Influences of ultrasound and Ohmic heating on growth of Sake yeast

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ABSTRACT

The influences of low intensity ultrasound and Ohmic heating on fermentation of Sake yeast were investigated. The results showed that the low intensity ultrasound promoted the growth of Sake yeast K-7 and increased the glucose consumption rate and ethanol production. After ultrasonic irradiation, a significant increase of intracellular uptake was observed as a result of temporary reversible permeabilization. The promotion effect of growth was frequency dependent, a larger effect was observed at lower frequency within the range (0.3–2.4MHz). In addition, the influences of ultrasonic irradiation in different phases on growth of Sake yeast were quite different. The ultrasonic irradiation during the exponential phase showed the strongest influence on growth of sake yeast and during the stationary phase the influences were not obvious. During the Ohmic heating treatment, the glucose consumption rate and the ethanol production were increased and the promotion effect of cell growth was higher at 3 KHz than that of 60 Hz. However, the influence of electricity consumption on growth of Sake yeast was not obvious. Meanwhile, the combined effect of ultrasound and Ohmic heating on the growth of Sake yeast was approximately as same as those of separate treatments. These results show that the low intensity ultrasound and Ohmic heating can be applied to improve the efficiency and quality of fermentation as one of the more advanced food technologies.

Keywords: Low intensity ultrasound; Ohmic heating; Sake yeast Kyokai No.7 (K-7);

INTRODUCTION

The diffusion boundary layer close to the cell surface is considered to be the barrier of mass transportation in or out the cell and so that the cell growth might be inhibited. On the other hand, the effects of ultrasound on propagation media were considered to be thermal effect, vibrational effect, pressure fluctuation, etc. The vibrational effect of ultrasound causes a vibration of cells and medium, and the minimization of the boundary layer can be expected. Moreover, the pressure fluctuation by ultrasound can induce stresses on the cells, and it is possible to promote the proliferation and metabolism of the microorganisms. Several other studies have demonstrated that the low intensity ultrasound activated the proliferation of lactic acid bacteria, fungi and animal cells [1,2,3,4,5]. Ohmic heating occurs when an electric current is passed through food, resulting in a temperature rise in the product due to the conversion of the electric energy into heat (Joule effect). Advantages of that are uniform heating and short time process [6]. Ohmic heating by alternating current also have shown some effects on the growth of microorganisms [7,8]. In this research, the influences of ultrasound and Ohmic heating on the growth of Sake yeasts were investigated at various frequencies and power output.

MATERIALS & METHODS

Microorganism

The Sake yeast (Saccharomyces cerevisiae, registered as Kyokai No.7 (K-7) for Japanese Sake making to the Brewing Society of Japan) obtained from Industrial Research Institute of Ishikawa was used.

Culture medium

The composition of the modified medium (YM-10) was as follows (per liter of deionized water): glucose (100g), yeast extract (3g), malt extract (3g), polypeptone (5g).

Experimental apparatus

As shown in Fig.1, a new experimental apparatus in combination with ultrasonic irradiation system and Ohmic heating system was developed. The apparatus is capable of conduct of ultrasonic irradiation and Ohmic heating separately or simultaneously. The ultrasonic irradiation system is constituted of a signal generator (WF1974, NF Corporation), an amplifier (HSA4052, NF Corporation), an ultrasonic transducer installed in one side of the water tank, and a small culture vessel with two holes covered with a thin
polyethylene film through which the ultrasound wave can propagate into the culture media. In order to avoid the influences of reflection waves, an absorber was set up at the opposition side of the water tank to ultrasonic transducer. For Ohmic heating, two titanium square electrodes were inserted in the small culture vessel described above. The on/off regulation of electric current was conducted by a temperature controller (KT-4, Matashita Electric works., Ltd) in order to maintain the requested culture temperature. And the actual temperature in the small culture vessel was recorded with a temperature logger. The frequencies and applied voltages can be modified by a signal generator and an amplifier.

**Ultrasound treatment**

Sake yeast K-7 was cultured in the small culture vessel described above. The culture temperature was maintained at 30°C by a low temperature circulating bath shown in Fig.1. The ultrasonic irradiations were performed at different frequencies (0.3~2.4 MHz) and applied voltages (3~24 V) for various irradiation durations. The ultrasound intensity distributions in the culture vessel were measured in advance with a sonic monitor (HUS-3, Honda Electronics Co., Ltd). During fermentation, the yeast cell density was determined by counting in a hemacytometer and the chemical composition changes of culture media were also determined by 13C-NMR.

**Intracellular uptake**

To study intracellular uptake, Sake yeast K-7 were culture in YM-10 medium in the presence of 10 μM calcein. After ultrasonic irradiation of 2.4MHz at 24V for 48h, cells were recovered for 5min at 500×g to remove excess calcein from the extracellular medium, followed by washing at least twice in phosphate buffered saline (PBS). To determine cell viability, 7.5μM propidium iodide (PI) was added to cell samples prior to assaying via fluorescence microscopy.

