

# The Studies on the Enzymatic Replacement of Traditional Koji in Sake Brewing

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## ABSTRACT

Sake is a traditional alcohol beverage of Japan produced from steamed rice by utilization of two microorganisms, *Aspergillus oryzae* and *Saccharomyces cerevisiae*. The traditional koji-brewing of sake has many factors that are difficult to maneuver and need experienced people to execute a suitable adjustment. These factors had become obstacles to large scale brewing of sake. The aim of this study was to study the feasibility of replacing koji with enzymes in the brewing of sake and to optimize the conditions of sake brewing process that employs enzymatic treatment. The D.E. value of rice mash reached 13 degrees after the addition of 2 mL  $\alpha$ -amylase (Spezyme R AA) per kilogram of rice and liquefied for 90 min. The D.E. value of liquefied rice mash reached 77.86 after saccharification with Optimax R HP7525 for 72 hrs. However, using Fermentzyme R L-400 in mash making can increase the rate of yeast growth. Nonetheless, the sensory results of sake brewed with Optimax R HP7525 were better. Addition of cellulase had assisted saccharification enzymes in digesting rice, and increased 11 degree of D.E. value of rice mash after 72 hrs. The addition of cellulase to sake brewing process improved the fermentation rate, and the alcohol content reached 15% (v/v) on the 10th day of fermentation. The brewing of sake with six commercial yeast strains had no manifest difference, and all alcohol contents reached 15% (v/v) after fermenting for 14 days. The addition of 6 grams of yeast nutrient per kilogram of rice resulted in best brew with yeast count reaching  $1.01 \times 10^{10}$  CFU/mL on the sixth day. The average sensory scores of sake brewed with Italian Zymoform strain were the best. The rate of sake mash fermentation was the fastest at 35 °C, but its sensory acceptability was bad. Therefore, the fermentation temperature was deemed suitable at 25 °C. Additionally, the Sparkolloid R combined with gelatin and silica gel had the best clarification effect and its sensory result was also the most encouraging. The flavor, taste and color of sake brewed with enzymes replacing koji were not changed significantly after 3 months of storage.

Keywords : sake, amylase, rice-koji, clarification, yeast

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