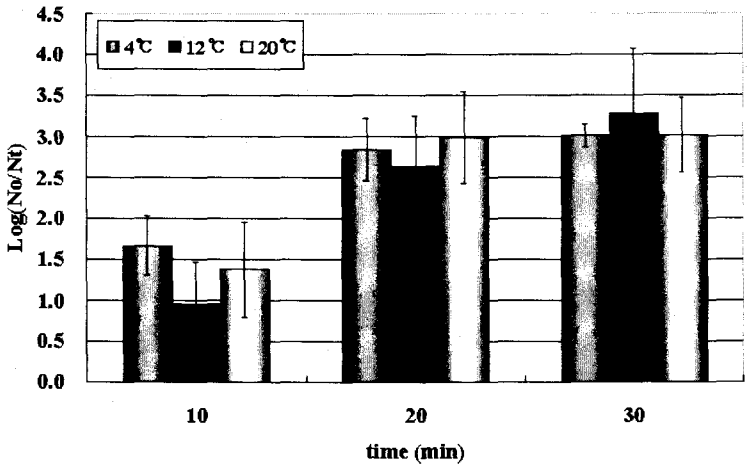

Fig. 1. Effect of HPLT time and temperature on inactivation of *S. cerevisiae* after HPLT at 200 MPa.

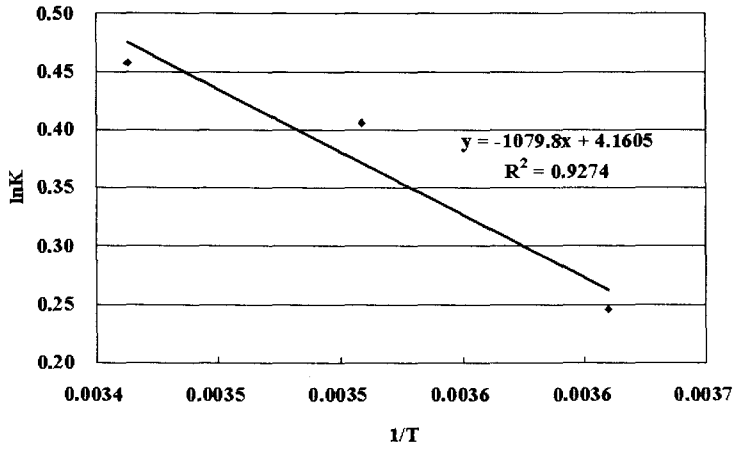

Fig. 2. Arrhenius plot for *S. cerevisiae* in HPLT.

at 200 MPa could sufficiently inactivate the cells. In conclusion, the pressure-inactivation of *S. cerevisiae* is strongly time dependent and *S. cerevisiae* was sensitive to high hydrostatic pressure.

The SEM image (Fig. 3) showed the resulting morphological changes and mechanical stresses generated in *S. cerevisae* cells through high pressure load. Shimada *et al.* (1993) observe disruption of the cell wall at pressures above 400 MPa. However, Ludwig *et al.* (2002) assumed that pressure load on the cell wall induces sever non-hydrostatic stress which might interact with inactivation mechanisms such as denaturation of membrane-bond protein. In this study, disruption of the cell wall at 200 MPa was not observed by SEM image. However, the surface of the cell after HPLT is uneven and showed changes like dimples.

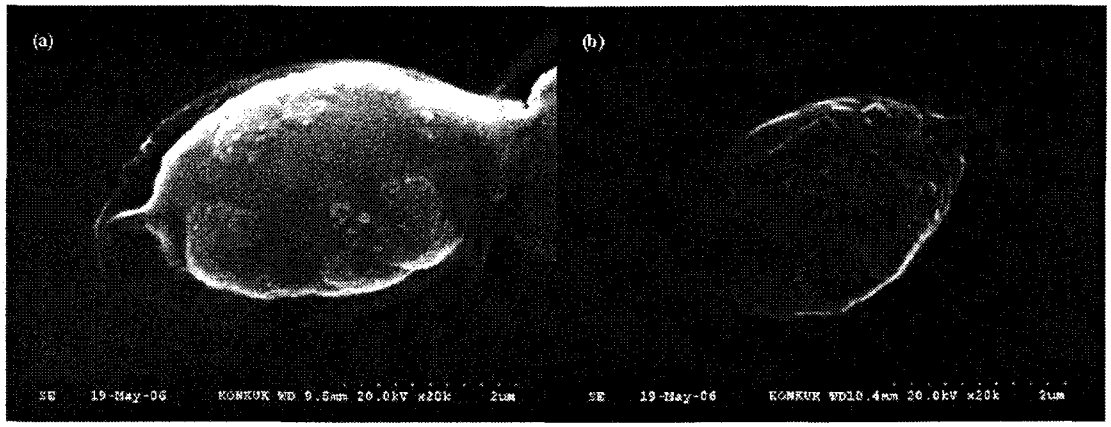

Fig. 3. SEM micrographs of *S. cerevisiae* non-pressurized (a) and pressurized at 200MPa for 30 min at 20℃ (b).

Summary

This study was carried out to investigate the effect of high pressure low temperature (HPLT) on the inactivation rates of *S. cerevisiae* in 0.9% saline solution depending on the pressurization time and temperature. *S. cerevisiae* was inoculated with UHT milk and submitted to HPLT of 200 MPa at 4, 12 and 20°C, respectively. Inactivation increased with pressurization time and HPLT of *S. cerevisiae* at 200 MPa was time dependent at any temperature. The morphological changes of yeast cells observed with a SEM after HPLT.

References

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