**Ohmic heating treatment**

In this experiment, the culture temperature of Sake yeasts K-7 was kept at 30°C through Ohmic heating at different frequencies of 60 Hz and 3 KHz at a fixed voltage of 3.2Vcm⁻¹. In order to control the electricity consumption during Ohmic heating, the temperature difference between the water bath and the small culture vessel was adjusted by the low temperature circulating bath shown in Fig.1. The cell density was determined as above during fermentation. And the glucose concentration in medium was measured with Glucose C2-Test Kit (Wako pure chemical industries, Ltd) according to the manufacturer's instructions. The ethanol productions were measured by Head-Space GC with a modified method according to Portari et al.[9].

**Combination of ultrasound and Ohmic heating treatment**

To study the combined effect of ultrasonic irradiation and Ohmic heating, Sake yeasts K-7 were cultured with an ultrasonic irradiation of 2.4MHz at 24V. The culture temperature was maintained at 30°C through Ohmic heating at frequency of 3 KHz while the temperature of the water bath was set up at 28°C. The cell density was determined as above.
RESULTS & DISCUSSION

Effects of low intensity ultrasound on Sake yeasts

The growth rates of Sake yeast increased by the ultrasound irradiation at applied voltages of 6V, 12V and 24V respectively with a fixed frequency of 2.4MHz, and the final cell numbers were larger than that of control (Fig.2A). Additionally, an increase of glucose consumption rate and ethanol production was observed by $^{13}$C-NMR compared with control (Fig.3). Considering the ultrasound frequency on cell growth, we have found a tendency that the promotion effect of cell growth increased with the frequency decrease in range of 0.3~2.4MHz at an applied voltage of 24V (Fig.2B). Therefore, the mechanism might be related to mechanical stimulation of cells or increased uptake of extracellular molecules [10] rather than vibration, which increases as the ultrasound frequency increases. Several other studies have demonstrated that the low intensity ultrasound promotes cell proliferation via activation of DNA and protein synthesis [5,11,12].

![Figure 2](image1.png)

**Figure 2.** Influence of ultrasound irradiation on growth of Sake yeast. (A) influence of ultrasound intensity (B) influence of ultrasound frequency.

![Figure 3](image2.png)

**Figure 3.** $^{13}$C-NMR spectrum of culture medium of Sake yeast. (A) medium before fermentation (B) medium after fermentation without ultrasonic irradiation for 48h (C) medium after fermentation with ultrasonic irradiation at 2.4MHz for 48h.

The results shown in Fig.4 have suggested that the influences of ultrasonic irradiation in different phases on growth of Sake yeast is quite different, which was probably because the shapes, structures, and physiological conditions of Sake yeast were quite different. The ultrasonic irradiation during the exponential phase showed the strongest influence on growth of sake yeast and during the stationary phase the influences were not obvious.
Figure 4. Influence of ultrasonic irradiation in different phases on growth of Sake yeast.

Effects of low intensity ultrasound on permeability of Sake yeasts
After ultrasonic irradiation, a significant increase of green fluorescence corresponding to intracellular uptake of calcein was observed when compared with no irradiation, but there was no significant difference in PI red fluorescence, so it suggests that the Sake yeasts were still alive after ultrasonic irradiation. These results indicate that the ultrasonic irradiation might enhance diffusion of nutrients and metabolites across cell membranes as a result of temporary reversible permeabilization (Fig. 5).

Figure 5. Changes of fluorescence microscopic images of Sake yeast. (A) control treatment (B) ultrasonic irradiation at 2.4MHz for 48 h.

Effects of Ohmic heating treatment on Sake yeast
The culture temperature of Sake yeast has been successfully controlled through Ohmic heating (Data not shown). During the Ohmic heating treatment, the growth rates were increased and the final cell numbers were larger than that of control. We also found that the promotion effect of cell growth was higher at 3 KHz than that of 60 Hz (Fig.6A). It is now well known that the impedance of cell membrane is frequency dependent, decreasing as the electric frequency increases. So that a more fraction of the currents penetrate the cells at higher frequency and that in consequence, cells growth was promoted greatly.

Figure 6. Influence of Ohmic heating on growth of Sake yeast. (A) influence of electric frequency (B) influence of electricity consumption.
Meanwhile as shown in Fig.6B, the influence of electricity consumption on growth of Sake yeast was not obvious. In addition, there was an increase of glucose consumption rate and ethanol production observed during the Ohmic heating treatment (Fig.7).

![Graph showing glucose consumption and ethanol production](image)

**Figure 7.** Influence of Ohmic heating on glucose consumption and ethanol production during Sake yeast fermentation.

**Combined effect of ultrasound and Ohmic heating on the growth of Sake yeasts**

As shown in Fig.8, the combined effect of ultrasound and Ohmic heating on the growth of Sake yeast was approximately as same as those of separate treatments.

![Graph showing cell density](image)

**Figure 8.** Influence of combination of ultrasonic irradiation and Ohmic heating on Sake yeast.

**CONCLUSION**

The results of the present study show that the growth and metabolism of Sake yeast K-7 could be promoted by the low intensity ultrasound and Ohmic heating. As one of the more advanced food technologies, it can be applied not only to improve the efficiency and quality of fermentation but offers the potential for developing new products with little capital input.

In order to elucidate the mechanisms, further studies are under way to determine influences of ultrasound and Ohmic heating on DNA and protein synthesis of yeast.

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REFERENCES


